Biochem. J. (1990) 269, 61–64 (Printed in Great Britain)

The tubulin-binding sequence of brain microtubule-associated proteins, tau and MAP-2, is also involved in actin binding

Isabel CORREAS, Rodolfo PADILLA and Jesús AVILA
Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

The interaction of actin with a synthetic peptide which corresponds to one of the repeated tubulin-binding sites present in tau and MAP-2 (microtubule-associated protein 2) proteins has been analysed. The analysis, which uses affinity chromatography of G-actin on a column containing the synthetic peptide, and the co-sedimentation and co-localization of F-actin and the peptide (as determined by immunoelectron microscopy), indicates that the part of the amino acid sequence of tau involved in the binding of tubulin is also involved in actin binding.

INTRODUCTION

The knowledge of the interconnections between the major filament systems of the cytoskeleton is a necessary step for the understanding of the cytomatrix organization [1]. There is little evidence *in vivo* supporting the hypothesis that the two main types of filaments (microtubules and microfilaments) are interconnected, although there are some indications which suggest that such interactions take place. For example, it is known that actin-filament-disrupting agents inhibit fast axonal transport, a microtubule-dependent movement [2]. By contrast, a number of studies *in vitro* with purified actin and microtubule proteins have been carried out. Viscometry, photobleaching, co-sedimentation and electron-microscopic analysis have documented extensively the existence of interactions *in vitro* between microtubules and microfilaments [3–5]. It has been indicated that whereas tubulin is probably not involved in this interaction, microtubule-associated proteins (MAPs) do seem to associate with actin [3,6]. At least two MAPs, MAP-2 and tau, bind to actin by forming aggregates or bundles of microfilaments [5–8]. Other MAPs, such as MAP-1, probably also associate with actin [9]. Pollard *et al.* [10] have described the presence of two actin-binding sites on MAP-2, one located on, or close to, the tubulin-binding domain [5,10]. More recently, Sattilaro [11] has reported that actin binds to a 32 kDa fragment of MAP-2 which also contains the tubulin-binding site. The tubulin-binding domain of MAP-2 has recently been reported [12] and is composed of three repeated sequences. Almost identical sequences have been reported for tau protein, although in this case three or four repeats are present [13,14]. A single repeat is sufficient for tubulin binding [15].

We have synthesized a peptide corresponding to one of the tubulin-binding-site repeats of tau protein and analysed its interaction with actin. Our results indicate that this peptide binds to tubulin as well as to actin.

MATERIALS AND METHODS

CNBr-activated Sepharose 4B and *Staphylococcus aureus* Protein A were purchased from Pharmacia (Piscataway, NJ, U.S.A.). BSA and ovalbumin were obtained from Sigma (St. Louis, MO, U.S.A.). The peptide KVTSKCGSLGNIHHKPGGG, which contains the tau-protein tubulin-binding site [13,14], was obtained by chemical synthesis using an automatic solid-phase peptide synthesizer (430A Applied Biosynthesis). The peptide was further purified by reverse-phase h.p.l.c. using a Nova-Pak C_{18} column.

Microtubule protein was obtained from bovine brain by two temperature-dependent assembly–disassembly cycles [16] and stored as pellets at −70 °C. Immediately before use, the microtubule pellets were resuspended in 0.1 M-Mes (pH 6.4)/0.5 mM-$MgCl_2$/2 mM-EGTA/1 mM-GTP (buffer A), and a third assembly cycle was performed. Tubulin depleted of MAPs was obtained by phosphocellulose chromatography [17], and tau protein was isolated as indicated by Herzog & Weber [18].

Actin was isolated from rabbit skeletal muscle by the method of Spudich & Watt [19]. G-actin was stored at −70 °C in 2 mM-Tris/HCl (pH 8.0)/0.2 mM-ATP/0.2 mM-$CaCl_2$/0.5 mM-dithiothreitol/25% (v/v) glycerol.

Polyclonal anti-tau antibodies were raised in rabbits as previously described [20]. Antibodies against the synthetic peptide were obtained after cross-linking of the peptide with keyhole-limpet hemocyanin by the method of Bulinski & Gundersen. [21].

Affinity chromatography

A portion (1 mg) of the synthetic peptide containing the tubulin-binding site was mixed with 0.3 g of CNBr-activated Sepharose 4B in a buffer which contained 0.1 M-$NaHCO_3$, pH 8.5, and 0.5 M-NaCl. The mixture was incubated and gently agitated for 2 h at 30 °C, and the coupling of the peptide to the resin was stopped by the addition of 0.1 M-Tris, pH 7.0. The resin was washed and equilibrated with buffer A (for tubulin chromatography) or with a 1/50 vol. of buffer A supplemented with 0.2 mM-$CaCl_2$, 0.5 mM-dithiothreitol and 0.2 mM-ATP (for actin chromatography). Protein bound to the column was eluted by addition of 0.5 M-NaCl in buffer A (for tubulin chromatography) or by decreasing the pH to 2.9 by addition of 0.2 M-glycine/HCl for actin chromatography, since the interaction between MAPs and actin is pH-dependent [8].

Co-polymerization studies

A constant amount of actin (140 μg) was incubated with either tau (37 μg) or the synthetic peptide of tau which contained the tubulin-binding site (2.6 μg) in a total volume of 200 μl in buffer A. Separate samples containing only actin or the synthetic tau peptide were also included. The incubation mixtures were then warmed to 37 °C for 30 min to assemble the actin filaments. For sedimentation studies, 100 μl of each sample was layered on a 100 μl cushion of 10% (w/v) sucrose in buffer A and centrifuged

Abbreviations used: MAPs, microtubule-associated proteins; PBS, phosphate-buffered saline (0.010 M-sodium phosphate/0.140 M-NaCl, pH 7.4).

at room temperature for 20 min at 150000 *g* in a Beckman Airfuge. Equivalent portions of the supernatant and the pellet protein fractions were treated as follows.

(*a*) They were dotted on to nitrocellulose sheets, blocked with 0.05% Tween-20 in phosphate-buffered saline (PBS), incubated with the anti-(synthetic tau peptide) antibody (1:200 dilution in PBS, containing 0.05% Tween-20), followed by incubation with ^{125}I-Protein A diluted in PBS containing 0.05% Tween-20. Samples were autoradiographed, and the radioactivity in each sample was quantified in a γ-radiation counter.

(*b*) They were analysed by gel electrophoresis as described by Hashimoto *et al.* [22], transferred to nitrocellulose paper and immunoblotted according to the method of Towbin *et al.* [23].

Electronmicroscopy analyses

Samples containing actin filaments were placed on carbon/collodion-coated grids and stained for 30 s in aq. 1% uranyl acetate.

For immunoelectron-microscopic analyses, aliquots of actin/MAP mixtures were fixed in solution in the presence of 0.5% glutaraldehyde for 10 min at room temperature. The reaction was stopped by addition of 0.2 M-NH_4Cl. Samples were then applied to carbon/collodion-coated grids, blocked with 2% ovalbumin, followed by incubations with polyclonal antibodies against tau factor (1:100 dilution in buffer A, containing 2% ovalbumin) or the synthetic tau peptide (1:100 dilution). Samples were further incubated with Protein A labelled with 5 nm gold particles for 30 min at room temperature and examined under a Jeol 100B electron microscope.

RESULTS

To analyse the possible association of actin with the peptide of tau protein which contains one of the tubulin-binding sequences, we first performed affinity-chromatography studies with the synthetic peptide coupled to Sepharose. Purified G-actin was retained in the column, as determined by measurements of the A_{280} (Fig. 1*a*). Tubulin, used as a positive control for the experiment, was also retained in the column (Fig. 1*c*), whereas BSA and ovalbumin, the negative controls, were not (Fig. 1*b*). After an increase in the ionic strength (in the case of tubulin) or a decrease in the pH (in the case of actin), the bound proteins were eluted and characterized by gel electrophoresis (insets in Fig. 1). These results suggest that the peptide containing the tubulin-binding site is also capable of binding to actin.

The association of the synthetic tau peptide with actin was also demonstrated by co-polymerization experiments carried out with purified F-actin. Fig. 2 shows that both the synthetic tau peptide and the whole tau protein co-sediment with F-actin with similar efficiency. Fig. 3 indicates the characterization by gel electrophoresis of the protein present in the soluble or the polymerized fraction after mixing the synthetic tau peptide and actin. Tau peptide moves on the gel as a wide band (as is usually found for small peptides), and it was observed in the sedimented protein fraction only when actin was present (Fig. 3, lane 2).

The binding of the synthetic tau peptide with F-actin was further confirmed by electron-microscopic analyses. Fig. 4 shows micrographs of negatively stained F-actin incubated in absence (Fig. 4, part c) or in the presence of tau protein (Fig. 4, part b) and those of F-actin incubated in the presence of the synthetic tau peptide (Fig. 4, part a). Tau protein aggregates actin filaments into bundles (Fig. 4, part b), as previously described [6], whereas the synthetic tau peptide does not (Fig. 4, part a). This suggests that the synthetic tau peptide may bind to F-actin, but, since it contains only one actin-binding site, no bundle formation was observed, since this may require the binding of a tau molecule to

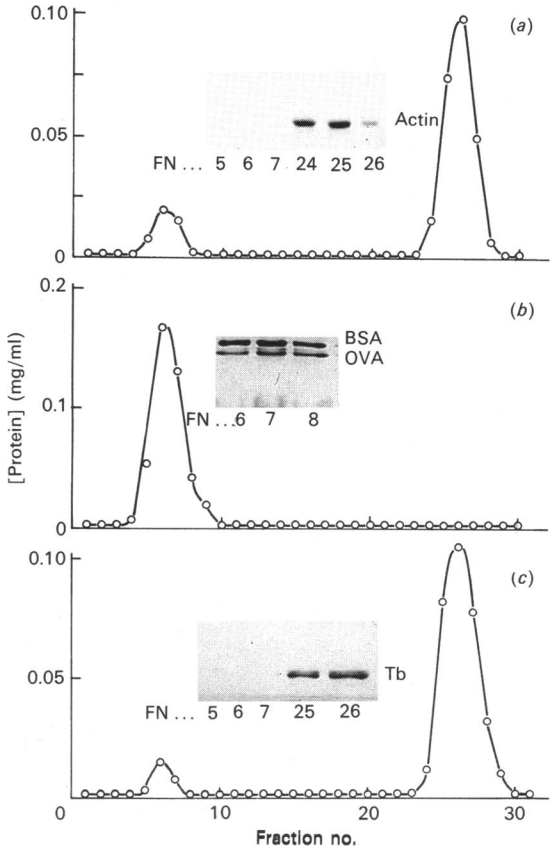

Fig. 1. Binding of the synthetic tau peptide to G-actin

The synthetic tau peptide was coupled to CNBr-activated Sepharose 4B. A 100 μg portion of G-actin (*a*), 100 μg each of BSA and ovalbumin (OVA) (*b*) or 100 μg of tubulin (Tb) (*c*) were loaded on the column as described in the Materials and methods section. Each protein sample was used at a concentration of 1 mg/ml. Bound proteins were eluted by addition of 0.5 M-NaCl in buffer A, except in the case of G-actin, which was eluted by lowering the pH. Insets represent the electrophoretic profiles of the indicated fractions. Abbreviation: FN, fraction no.

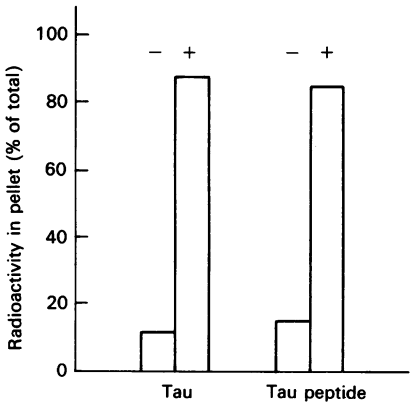

Fig. 2. Binding of the synthetic tau peptide to F-actin

Tau protein (37 μg) and the synthetic tau peptide (2.6 μg) were separately incubated in the absence (−) or in the presence (+) of actin (140 μg) under the conditions described in the Materials and methods section. Samples were loaded on to a 10%-(w/v)-sucrose cushion in buffer A and centrifuged at 150000 *g* for 20 min in a Beckman Airfuge. Pellet fractions were dotted on to nitrocellulose paper, and incubated with a polyclonal antibody against the synthetic tau peptide and with ^{125}I-Protein A. Radioactivity was quantified in a γ-radiation counter. The total radioactivity in the pellet was: for the tau protein, 305 c.p.m.; for the synthetic peptide, 807 c.p.m.

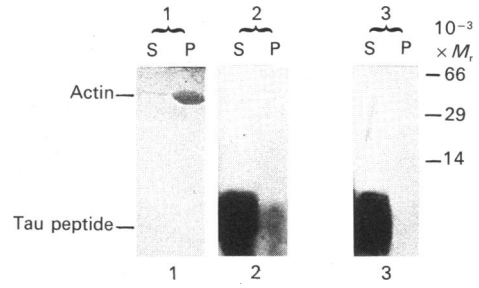

Fig. 3. Sedimentation analysis of tau binding to actin

Actin alone (lanes 1) or the synthetic tau peptide incubated either in the presence of actin (lanes 2) or in its absence (lanes 3) were incubated under the conditions described in the Materials and methods section to allow the assembly of actin. Samples were loaded on a 10%-sucrose cushion in buffer A and centrifuged at 150000 *g* for 20 min in a Beckman Airfuge. Supernatants (S) and pellets (P) were loaded on a 10–18%-acrylamide-gradient/SDS gel containing 7 M-urea, with an acrylamide/bisacrylamide ratio of 20:1 (w/w) [22]. Proteins were transferred to nitrocellulose paper as described by Towbin *et al.* [23]. The paper was incubated with the anti-tau antibody and subsequently with ^{125}I-Protein A from *S. aureus*. 1, Amido Black-stained proteins; 2 and 3, autoradiogram of immunoblots.

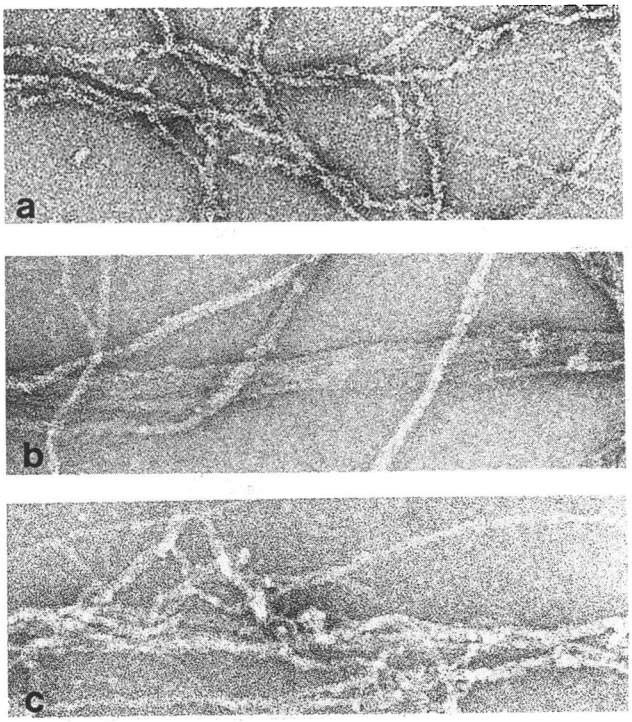

Fig. 4. Electron micrographs of negatively stained specimens of actin filaments alone (c), actin filaments exposed to tau protein (b) and actin filaments exposed to the synthetic tau peptide (a) (magnification × 150000)

two actin molecules. To corroborate the binding of the synthetic tau peptide to F-actin, a more direct analysis was performed by incubating the polymers with anti-(synthetic tau peptide) antibody, followed by addition of Protein A labelled with gold particles. Fig. 5, part a, shows that the synthetic tau peptide is bound to the actin filaments. Tau protein, the positive control, was also bound to F-actin, as determined by using the antibody against the synthetic tau peptide (Fig. 5, part b). A negative control in which the preimmune serum of the rabbit was used is also shown (Fig. 5, part c).

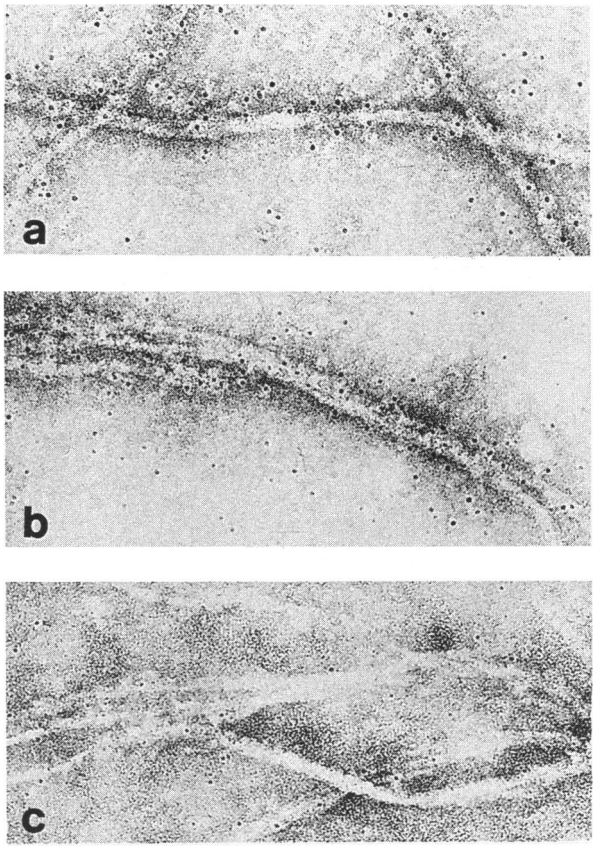

Fig. 5. Immunoelectron-microscopic analysis of synthetic tau peptide binding to actin

Actin was incubated with the synthetic tau peptide (a) or with tau protein (b) or alone (c) under the conditions described in the Materials and methods section. Samples were loaded on a grid and incubated with a polyclonal antibody against the synthetic tau peptide for 1 h. Protein A labelled with 5 nm gold particles was then added and incubated for 30 min. The number of gold particles/0.1 μm was counted in seven different samples and the following values were obtained: a, 13±2; b, 16±4; and c, 2±1. (Magnification ×250000.)

DISCUSSION

Both MAP-2 and tau proteins have been shown to bind to actin filaments [5–8]. The site at which MAP-2 binds to actin has been located on a 32 kDa fragment of the MAP-2 molecule which contains the tubulin-binding site [11]. The molecular cloning of both MAP-2 and tau has allowed the localization of the tubulin-binding site in these two proteins to a small region of the molecule which comprises a consensus motif repeated several times in both MAP-2 and tau molecules [12–15]. This study defines that the peptide sequence KVTSKCGSLGNIHHKPGGG, which corresponds to one of these tubulin-binding motifs, also binds to actin. As this sequence, which is shared by tau and MAP-2, is involved in the binding of tubulin as well as of actin, it might be suggested that actin and tubulin share a similar sequence responsible for the binding of tau and MAP-2 proteins. As the tau [24,25] and MAP-2 [25–27] binding sites on tubulin have been described, we have searched for a similar sequence on the actin molecule. Fig. 6 shows the result of the comparison of tubulin [28,29] and actin [30,31] sequences. The *C*-terminal regions of these two proteins (amino acids 433–440 of tubulin and 362–368 of actin) show a certain identity, since five amino acids are identical and two show conservative changes. This

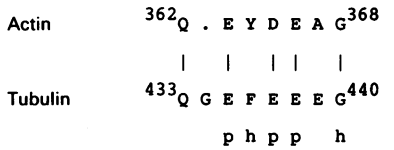

Fig. 6. Sequence similarity between the *C* terminal sequences of actin and tubulin molecules

h, Hydrophobic amino acids; p, polar amino acids.

suggests that MAP-2 and tau may bind to that region of actin as they do to the corresponding tubulin region. Interestingly, gelsolin, an actin-binding protein, associates with actin at the region containing amino acids 329–369 [32,33].

The synthetic tau peptide binds to G-actin (Fig. 1) as well as to F-actin (Figs. 2, 3 and 5); however, it is unable to associate actin filaments into bundles (Fig. 4). By contrast, MAP-2 and tau proteins are capable of bundling microfilaments [5–8], which suggests that bundle formation may require more than one actin-binding site. Since the existence of more than one binding site on tau or MAP-2 molecules may be required to establish the interconnections between cytoskeletal polymers, the saturation of those binding sites by large numbers of actin or tubulin molecules could prevent the establishment of such connections. Competition of MAPs for microtubules or microfilaments has been suggested in some systems [11,34]. It has also been proposed that the presence of gelsolin prevents fast axonal transport, a microtubule-dependent movement [35].

The presence of MAPs in association with actin has been described in physiological situations, as in the case of MAP-2, which could act as an actin-binding protein during the initial phase of dendritic growth in neurons [36]. In pathological situations, MAP-2 and tau proteins have been detected in structures composed of actin but lacking tubulin, such as the Hirano bodies [37,38].

These findings indicate that MAP-2 and tau could be considered not only as microtubule-connecting proteins, but also as actin-binding proteins, microfilament-connecting (bundling) proteins or microtubule–microfilament-connecting proteins [3–11].

This work was supported by the Spanish Comision Asesora para la Investigación Cientifica y Técnica. We thank J. E. Domínguez for his generous help.

REFERENCES

1. Satir, P. (1984) J. Cell Biol. **99**, 235s–238s
2. Isenberg, G., Schubert, P. & Kreutzberg, G. W. (1980) Brain Res. **194**, 488–593
3. Griffith, L. & Pollard, T. (1984) J. Cell Biol. **78**, 958–965
4. Arakawa, T. & Frieden, C. (1984) J. Biol. Chem. **259**, 11730–11734
5. Sattilaro, R. F., Dentler, W. L. & Lecluyse, E. L. (1981) J. Cell Biol. **90**, 467–473
6. Griffith, L. & Pollard, T. (1982) J. Biol. Chem **257**, 9143–9151
7. Selden, C. S. & Pollard, T. (1983) J. Biol. Chem. **258**, 7064–7071
8. Nishida, E., Kuwaki, T. & Sakai, H. (1981) J. Biochem. (Tokyo) **90**, 575–578
9. Asai, D. J., Thompson, W. C., Wilson, L., Dresden, C. F., Schulman, H. & Purich, D. L. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 1434–1438
10. Pollard, T., Selden, C. & Maupin, P. (1984) J. Cell Biol. **99**, 33s–37s
11. Sattilaro, R. F. (1986) Biochemistry **25**, 2003–2009
12. Lewis, S. A., Wang, D. & Cowan, N. J. (1988) Science **242**, 936–939
13. Lee, G., Cowan, N. & Kirschner, M. (1988) Science **239**, 285–288
14. Goedert, M., Spillantini, M. G., Potier, M. C., Ulrich, J. & Crowther, R. A. (1989) EMBO J. **8**, 393–399
15. Lee, G., Neve, R. & Kosik, K. (1989) Neuron **2**, 1615–1624
16. Shelanski, M. L., Gaskin, F. & Cantor, C. R. (1973) Proc. Natl. Acad. Sci. U.S.A. **70**, 765–768
17. Weingarten, M. D., Lockwood, A., Hwo, S. Y. & Kirschner, M. W. (1975) Proc. Natl. Acad. Sci. U.S.A. **72**, 1858–1862
18. Herzog, W. & Weber, K. (1978) Eur. J. Biochem. **92**, 1–8
19. Spudich, J. E. & Watt, S. (1971) J. Biol. Chem. **246**, 4866–4871
20. Montejo, E., Díez, J. C. & Avila, J. (1986) Biochem. Biophys Acta **881**, 456–461
21. Bulinski, J. C. & Gundersen, G. G. (1986) Methods Enzymol. **134**, 453–460
22. Hashimoto, T., Horigome, T., Kanbayashi, M., Yoshida, K. & Sugano, H. (1983) Anal. Biochem. **129**, 192–199
23. Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
24. Serrano, L., Montejo de Garcini, E., Hernandez, M. A. & Avila, J. (1985) Eur. J. Biochem. **153**, 595–600
25. Littauer, U. Z., Giveon, D., Thierauf, M., Ginzburg, I. & Postingl, H. (1985) in Microtubules and Microtubule Inhibitors (De Brabander, M. & De Mey, J., eds.), pp. 171–176, Elsevier, Amsterdam
26. Serrano, L., de la Torre, J., Maccioni, R. B. & Avila, J. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 5959–5993
27. Serrano, L., Avila, J. & Maccioni, R. B (1984) Biochemistry **23**, 4675–4681
28. Wang, D., Villasante, A., Lewis, S. A. & Cowan, N. J. (1986) J. Cell Biol. **103**, 1903–1910
29. Krauhs, E., Little, M., Kempf, T., Hofer-Warbinek, R., Ade, W. & Postingl, H. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 4156–4160
30. Collins, J. H. & Elzinga, M. (1975) J. Biol. Chem. **250**, 5915–5920
31. Vandekerckhove, J. & Weber, K. (1978) Eur. J. Biochem. **90**, 451–462
32. Tellam, R. L., Morton, D. J. & Clarke, F. M. (1989) Trends Biochem. Sci. **14**, 130–133
33. Vandekerckhove, J. (1989) Curr. Opinions Cell Biol. **1**, 15–22
34. Danowski, B. A. (1989) J. Cell Sci. **93**, 255–266
35. Goldberg, D. J., Harris, D. A., Lubit, B. W. & Schwartz, J. H. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 7448–7452
36. Bernhardt, R. & Matus, A. (1982) J. Cell Biol. **92**, 589–593
37. Peterson, C., Kress, Y., Vallee, R. & Goldman, J. E. (1988) Acta Neuropathol. **77**, 168–174
38. Galloway, P. G., Perry, G., Kosik, K. & Gambetti, P. (1987) Brain Res. **403**, 337–340

Received 24 October 1989/4 January 1990; accepted 10 January 1990