Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1561–1565, February 1990
Neurobiology

Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport

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Communicated by Keith Porter, November 27, 1989

ABSTRACT **In the brains of aged humans and cases of Alzheimer disease, deposits of amyloid in senile plaques are located in proximity to nerve processes. The principal component of this extracellular amyloid is β/A4, a peptide derived from a larger amyloid precursor protein (APP), which is actively expressed in brain and systemic organs. Mechanisms that result in the proteolysis of APP to form β/A4, previously termed β-amyloid protein, and the subsequent deposition of the peptide in brain are unknown. If β/A4 in senile plaques is derived from neuronally synthesized APP and deposited at locations remote from sites of synthesis, then APP must be transported from neuronal cell bodies to distal nerve processes in proximity to deposits of amyloid. In this study, using several immunodetection methods, we demonstrate that APP is transported axonally in neurons of the rat peripheral nervous system. Moreover, our investigations show that APP is transported by means of the fast anterograde component. These findings are consistent with the hypothesis of a neuronal origin of β/A4, in which amyloid is deposited in the brain parenchyma of aged individuals and cases of Alzheimer disease. In this setting, we suggest that APP is synthesized in neurons and delivered to dystrophic nerve endings, where subsequent alterations of local processing of APP result in deposits of brain amyloid.**

A pathological hallmark of the brains of individuals with Alzheimer disease (AD) is the formation of senile plaques, which consist of abnormal axonal and dendritic processes (neurites) surrounding extracellular deposits of amyloid fibrils (1, 2). The amyloid in plaque cores is composed of a 4-kDa peptide, β/A4 (3, 4), which is derived by proteolysis from the transmembrane amyloid precursor protein (APP). At least three APP cDNAs have been identified: APP-695, APP-751, and APP-770 (5–8). The latter two APP transcripts encode additional domains that share structural homologies with the Kunitz family of serine protease inhibitors (6–8). In cerebral cortex, APP mRNAs are transcribed actively in neurons (9, 10), but the function of APP isoforms is largely unknown. Moreover, proteolytic events that cleave β/A4 from APP, leading to amyloid deposition, are not clear (11). However, two prevailing hypotheses, not mutually exclusive, argue for neuronal (4, 12) and vascular (3, 13) origins of β/A4, previously termed β-amyloid protein. Evidence for a neuronal origin centers on two principal findings: the topographic distribution of senile plaques within gray matter (4, 12) and the neuronal localization of all APP mRNAs, including high levels of expression of the brain-enriched transcript (APP-695) (9, 14). However, to date, a consistent correlation between levels of APP expression in brain and the distribution of deposits of amyloid has not been clearly established (10, 15–19).

The rationale for the present investigation is based on the hypothesis that, if β/A4 is derived from neuronal APP and is deposited at locations remote from the site of synthesis, APP must be translocated from perikarya to distal terminals in proximity to sites of amyloid deposition. Because APP is a membrane-associated glycoprotein (5, 20, 21), the fast axonal transport of membrane components (22) is a mechanism by which APP can reach neurites that surround extracellular deposits of amyloid. Therefore, we suggest that APP, axonally transported from perikarya to neuritic endings, is aberrantly processed at sites of amyloid deposition to form parenchymal β/A4 (11). In this study, we used an experimental paradigm that involved axonal blockage by nerve ligation in combination with three immunodetection methods to examine the anterograde transport of APP within peripheral nerves. Our findings suggest that APP, synthesized in rodent peripheral sensory neurons, is transported within axons by the fast anterograde system.

MATERIALS AND METHODS

Adult Sprague–Dawley rats (200–250 g) were anesthetized with 4% chloral hydrate i.p., and the sciatic nerve was exposed at the level of the hip flexure ≈3 cm distal to the L4–L5 dorsal root ganglia (DRG). Two ligations, 8–10 mm apart, were placed on the sciatic nerve with surgical sutures. The wound was closed, and animals were allowed to recover for various times. Subsequently, ligated sciatic nerves were used for immumoblotting or immunocytochemistry. In addition, the sciatic nerve was transected before nerve ligation in one group of animals. In these animals, a 5-mm segment of sciatic nerve was transected ≈1 cm distal to the L4–L5 DRG. Six days later, the sciatic nerve distal to the transection was ligated as before, and the tissue was harvested 24 hr later for analysis.

For immunoblotting, two to five animals from the ligated group were sacrificed at 6, 12, 24, or 48 hr after ligations. Equal segments of sciatic nerve (4–5 mm) were homogenized in 2× Laemmli buffer, fractionated in denaturing polyacrylamide gels (7% isocratic or 3–20% gradient gels), and transferred to nitrocellulose membranes. The membranes were incubated with an anti-APP monoclonal antibody (22C11) specific to the N-terminal region of all APP isoforms (21) or an anti-β-tubulin monoclonal antibody (Amersham), followed by an ^{125}I-labeled rabbit anti-mouse antibody to detect the primary antibody. In several experiments, the peroxidase–antiperoxidase tech-

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Abbreviations: APP, amyloid precursor protein; DRG, dorsal root ganglia.

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nique (23) with diaminobenzidine as the chromogen was used to detect the primary antibody. Blots incubated with the radiolabeled antibody were exposed to x-ray films. Subsequently, the band corresponding to the APP signal in each nerve segment was excised from the membranes, and radioactivity (dpm) was determined in a γ counter.

Pulse labeling of lumbar sensory neurons was carried out by microinjecting the L4 and L5 DRGs with 100 Ci of [^{35}S]methionine (1 Ci = 37 GBq). Two hours later, sciatic nerve ligatures were placed as previously described. In animals sacrificed 20 hr after ligation, 5-mm nerve segments proximal and distal to ligatures were removed and analyzed by immunoprecipitation with a rabbit anti-APP polyclonal antibody (anti-Fd-PreA4) (21). The immunoprecipitation mixture was subsequently fractionated by SDS/PAGE as described above and exposed to x-ray film after fluorographic enhancement.

The rabbit anti-APP polyclonal antibody (anti-Fd-PreA4) was used for immunocytochemical localization of APP in sciatic nerves that had been doubly ligated as described above. After ligation, nerve segments proximal and distal to ligatures were frozen, and cross sections (10 μm) of these segments (mounted on glass slides) were incubated with the polyclonal antibody. A goat anti-rabbit secondary antibody, followed by peroxidase–antiperoxidase of rabbit origin and diaminobenzidine, detected APP immunoreactivity in the nerve. Staining was not seen in negative control sections in which the anti-APP primary antibody was replaced by normal rabbit serum.

Acetylcholinesterase activity in the sciatic nerve was analyzed by using [^{14}C]acetylcholine as substrate (24). Enzyme activity in consecutive 2-mm nerve segments proximal to the first ligature was assayed in three animals at 6, 12, and 24 hr after sciatic nerve ligation, respectively. Ethopropazine (0.1 mM) was added to the incubation mixture to inhibit pseudocholinesterase activity (25). [^{14}C]Acetate released in the reaction was recovered by tetraethylboron and toluene–acetonitrile extraction (24).

RESULTS

By immunoblotting, the anti-APP monoclonal antibody (22C11) detected a broad band between 100–135 kDa in homogenates of the DRG and sciatic nerve (Fig. 1). Heterogeneity in molecular masses of the immunoreactive APP species in these tissues is consistent with reports (21, 26, 27) that the protein is modified posttranslationally. In sciatic nerve, APP was most conspicuous in nerve segments proximal to a ligature placed 6–48 hr previously (Fig. 1). Specifically, up to 24 hr after ligation, APP accumulated progressively in the segment immediately proximal to the first ligature (Fig. 2*A*); at 48 hr after ligation, there was some attenuation of accumulation of APP. In addition, an extra band of ≈75–80 kDa was seen in the segment just proximal to the ligature at all postligation time points. It is not clear whether this band of lower intensity represented breakdown products of APP. Accumulation of APP was not observed up to 48 hr after ligation in nerve segments between the two ligatures or in the segment immediately distal to the second ligature, where retrogradely transported material would be detected. These observations are consistent with the fast anterograde transport of APP. The rate of transport was further confirmed by two additional control experiments with constituents carried by fast or slow axonal transport (i.e., acetylcholinesterase or β-tubulin, respectively) (22). Six hours after ligation, the activity of acetylcholinesterase was highest in the nerve segment immediately proximal to the ligature and progressively increased up to 24 hr after ligation (Fig. 2*B*). In contrast, β-tubulin did not accumulate in proximal nerve segments 24 hr after ligation (data not shown).

Three additional studies were performed to establish that DRG neurons were the ultimate source of APP detected in the sciatic nerve, rather than being derived from sources unrelated

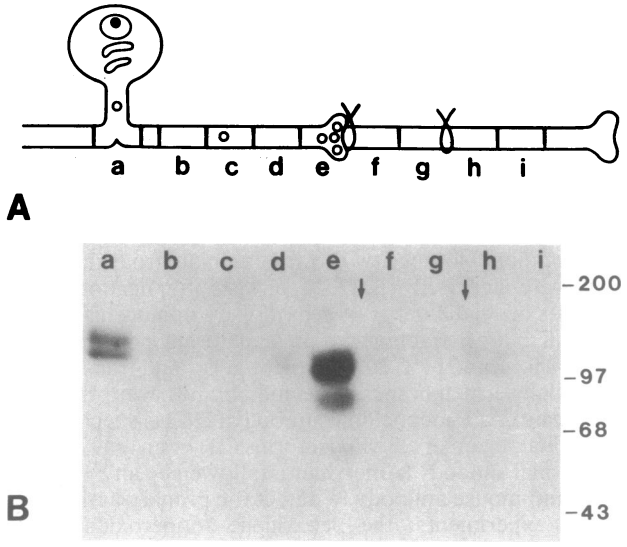

FIG. 1. Detection of APP accumulation in ligated sciatic nerve by immunoblotting. (*A*) Diagrammatic representation of the ligation paradigm. Lanes: a, L5 DRG; b–i, consecutive nerve segments from proximal to distal; arrows indicate ligature positions. (*B*) Immunoblot of equal segments of sciatic nerve corresponding to the diagram of *A* 24 hr after ligation. Molecular mass markers on right are in kDa.

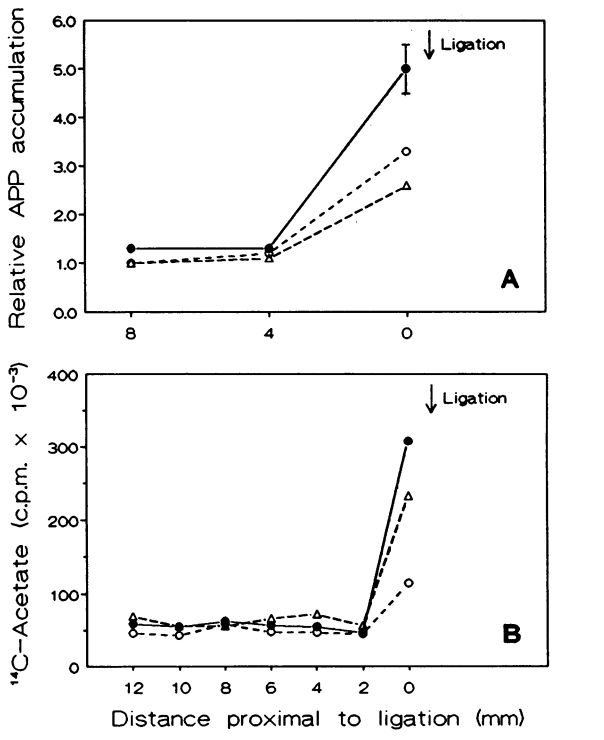

FIG. 2. Accumulation of APP (*A*) and acetylcholinesterase (*B*) activity in ligated sciatic nerves as a function of time: 0, 6 hr; Δ, 12 hr; ●, 24 hr. (*A*) Each point represents the relative amount of APP accumulation in a nerve segment (≈4 mm), from proximal to distal, expressed as a ratio of radioactivity of a single segment divided by the value of the most proximal segment. The average of two animals was used from the 6- and 12-hr time points; the average of five animals was used from the 24-hr time point (±SEM). (*B*) Acetylcholinesterase activity in each nerve segment (2 mm), from proximal to distal, is expressed as the amount of [^{14}C]acetate (cpm) released in the reaction. Values on the abscissa represent the distances of each sample from the first ligature.

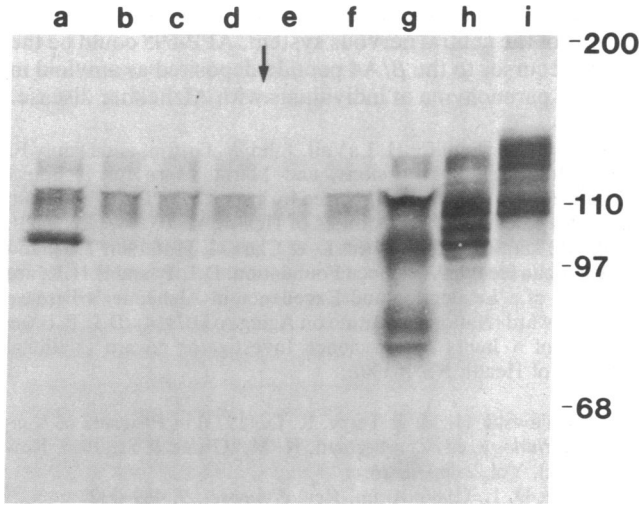

FIG. 3. Pattern of APP in ligated sciatic nerve preceded by nerve transection. Immunoblots show equal nerve segments corresponding to various levels of sciatic nerve transected 6 days before ligation. Nerve samples in this blot were harvested 24 hr after ligation. The detection method used in this experiment was a standard peroxidase technique. Lanes: a, control DRG; b–f, consecutive segments proximal and distal to the first ligature (arrow) in a sciatic nerve transected previously; g, segment immediately proximal to the first sciatic nerve ligature without prior transection (see Fig. 1*B* lane e); h and i, lysates of COS-1 cells transfected transiently with full-length human APP-695 and APP-770 cDNAs, respectively (27). Molecular mass markers on right are in kDa.

to axonal transport, such as from the circulation or from local synthesis by Schwann cells. In the first experiment, the proximal sciatic nerve was initially transected near the DRG, and the distal sciatic nerve stump was ligated 6 days later. Immunoblotting of these ligated nerve segments, which had undergone Wallerian degeneration, revealed two differences. (*i*) In contrast to the untransected nerve, APP did not accumulate in the nerve segment immediately proximal to the first ligature. This finding argues against significant local synthesis of APP by nonneuronal cells as a contributor to APP accumulation in nerve segments proximal to the ligature (Fig. 3). (*ii*) Moreover, in these preparations, the composition of proteins detected by the monoclonal antibody was somewhat different and was most readily appreciated in low percentage (7%) isocratic gels. In the ligated but untransected nerve (Fig. 3, lane g), four sets of bands (75–135 kDa) were found. In contrast, in transected nerves (Fig. 3, lanes b–f), only two sets of bands were observed, both of which overlapped the two upper bands present in the untransected nerve. Thus, in homogenates of whole nerve or ganglia, there may be two different species of APP derived from two separate sources—the axoplasm and the nonneuronal elements (Schwann cells, blood vessels, or other mesenchymal cells). Based solely on determinations by molecular mass, the lower bands (97–110 kDa) seen in untransected nerve corresponded to the APP-695 isoform (Fig. 3, lane h), whereas, in transected nerve (i.e., nonneuronal constituents), the bands (110–135 kDa) corresponded to the APP-770 isoform (Fig. 3, lane i).

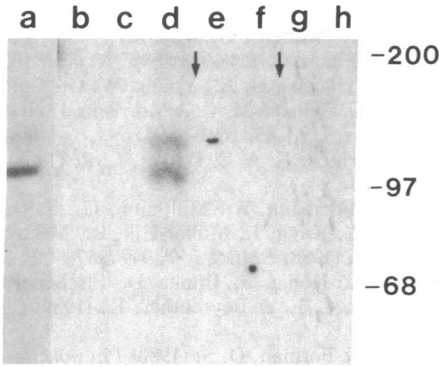

FIG. 4. Detection by immunoprecipitation of labeled APP from nerve segments proximal to ligation of sciatic nerve after pulse-labeling L5 DRG with [^{35}S]methionine. Lane a is from the injected DRG; lanes b–h are consecutive segments proximal and distal to ligatures (arrows). Molecular mass markers on right are in kDa.

In the second study, lumbar DRG were microinjected with [^{35}S]methionine, followed 2 hr later by ligations of the sciatic nerve as described previously. Twenty-four hours after ligation, immunoprecipitation of homogenized nerve segments demonstrated an accumulation of labeled APP proximal to the first ligature (Fig. 4), indicating that the accumulation of APP detected in ligated nerves must have originated from labeled neuronal perikarya.

In the third study, immunocytochemistry localized APP immunoreactivity within axons of the sciatic nerve. One day after sciatic nerve ligation, APP immunoreactivity localized within many swollen axons was observed proximal to the first ligature. Immunostaining was more intense in enlarged axons, which were distended, in part, due to the accumulation of anterogradely transported material (Fig. 5 *A* and *B*). Nonneuronal cells, myelin, and interstitial spaces did not show APP immunoreactivity. Moreover, immunostaining was not de-

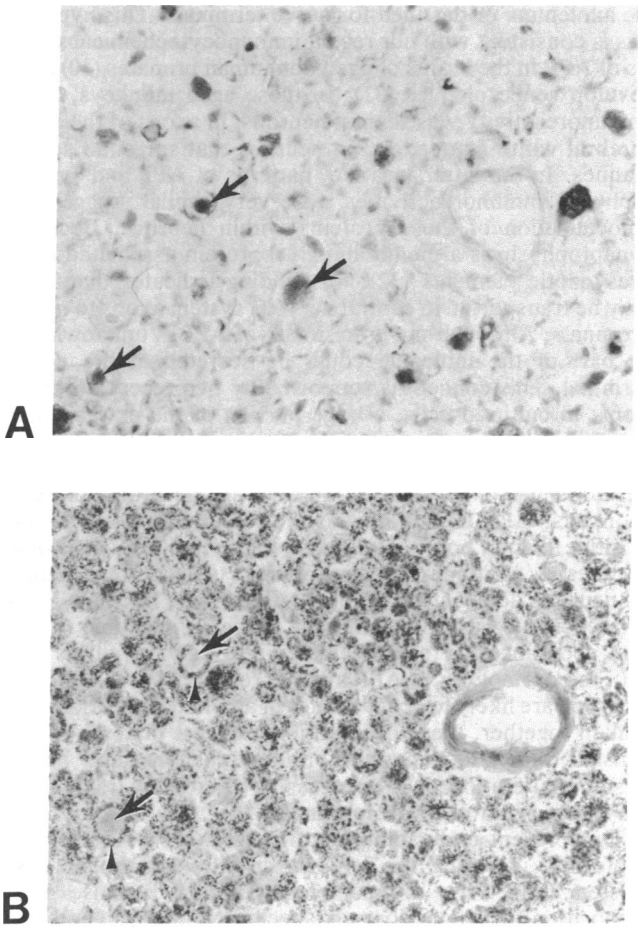

FIG. 5. APP immunoreactivity within sciatic nerve axons proximal to the first ligature. (*A*) In this cross-section of nerve taken immediately proximal to the first ligature, APP immunoreactivity appears as globular deposits of stained material (arrows). (×300.) (*B*) An adjacent section counterstained with Luxol fast blue, a myelin stain. Immunostained areas in *A* can be identified as distended axons (arrows) within myelin sheaths (arrowheads). (×300.)

xtected in axons within or distal to ligatures. Thus, these studies confirmed that the accumulation of APP proximal to a sciatic ligature was due to the axonal transport of proteins synthesized in lumbar sensory neurons.

DISCUSSION

In aged individuals and in cases of Alzheimer disease, amyloid appears to be deposited preferentially in proximity to neurites, some of which may be remote from perikaryal sites of synthesis (28, 29). Thus, if neuronally synthesized APP is a potential source of parenchymal β/A4, APP must be transported from neuronal cell bodies to distal terminals. Our results show that APP, synthesized in lumbar sensory neurons, is transported anterogradely in axons of the peripheral nervous system. If similar processing occurs with APP synthesized in neurons of the central nervous system, the difficulties encountered in correlating the distribution of amyloid deposits with levels of APP mRNAs may be partly explained (10, 14–19). In this setting, the relationship between expression of neuronal APP and formation of amyloid may be resolved only when a correlation between the amyloid deposition within a cortical region and the APP source is established.

This study focuses on APP transport within the peripheral nervous system and does not address the fate of this transported APP. Conceptually, in brain, neuronally synthesized APP could also be axonally transported and be inserted into the axolemma or destined for nerve terminals. This hypothesis is consistent with our recent immunocytochemical studies of APP in the brains of aged nonhuman primates (30) that develop senile plaques (31). In these aged monkeys, APP immunoreactivity was seen in neurons, in axons of the deep cerebral white matter, and in neurites that surround senile plaques. In the latter location, patterns of APP and synaptophysin immunoreactivities were very similar, suggesting colocalization of these proteins within neurites. Because synaptophysin is a membrane glycoprotein associated with presynaptic elements (32), this finding indicates that APP may be transported in central nervous system axons to nerve terminals. Although the precise fate of APP is unknown, all or part of the molecule could be secreted at the nerve terminal. This concept is supported by two recent observations: in cultured cells, a large portion of the extracellular domain of APP is secreted (21, 26, 27) and APP molecules lacking the carboxyl-terminal domain are present in human cerebrospinal fluid (21, 33). Finally, in the brains of aged monkeys, APP immunoreactivity is also present in β/A4-positive plaque cores (30). Interestingly, within occasional plaques, β/A4 immunostaining appears to decorate the edges of neurites adjacent to deposits of amyloid. Because β/A4 unassociated with the membrane is highly insoluble (4, 12) and spontaneously self-assembles into amyloid fibrils (34, 36), the proteolysis of APP and the release of β/A4 into the neuropil are likely to occur at sites of amyloid deposition (11). Taken together, these observations suggest that one mechanism of amyloidogenesis in brain parenchyma may be the result of alterations in the processing of axonally transported APP that occur at nerve terminals.

Our present understanding of the biological role of APP isoforms, including the isoform specific for the nervous system (APP-695), is incomplete (11, 35). Because β/A4 deposition in plaque cores is specific to brain, hypotheses that correlate amyloid formation with the biology of APP-695 are inherently appealing. Thus, it will be important to verify that APP-695 is selectively transported in neurons—a result that awaits confirmation pending the availability of antibodies specific to each APP isoform. Nonetheless, based solely on molecular-mass criteria, our data suggest that APP-695 is the predominant APP species transported axonally in the peripheral nervous system. If this observation is confirmed in neurons of the central nervous system, APP-695 could be the direct precursor to the β/A4 peptide deposited as amyloid in the brain parenchyma of individuals with Alzheimer disease.

We thank Drs. Jennifer H. LaVail, John W. Griffin, and Danny F. Watson for helpful discussions, and Maria Ysern for technical assistance. This work was supported by grants from the U.S. Public Health Service (National Institutes of Health AG 05146, NS 07179, AG 03359) and from The Robert L. & Clara G. Patterson Trust and the American Health Assistance Foundation. D.L.P. and E.H.K. are recipients of a Leadership and Excellence in Alzheimer's Disease (LEAD) award (National Institute on Aging AG 07914). D.L.P. is the recipient of a Javits Neuroscience Investigator Award (National Institutes of Health NS 10580).

1. Wisniewski, H. M. & Terry, R. D. (1973) in *Progress in Neuropathology*, ed. Zimmerman, H. M. (Grune & Stratton, New York), Vol. 2, pp. 1–26.
2. Price, D. L. (1986) *Annu. Rev. Neurosci.* **9,** 489–512.
3. Glenner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120,** 885–890.
4. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. & Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4245–4249.
5. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. & Müller-Hill, B. (1987) *Nature (London)* **325,** 733–736.
6. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. & Ito, H. (1988) *Nature (London)* **331,** 530–532.
7. Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. & Cordell, B. (1988) *Nature (London)* **331,** 525–527.
8. Tanzi, R. E., McClatchey, A. I., Lampert, E. D., Villa-Komaroff, L., Gusella, J. F. & Neve, R. L. (1988) *Nature (London)* **331,** 528–530.
9. Bahmanyar, S., Higgins, G. A., Goldgaber, D., Lewis, D. A., Morrison, J. H., Wilson, M. C., Shankar, S. K. & Gajdusek, D. C. (1987) *Science* **237,** 77–79.
10. Higgins, G. A., Lewis, D. A., Bahmanyar, S., Goldgaber, D., Gajdusek, D. C., Young, W. G., Morrison, J. H. & Wilson, M. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1297–1301.
11. Müller-Hill, B. & Beyreuther, K. (1989) *Annu. Rev. Biochem.* **58,** 287–307.
12. Masters, C. L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R. N. & Beyreuther, K. (1985) *EMBO J* **4,** 2757–2763.
13. Selkoe, D. J. (1989) *Annu. Rev. Neurosci.* **12,** 463–490.
14. Palmert, M. R., Golde, T. E., Cohen, M. L., Kovacs, D. M., Tanzi, R. E., Gusella, J. F., Usiak, M. F., Younkin, L. H. & Younkin, S. G. (1988) *Science* **241,** 1080–1084.
15. Goedert, M. (1987) *EMBO J.* **6,** 3627–3632.
16. Cohen, M. L., Golde, T. E., Usiak, M. F., Younkin, L. H. & Younkin, S. G. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1227–1231.
17. Johnson, S. A., Pasinetti, G. M., May, P. C., Ponte, P. A., Cordell, B. & Finch, C. E. (1988) *Exp. Neurol.* **102,** 264–268.
18. Lewis, D. A., Higgins, G. A., Young, W. G., Goldgaber, D., Gajdusek, D. C., Wilson, M. C. & Morrison, J. H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1691–1695.
19. Neve, R. L., Finch, E. A. & Dawes, L. R. (1988) *Neuron* **1,** 669–677.
20. Dyrks, T., Weidemann, A., Multhaup, G., Salbaum, J. M., Lemaire, H.-G., Kang, J., Müller-Hill, B., Masters, C. L. & Beyreuther, K. (1988) *EMBO J.* **7,** 949–957.
21. Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J. M., Masters, C. L. & Beyreuther, K. (1989) *Cell* **57,** 115–126.
22. Grafstein, B. & Forman, D. S. (1980) *Physiol. Rev.* **60,** 1167–1283.
23. Sternberger, L. A. (1979) *Immunocytochemistry* (Wiley, New York), 2nd Ed.
24. Fonnum, F. (1969) *Biochem. J.* **115,** 465–472.
25. Couraud, J. Y. & Di Giamberardino, L. (1980) *J. Neurochem.* **35,** 1053–1066.

26. Schubert, D., LaCorbiere, M., Saitoh, T. & Cole, G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2066–2069.
27. Sisodia, S. S., Koo, E. H., Martin, L. J., Unterbeck, A. J., Beyreuther, K., Weidemann, A. & Price, D. L. (1989) *Soc. Neurosci. Abstr.* **15,** 23.
28. Kitt, C. A., Price, D. L., Struble, R. G., Cork, L. C., Wainer, B. H., Becher, M. W. & Mobley, W. C. (1984) *Science* **226,** 1443–1445.
29. Powers, R. E., Struble, R. G., Casanova, M. F., O'Connor, D. T., Kitt, C. A. & Price, D. L. (1988) *Neuroscience* **25,** 401–417.
30. Martin, L. J., Cork, L. C., Koo, E. H., Sisodia, S. S., Weidemann, A., Beyreuther, K., Masters, C. & Price, D. L. (1989) *Soc. Neurosci. Abstr.* **15,** 23.
31. Struble, R. G., Price, D. L., Jr., Cork, L. C. & Price, D. L. (1985) *Brain Res.* **361,** 267–275.
32. Wiedenmann, B. & Franke, W. W. (1985) *Cell* **41,** 1017–1028.
33. Palmert, M. R., Podlisny, M. B., Witker, D. S., Oltersdorf, T., Younkin, L. H., Selkoe, D. J. & Younkin, S. G. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6338–6342.
34. Castaño, E. M., Ghiso, J., Prelli, F., Gorevic, P. D., Migheli, A. & Frangione, B. (1986) *Biochem. Biophys. Res. Commun.* **141,** 782–789.
35. Price, D. L., Koo, E. H. & Unterbeck, A. (1989) *BioEssays* **10,** 69–74.
36. Kirschner, D. A., Inouye, H., Duffy, L. K., Sinclair, A., Lind, M. & Selkoe, D. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6953–6957.