

## The Alzheimer's Disease Sphinx: A Riddle with Plaques and Tangles

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**U**NLIKE other degenerative diseases of the nervous system, such as schizophrenia and Huntington's disease, Alzheimer's disease (AD)<sup>1</sup> leaves behind in the brain two clues that have provided a starting point for unraveling the pathogenesis of this illness. One clue is the senile plaque, which consists principally of extracellular fibrillar aggregates in a dense plaque core or as a diffuse infiltrate through portions of the neuropil. The second clue is the neurofibrillary tangle, an intraneuronal structure affecting discrete populations of neurons. It has been almost a faith among Alzheimer researchers that a complete understanding of how these pathological structures arise will solve the disease. The rapid pace of research in the field stems directly from the identification and cloning of the constituent proteins within these structures.

### *The Amyloid Precursor Protein (APP): Its Products and Mutations*

In 1984, Glenner (15) made what turned out to be a correct assumption: that amyloid in the cerebrovasculature of Alzheimer's patients contained the same protein as that of the senile plaque. The relative ease with which vascular amyloid could be purified and his success in obtaining the first direct sequence of the  $\beta$ -amyloid peptide ( $A\beta$ ) led directly to the cloning of the larger parent gene, the APP by other laboratories. This 100–140-kD glycoprotein spans the membrane one time with a short carboxy terminal cytoplasmic tail and a long ectodomain (see Fig. 1). Remarkably,  $A\beta$  is cleaved from a site within the membrane ( $\gamma$  site), and at a site just outside the membrane ( $\beta$  site) to generate the peptide (see Fig. 1).  $A\beta$  is heterogeneous in length ranging from 39 to 43 residues, and can aggregate in vitro and in vivo into 6–10-nm fibrils.

Interest in AD soared in 1991, when the first of several APP mutations were linked to the disease by Alison Goate and John Hardy. Previously, a genetic basis for the disease was suspected because trisomy of chromosome 21 strongly predisposes to the development of Alzheimer's pathology,

and the APP gene is located on chromosome 21. The known mutations are: (a) codon 717, which occurs in several unrelated families; (b) codons 670/671, a double mutation, which occurs in Swedish kindred; and (c) codons 692 or 693, which shift, to varying degrees, the principle site of amyloid deposition from the brain parenchyma to the cerebrovasculature (reviewed in reference 14). In the case of the 693 mutation, which causes the variant condition, hereditary cerebral hemorrhage with amyloidosis of the Dutch type, deposition is principally in the cerebrovasculature. Another as yet unidentified locus responsible for the AD phenotype has been identified by linkage on the long arm of chromosome 14 (39). In addition to these rare genetic causes of the disease, a genetic risk factor for Alzheimer's disease is present on chromosome 19. The locus on chromosome 19 is believed to be the apolipoprotein E gene because homozygotes with the  $\epsilon 4$  allele of the apolipoprotein gene have an eightfold increased risk of developing the disease (38).

### *Domain Organization of APP and Its Trafficking*

The ectodomain of APP consists of an amino-terminal 17-residue signal sequence followed by a cysteine-rich domain, and an anionic domain of  $\sim 100$  residues (reviewed in reference 26). The ectodomain undergoes N- and O-glycosylation, tyrosine sulfation, and an unusual phosphorylation (20). APP undergoes alternative splicing that results in a variety of isoforms. These splice variants include exons in the ectodomain, one with homology to the Kunitz family of serine protease inhibitors and another with homology to the OX-2 surface antigen in leukocytes. A shorter 695-residue isoform predominates in neurons; whereas a longer isoform with these exons tends to be expressed in nonneural tissue including glia (46). Another exon, exon 15, is alternatively spliced in white cells, where it may have a role in cell adhesion (24). In addition to APP, at least two APP-like gene products are widely expressed and have homologues in *Drosophila* and *Caenorhabditis elegans* (7, 47). Notable among these homologues is the absence of the  $A\beta$  sequence.

The holoprotein is internalized via clathrin-coated vesicles (13, 36), probably using the NPXY consensus sequence in the cytoplasmic tail. APP trafficks to the endosomal-lysosomal pathway, where a series of carboxy terminal fragments are generated (11). The function of this internalization pathway is not known. Although a receptor function has been suggested, no ligand has been identified.

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1. *Abbreviations used in this paper:*  $A\beta$ ,  $\beta$ -amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; PHF, paired helical filaments.

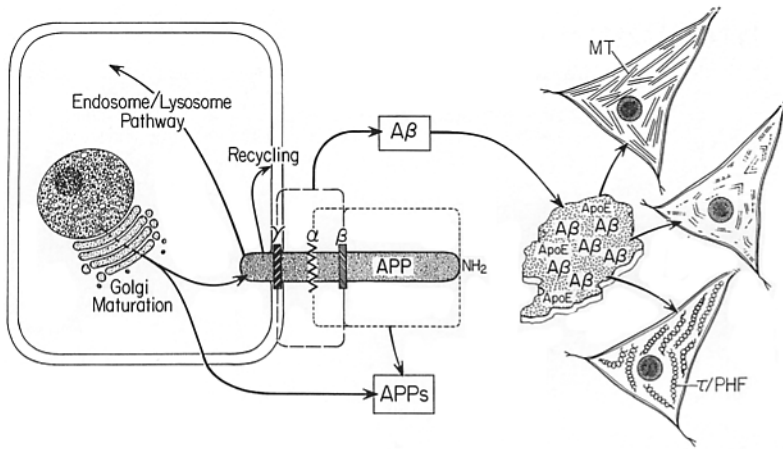


Figure 1. A diagrammatic representation of some possible steps involved in the pathogenesis of Alzheimer's disease. The  $\beta$  and  $\gamma$  cleavage sites of APP generate the  $A\beta$  fragment. ApoE has been identified among the components of the senile plaque. MT, microtubules that undergo dissolution with disease progression, and ultimately tau protein aggregates to form PHF.

APP generates specific fragments with distinct fates in discrete cellular compartments. The most abundant secreted fragment is a 100–110-kD polypeptide cleaved between residues Lys16 and Leu17 of the  $A\beta$  peptide, which precludes the formation of  $A\beta$  (10). The enzyme activity responsible for this cleavage, known as  $\alpha$ -secretase, awaits discovery; but because it cuts in the middle of the  $A\beta$  fragment, it has been dubbed a “good” enzyme. Less abundant secretory cleavages release both truncated fragments and longer fragments that may contain the  $A\beta$  region (reviewed in reference 41). Possible functions of APP, are a serine protease inhibitor identified first as protease nexin II, and later as Factor XI<sub>i</sub>-inhibitor (reviewed in reference 41). APP, also has growth-promoting properties (34), which may follow from its ability to activate the mitogen-activated kinase in a ras-dependent manner (16). In the extracellular matrix, APP, can bind to heparin (40), laminin (22), and a basement membrane HSPG (33), where it may mediate adhesiveness.

$A\beta$  occurs as a soluble peptide released into culture medium, into normal human cerebrospinal fluid at  $\sim 10$  ng/ml, and into serum in vanishingly small amounts (42). One site of  $A\beta$  production is early in the endocytic pathway, a conclusion based on cell surface radioiodination of APP that indicated a precursor relationship to  $A\beta$ , and a reduction in  $A\beta$  production with a COOH-terminal-deleted APP (25). Messengers that activate PLC such as bradykinin or that activate protein kinase C such as phorbol ester decrease  $A\beta$  release and increase APP<sub>s</sub> (5, 35). On the other hand, the calcium ionophore A23187 increased  $A\beta$  production (37).

The site of  $A\beta$  deposition on the abluminal side of blood vessel walls has raised the question of polarized release. In MDCK cells, 80–90% of both APP<sub>s</sub> and  $A\beta$  are released from the basolateral surface; likewise, the holoprotein is inserted preferentially in the basolateral membrane (17). Deletion of the cytoplasmic tail and mutagenesis of certain sequences within the tail can shift a portion of these secreted products apically. The site of  $A\beta$  secretion appears to follow the holoprotein. These findings are consistent with both the abluminal deposition of  $A\beta$ , as well as an appropriate basolateral secretion of APP<sub>s</sub>, in any of its putative roles as a growth promoter, an adhesion molecule, or a factor in blood coagulation. The cellular basis for the observed topography of  $A\beta$  deposition within the neuropil is more complex. In this regard, recent results raise the possibility that, in con-

trast to MDCK cells, APP may move transcytotically in neurons from the axon to the dendrite (Dotti, C., personal communication; Koo, E., personal communication).

### Seeding the Plaque

The finding of soluble  $A\beta$  under normal conditions and the suggestion that aggregated  $A\beta$  is toxic opened the search for factors that enhance the aggregation and precipitation of this fragment. Knowledge of these factors will be important because once  $A\beta$  aggregates, it appears to induce free radical damage to neurons (3). A potent theoretical approach to protein self association is the concept of nucleation-dependent polymerization (21). Briefly, to seed the macromolecular aggregate, an initial thermodynamically unfavorable nucleation step must precede the thermodynamically favorable addition of monomers. The interval between the formation of protein nuclei in trace amounts and the detection of aggregates bears an exponential relationship to protein concentration. Small differences in protein concentration can change this lag time from years to milliseconds.

Most of the soluble  $A\beta$  is 1-40.  $A\beta$  deposited in Alzheimer's disease is heterogeneous at its carboxyl terminus: the two principle moieties are 1-40 and 1-42. When APP with the Swedish mutation was transfected into cells,  $A\beta$  increased in the medium (41), creating a concentration effect that could lead to aggregation. When the 717 mutation was expressed in cells, there was an increase in the percentage of  $A\beta$ 1-42 (43), a finding that, combined with the known increased in vitro polymerization of  $A\beta$ 1-42 compared to  $A\beta$ 1-40 (21), could provide another mechanism of amyloidogenesis.  $A\beta$ 1-42 is also more abundant in plaques from patients with the 717 mutation (19).

The frequent occurrence of  $A\beta$  deposition in the aged may be related to a common age-related modification that affects many proteins: glycation. Glycation is a series of nonenzymatic reactions leading to irreversibly cross-linked advanced glycosylation end products. When  $A\beta$  undergoes glycation, it can seed the accelerated aggregation of soluble  $A\beta$  in vitro (44). Similar mechanisms may underlie the progressive insolubility of tau aggregates in the form of paired helical filaments (PHF) (28). It has also been suggested that elements from the extracellular matrix (HSPG) or heavy metals (31) induce aggregation.

Once aggregated, A $\beta$  appears to induce extensive damage to membrane systems. This damage is thought to be mediated by free radicals because the A $\beta$  increases H<sub>2</sub>O<sub>2</sub> and lipid peroxides in cells, and because antioxidants have a protective effect (3). Alternatively, toxicity may be mediated by the spontaneous assembly of A $\beta$  subunits into a membrane structure with some properties of an ion channel (2, 12).

### Neurofibrillary Degeneration

Neurofibrillary tangles consist of highly ordered intraneuronal filaments called PHF assembled from the microtubule-associated protein, tau (30). These structures lie within neuronal cell bodies and neurites, where they induce processes to become swollen and dystrophic. The link between APP and tangles is not understood, and it is complicated by a poor anatomical correlation between the sites of amyloid deposition and PHFs (9), and because PHFs occur in disease states devoid of amyloid. In cell culture, tau has a role in the establishment of polarity and, in this capacity, can serve as a rapid elongator of a single process (6, 23). On the other hand, neurons from a tau knockout mouse do become polarized (18). Tau mRNA is targeted to the axon hillock (29), and the protein is enriched in axons (27). A complex splicing pattern results in the appearance of certain exons as a function of development; the tau isoforms also show spatial restrictions to either the central or peripheral nervous systems (1). Tau undergoes multiple phosphorylations (45) on serines and threonines, several of which are amino to a proline, a finding that has triggered a search for tau kinases among both proline-directed and nonproline-directed kinases. Although the effects of phosphorylation at specific sites are unknown, increased tau phosphorylation can decrease the affinity of tau for microtubules, and it can inhibit tau's tendency to suppress microtubule dynamics (8).

Tau protein from PHFs is unable to bind to microtubules, (4) and it is aberrantly phosphorylated (30). The additional phosphorylation sites in PHF tau coincide with consensus sequences for both proline-directed and nonproline-directed kinases (32). Because phosphate cannot be detected on these sites under normal conditions, it is more likely that deregulation of a kinase, rather than a phosphatase, is responsible for the disease-related modifications of tau protein. Furthermore, it appears that several different kinases get activated in the course of the disease process, raising a more global cellular problem involving the dysregulation of the cell's phosphorylation state. While tau protein in the PHF is a clue to the nature of the damage incurred, its vestigial presence may represent no more than a molecular accident of its ability to assemble into highly resistant filaments during the degeneration of the neuron. Neuronal degeneration in AD involves a disassembly of several cytoskeletal systems, including microtubules and neurofilaments, as well as the loss of other structures such as synapses. This form of cell death is reminiscent of the neuron's response to injury or a necrotic death, rather than apoptosis. Injury in many cells induces mitosis; however, the irretrievably postmitotic state of neurons may activate enzymes related to cell division without accomplishing mitosis.

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### References

1. Andreadis, A., W. M. Brown, and K. S. Kosik. 1992. Structure and novel exons of the human tau gene. *Biochemistry*. 31:10626-10633.
2. Arispe, N., E. Rojas, and H. B. Pollard. 1993. Giant multilevel cation channels formed by Alzheimer disease amyloid beta-protein [A beta P-(1-40)] in bilayer membranes. *Proc. Natl. Acad. Sci. USA*. 90:10572-10577.
3. Behl, C., J. B. Davis, R. Lesley, and D. Schubert. 1994. Hydrogen peroxide mediates amyloid  $\beta$  protein toxicity. *Cell*. 77:817-827.
4. Bramblett, G. T., J. Q. Trojanowski, and V. M-Y. 1993. Regions with abundant neurofibrillary pathology in human brain exhibit a selective reduction in levels of binding-competent tau and accumulations of abnormal tau-isoforms (A68 proteins). *Lab. Invest*. 66:212-222.
5. Buxbaum, J. D., E. H. Koo, and P. Greengard. 1993. Protein phosphorylation inhibits production of Alzheimer amyloid  $\beta$ /A4 peptide. *Proc. Natl. Acad. Sci. USA*. 90:9195-9198.
6. Caceres, A., and K. S. Kosik. 1990. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature (Lond.)*. 343:461-463.
7. Daigle, I., and C. Li. 1993. *APL-1*, *Caenorhabditis elegans* gene in coding of protein related to  $\beta$ -amyloid protein precursor. *Proc. Natl. Acad. Sci. USA*. 90:12045-12049.
8. Drechsel, D. N., A. A. Hyman, M. H. Cobb, and M. W. Kirschner. 1992. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated tau. *Mol. Biol. Cell*. 3:1141-1154.
9. Einstein, G., R. Buranosky, and B. J. Crain. 1994. Dendritic pathology of granule cells in Alzheimer's disease is unrelated to neuritic plaques. *J. Neurosci*. 14:5077-5088.
10. Esch, F. S., P. S. Kiem, E. C. Beattie, R. W. Blacher, A. R. Culwell, T. Oltersdorf, D. McClure, and P. J. Ward. 1990. Cleavage of amyloid  $\beta$ -peptide during constitutive processing of its precursor. *Science (Wash. DC)*. 248:1122-1124.
11. Estus, S., T. E. Golde, T. Kunishita, D. Blades, D. Lowery, M. Eisen, M. Usiak, X. Qu, T. Tabira, B. D. Greenberg, and S. G. Younkin. 1992. Potentially amyloidogenic carboxyl-terminal derivatives of the amyloid protein precursor. *Science (Wash. DC)*. 255:726-728.
12. Etcheberrigaray, R. E. Ito, C. S. Kim, and D. L. Alkon. 1994. Soluble  $\beta$ -amyloid induction of Alzheimer's phenotype for human fibroblast K<sub>1</sub> channels. *Science (Wash. DC)*. 264:276-279.
13. Ferreira, A., A. Caceres, and K. S. Kosik. 1993. Intraneuronal compartments of the amyloid precursor protein. *J. Neurosci*. 13:3122-3123.
14. Ghiso, J., T. Wisniewski, and B. Frangione. 1994. Unifying features of systemic and cerebral amyloidosis. *Mol. Neurobiol*. 8:49-64.
15. Glenner, G. G., and C. W. Wong. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun*. 120:885-890.
16. Greenberg, S. M., E. H. Koo, D. J. Selkoe, W. Q. Qiu, and K. S. Kosik. 1994. Stimulation of MAP kinase and tau phosphorylation by secreted  $\beta$ -amyloid precursor protein. *Proc. Natl. Acad. Sci. USA*. 91:7104-7108.
17. Haass, C., E. H. Koo, D. B. Teplow, and D. J. Selkoe. 1994. Polarized secretion of  $\beta$ -amyloid precursor protein and amyloid  $\beta$ -peptide in MDCK cells. *Proc. Natl. Acad. Sci. USA*. 91:1564-1568.
18. Harada, A., K. Oguchi, S. Okabe, J. Kuno, S. Terada, T. Ohshima, R. Sato-Yoshitake, Y. Takei, T. Noda, and N. Hirokawa. 1994. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature (Lond.)*. 369:488-491.
19. Iwatsubo, T., A. Odaka, N. Suzuki, H. Mizusawa, N. Nukina, and Y. Ihara. 1994. Visualization of A $\beta$ 42(43) and A $\beta$ 40 in senile plaques with end-specific A $\beta$  monoclonals: evidence that an initially deposited species is A $\beta$ 42(43). *Neuron*. 13:45-53.
20. Hung, A. Y., and D. J. Selkoe. 1994. Selective ectodomain phosphorylation and regulated cleavage of  $\beta$ -amyloid precursor protein. *EMBO (Eur. Mol. Biol. Organ.) J*. 13:534-542.
21. Jarrett, J. T., and P. T. Lansbury, Jr. 1993. Seeding "one-dimensional crystallization of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell*. 73:1055-1058.
22. Kibbey, M. C., M. Jucker, B. S. Weeks, R. L. Neve, W. E. Van Nostrand, and H. K. Klienman. 1993.  $\beta$ -Amyloid precursor protein binds to the neurite-promoting IKVAV site of laminin. *Proc. Natl. Acad. Sci. USA*. 90:10150-10153.
23. Knops, J., K. S. Kosik, G. Lee, J. D. Pardee, L. Cohen-Gould, and L. McConlogue. 1991. Overexpression of tau in a non-neuronal cell induces long cellular processes. *J. Cell Biol*. 114:725-733.
24. Koenig, G., U. Monning, C. Czech, R. Prior, R. Banati, U. Schreiter-Gasser, J. Bauer, C. L. Masters, and K. Beyreuther. 1992. Identification and differential expression of a novel alternative splice isoform of the  $\beta$ A4 amyloid precursor protein (APP) mRNA in leukocytes and brain microglial cells. *J. Biol. Chem*. 267:10804-10809.
25. Koo, E. H., and S. L. Squazzo. 1994. Evidence that production and release of amyloid  $\beta$ -protein involves the endocytic pathway. *J. Biol. Chem*. 269:17386-17389.
26. Kosik, K. S. 1992. Alzheimer's disease from a cell biological perspective. *Science (Wash. DC)*. 256:780-783.

27. Kowall, N. W., and K. S. Kosik. 1987. Axonal disruption and aberrant localization of tau protein characterize the neuropil pathology of Alzheimer's disease. *Ann. Neurol.* 22:639-643.
28. Ledesma, M. D., P. Bonay, C. Colaco, and J. Avila. 1994. Analysis of microtubule associated protein tau glycation in paired helical filaments. *J. Biol. Chem.* In press.
29. Litman, P., J. Barg, L. Rindzoonski, and I. Ginzburg. 1993. Subcellular localization of tau mRNA in differentiating neuronal cell culture: implications for neuronal polarity. *Neuron.* 10:627-638.
30. Mandelkow, E.-M., and E. Mandelkow. 1993. Tau as a marker of Alzheimer's disease. *Trends Biochem. Sci.* 18:480-483.
31. Mantyh, P. W., J. R. Ghilardi, S. Rogers, E. DeMaster, C. J. Allen, E. R. Stimson, and J. E. Maggio. 1993. Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of  $\beta$ -amyloid peptide. *J. Neurochem.* 61:1171-1174.
32. Morishima-Kawashima, M., and Y. Ihara. 1994. Posttranslational modifications of the tau in PHF: phosphorylation and ubiquitination. IPSEN Symposium. Aging and Alzheimer's Disease. Springer-Verlag, Berlin.
33. Narindrasorasak, S., D. Lowery, P. Gonzalez-DeWhitt, R. A. Poorman, B. Greenberg, and R. Kisilevsky. 1991. High affinity interactions between the Alzheimer's  $\beta$ -amyloid precursor proteins and the basement membrane form of heparan sulphate proteoglycan. *J. Biol. Chem.* 266:12878-12883.
34. Ninomiya, H., J. Roch, M. P. Sundsmo, D. A. C. Otero, and T. Saitoh. 1993. Amino acid sequence RERMS represents the active domain of amyloid  $\beta$ /A4 protein precursor that promotes fibroblast growth. *J. Cell Biol.* 121:879-886.
35. Nitsch, R. M., S. A. Farber, J. H. Growdon, and R. J. Wurtman. 1993. Release of amyloid beta-protein precursor derivatives by electrical depolarization of rat hippocampal slices. *Proc. Natl. Acad. Sci. USA.* 90:5191-5193.
36. Nordstedt, C., G. L. Caporaso, J. Thyberg, S. E. Gandy, and P. Greengard. 1993. Identification of the Alzheimer  $\beta$ /A4 amyloid precursor protein in clathrin-coated vesicles purified from PC12 cells. *J. Biol. Chem.* 268:608-612.
37. Querfurth, H. W., and D. J. Selkoe. 1994. Calcium ionophore increases amyloid  $\beta$  peptide production by cultured cells. *Biochemistry.* 33: 4550-4561.
38. Saunders, A. M., W. J. Strittmatter, D. Schmechel, P. H. St. George-Hyslop, M. A. Pericak-Vance, S. H. Joo, B. L. Rosi, J. F. Gusella, D. R. Crapper-Machlachlan, M. J. Alberts et al. 1993. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology.* 43:1467-1472.
39. Schellenberg, G. D., T. D. Bird, E. M. Wijsman, H. T. Orr, L. Anderson, E. Nemens, J. A. White, L. Bonnycastle, J. L. Weber, M. E. Alonso et al. 1992. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science (Wash. DC).* 258:668-671.
40. Schubert, D., M. LaCorbiere, T. Saitoh, and G. Cole. 1989. Characterization of an amyloid  $\beta$  precursor protein that binds heparin and contains tyrosine sulfate. *Proc. Natl. Acad. Sci. USA.* 86:2066-2069.
41. Selkoe, D. J. 1994. Normal and abnormal biology of the  $\beta$ -amyloid precursor protein. *Ann. Rev. Neurosci.* 17:489-517.
42. Seubert, P., C. Vigo-Pelfrey, F. Esch, M. Lee, H. Dovey, D. Davis, S. Sinha, M. Schlossmacher, J. Whaley, C. Swindlehurst et al. 1992. Isolation and quantification of soluble Alzheimer's  $\beta$ -peptide from biological fluids. *Nature (Lond.).* 359:325-327.
43. Suzuki, N., T. T. Cheung, X.-D. Cai, L. Otvos, C. Eckman, T. Golde, and S. G. Younkin. 1994. An increased percentage of long amyloid  $\beta$  protein secreted by familial amyloid  $\beta$  protein precursor ( $\beta$ APP<sub>717</sub>) mutants. *Science (Wash. DC).* 264:1336-1340.
44. Vitek, M. P., K. Bhattacharya, J. M. Glendening, E. Stopa, H. Vlassara, R. Bucala, K. Monogue, and A. Cerami. 1994. Advanced glycation end products contribute to amyloidosis in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA.* 91:4766-4770.
45. Watanabe, A., M. Hasegawa, M. Suzuki, K. Takio, M. Morishima-Kawashima, K. Titani, T. Arai, K. S. Kosik, and Y. Ihara. 1993. In vivo phosphorylation sites in fetal and adult tau. *J. Biol. Chem.* 268:25712-25717.
46. Wertkin, A. M., R. S. Turner, S. J. Pleasure, T. E. Golde, S. G. Younkin, J. Q. Trojanowski, and V. M. Lee. 1993. Human neurons derived from a teratocarcinoma cell line expresses solely the 695-amino acid amyloid precursor protein and produce intracellular  $\beta$ -amyloid of A4 peptides. *Proc. Natl. Acad. Sci. USA.* 90:9513-9517.
47. White, K., L. Luo, T. Aigaki, and M. Monastirioti. 1994. *Drosophila* Appl gene and APPL protein: a model system to study the function of the APP protein family. In *Amyloid Protein Precursor in Development, Aging, and Alzheimer's Disease.* C. L. Masters et al., editors. Springer-Verlag, Berlin.