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Intracellular A-Beta Amyloid, A Sign for Worse Things to Come?

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Abstract

In this review the authors discuss the possible neuropathological role of intracellular amyloid-β accumulation in Alzheimer's disease (AD) pathology. There is abundant evidence that at early stages of the disease, prior to Aβ amyloid plaque formation, Aβ peptides accumulate intraneuronally in the cerebral cortex and the hippocampus. The experimental evidence would indicate that intracellular amyloid-β could originate both by intracellular biosynthesis and also from the uptake of amyloidogenic peptides from the extracellular milieu. Herein the aspects of the possible impact of intracellular amyloid-β in human AD pathology are discussed, as well as recent observations from a rat transgenic model with a phenotype of intracellular accumulation of Aβ fragments in neurons of the hippocampus and cortex, without plaque formation. In this model, the intracellular amyloid-β phenotype is accompanied by increased MAPK/ERK activity and tau hyperphosphorylation. Finally, the authors discuss the hypothesis that, prior to plaque formation, intracellular Aβ accumulation induces biochemical and pathological changes in the brain at the cellular level priming neurons to further cytotoxic attack of extracellular amyloidogenic peptides.

Index Entries: Alzheimer's disease; tau; phosphorylation; Aβ; transgenic rats.

Introduction

Alzheimer's disease (AD) is the most prevalent form of late-life mental failure in humans. It is characterized by disordered cognitive function, altered behavior, and progressive memory impairment. It strikes at least 35% of those over 85 yr old, and major risk factors include genetic components and age (for review *see* refs. *1*,*2*). The brains of AD patients are characterized by the presence of extracellular amyloid deposits called senile plaques (SP), comprised mainly of fibrillar

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amyloid-β peptides (Aβ) and neurofibrillary tangles (NTF). The core of the NTF is composed of abnormally hyperphosphorylated forms of the microtubule-associated protein tau, referred to as paired helical filaments (PHF) (for review *see* refs. *3, 4*). The Aβ peptides are products of the enzymatic cleavage of the so-called amyloid precursor protein (APP) *(5,6);* (for review *see* ref. *7*). The enzymes responsible for this APP cleavage are the β and γ secretases which act at the N- and C-terminus of the Aβ fragment (for review *see* ref. *8*). The γ-secretase site is heterogeneous and produces Aβ fragments which are 39 to 43 residues in length. The longer 4.5Kda Aβ peptide, which spans residues 1–42 (Aβ1–42), is the more amyloidogenic species *(9).* This increase in Aβ production, resulting from autosomal dominant mutations of the APP gene in the neighborhood of the β or γ sites, is sufficient to unleash the AD neuropathology *(10–12).* The disease has a complex etiology, existing in early and late-onset familial forms (familial Alzheimer's disease or FAD). Three genes have so far been clearly associated with FAD: APP on chromosome 21 *(13),* presenilin 1 (PS1) on chromosome 14 *(14),* and presenilin 2 (PS2) on chromosome 1 *(15,16).* In addition to these genetic causes, the ε4 allele of the apolipoprotein (APO) on chromosome 19 is considered a strong risk factor for late onset of AD *(17–20);* (for review *see* refs. *18*,*21*). Other polymorphisms in additional genes, including the α 2 macroglobuline gene *(22),* Interleukin 1 *(23),* Cathepsin D *(24),* LRP-1 *(25),* VLDL-R *(26),* Fe65 *(27),* bleomycin hydrolase *(28),* and cystatin C *(29)* are suspected of increasing AD incidence. Aβ production is also influenced by other factors such as hormones *(30–34)* as well as environmental risk factors such as high calorie diets, folic acid insufficiency, and low intellectual activity *(35);* (for review *see* ref. *36*).

The concept of the primary role of Aβ in AD neuropathology is reinforced by the well-established Aβ accumulation in the brains of AD sufferers with FAD mutations *(37);* (for review *see* ref. *38*) and also in individuals with Down's syndrome, conditions which have an increase in the production of soluble Aβ42 in common *(10, 39–41);* (for review *see* ref. *42*). It is known that Down's syndrome patients—who have an extra copy of chromosome 21 encoding for APP—develop AD pathology in their third to fourth decade *(43–52).* A number of molecular changes at the cellular level have also been attributed to extracellular Aβ, including changes in protein kinases activity *(53–57),* proteasome activity *(58),* the complement cascade (for review *see* refs. *59,60*), the redox status of the cell (for review *see* refs. *61–63*), alterations in the neuronal endosomal-lysosomal system (for review *see* ref. *64*), mitochondrial respiration *(65–68),* neurotransmitter modulation *(69–71);* (for review *see* ref. *70*), calcium homeostasis *(72,73);* (for review *see* refs. *35,74,75*), and gene expression *(76,77).* These processes are triggered differentially during the progress of the disease, and most probably contribute to different stages of the pathology. Indeed, synergistic effects amongst them could ultimately provoke structural–functional changes leading to neuronal dysfunction and death.

Numerous studies have established that AD is associated with selective lesions of neuronal circuits in the neocortex, hippocampus, and the basal forebrain cholinergic system. These lesions diminish synaptic inputs in cortical regions of the brain, leading to cognitive impairments. There are also reports, both in the human brain and in transgenic models, which establish a good correlation between elevated levels of Aβ in the brain and cognitive decline *(78–82).* The application of exogenous β-amyloid in the central nervous system (CNS) induces abnormal tau phosphorylation and neuronal loss in the hippocampal layers *(83),* provokes a deterioration of cholinergic neurons *(84–86),* and causes a decline in the ability to learn spatial memory tasks tested using the Morris Water Maze (MWM) *(84).*

Furthermore, intracerebral β-amyloid *(25–35)* or beta 1–40 induces tissue loss and neurodegeneration *(87);* (for review *see* ref. *69*). However, despite the relative contribution of

soluble forms of Aβ, the relative role of fibrillar Aβ aggregated in plaques or in intracellular Aβ remains unclear. For example, whether Aβ plaques are pathogenic or part of a beneficial mechanism sequestering toxic Aβ fragments has yet to be resolved. Evidence from a transgenic mouse model which over-expresses the β-APP 751 transgene shows an age-dependent and plaque-independent deficit in the MWM task *(88).* On the other hand, an effect of plaque load on cognitive decline is supported by other studies *(79,89,90)* as well as by evidence that the vaccine-induced reduction of amyloid in the brain alleviates behavioral impairment in transgenic models of AD pathology *(91,92).*

Amyloid Aβ Generation and Its Significance in the Cellular Pathology Observed in Alzheimer's Disease

The regulation of APP metabolism is controlled by complex physiological and pathophysiological mechanisms including NGF *(93);* (for review *see* ref. *94*), basic fibroblast growth factor *(95),* activation of PKC *(96–99),* and hormonal levels *(33).* The different sensitivities to Aβ toxicity expressed by different neuronal types could perhaps be explained by the characteristics of Aβ production, and by the degradation capacity in diverse brain regions *(100).*

Several cell lines expressing wild-type APP can produce and release Aβ after internalization of APP from the cell surface. The main pathway for Aβ production appears to involve endocytic recycling of APP from the cell surface *(101–103).* Strong activation of the neuronal endocytic pathway and lysosomal system is a prominent neuropathological characteristic of AD. In fact, one of the earliest pathological changes described includes a population of neurons packed with swollen lysosomes or granules *(64,104),* which also stain with antibodies against Aβ *(105,106).* The generation of both Aβ40 and Aβ42 in endosomes is also well-established *(103).* In addition, the prevention of endocytosis appears to decrease the ratio of secreted Aβ42/40 *(101).* Besides the endocytic pathway, it appears that Aβ42 is also generated in other cell compartments such as the endoplasmic reticulum (ER) and intermediate compartment (ER/IC), as well as in the Golgi compartment *(32,101,107–111).* Figure 1 summarizes schematically the Aβ processing in the endocytic pathway and Figure 2 illustrates its subcellular compartimentalization in the rough endoplasmic reticulum, Golgi apparatus, and endosomal vesicles.

Presently, there is a current of opinion that the Aβ40 peptide is generated exclusively within the transgolgi network (TGN), while Aβ42 is generated in the ER *(112)* and TGN *(32,108,113).* Apparently, the Aβ42 produced within the ER is not packaged into ER-derived vesicles and remains as a detergent insoluble fraction that is not a source of secreted Aβ. The TGN is a major reservoir of peptides from which the secreted Aβ is packaged into secretory vesicles *(32,111,114).* APP harboring the Swedish mutation is processed to Aβ at an early step in the secretory pathway, giving rise to a stable intracellular pool of Aβ *(102).* There is evidence that secreted Aβ is produced in a postGolgi compartment different from the Golgi generation site of intracellular Aβ *(114–118).*

Until recently, extracellular Aβ was considered to be the only toxic form of this peptide *(119,120);* (for review *see* ref. *121*). It is only in the last few years that attention has been focused on the intracellular accumulation of the amyloid Aβ peptide, and its effects in brains with early AD pathology *(110,114,122–124);* (for review *see* refs. *42,109*).

As the origin of Aβ deposited in brain tissues remains uncertain, all sources need to be investigated further. Thus, intracellular or extracellular Aβ monomer, dimers, and oligomers *(125)* can easily diffuse from and to intracellular and extracellular compartments to induce toxic effects. Our knowledge of the

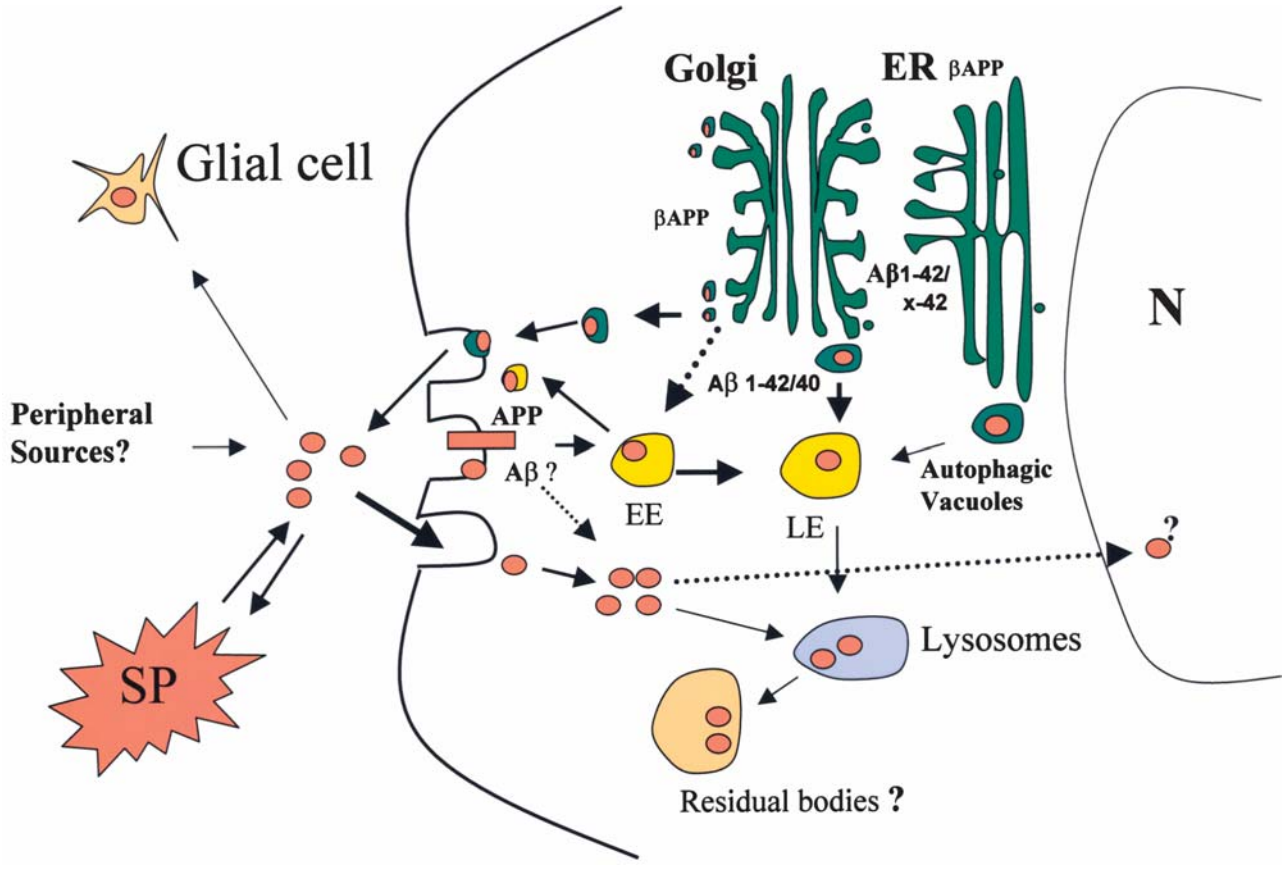

Fig. 1. Schematic representation of Aβ synthesis, intracellular trafficking, and uptake from the extracellular milieu. APP, Amyloid Precursor Protein; Aβ, amyloid-β peptide; SP, senile plaques; N, nucleus; AD, Alzheimer's disease; ER, endoplasmic reticulum; EE, early endosomes; LE, late endosomes.

neurotoxicity of extracellular Aβ has been obtained by the application of synthetic Aβ peptide extracellularly in vitro to a variety of cells systems *(126,127);* (for review *see* ref. *128*), and also in vivo in animal models *(83,85,129–131);* (for review *see* ref. *69*). On the other hand, LeBlanc and collaborators have recently reported toxic effects from very low concentrations of Aβ peptides when injected intracellularly into cultured primary human neurons; this process involves p53 and Bax apoptotic proteins *(33).* Grant et al. *(66)* have also shown that cells bearing intracellular Aβ display mitochondrial structural abnormalities and reduced membrane potential. It has also been demonstrated that the Aβ produced intracellularly is secreted, while significant levels remain inside the cell and exogenous Aβ 1–42 is internalized *(113,122,132).* Furthermore, in NT2N neuronal and other cells systems, the Aβ42 produced in the ER/IC accumulates into insoluble amyloid Aβ protein fractions in a time-dependent manner *(122)* (for a diagrammatic view of Aβ peptide cellular biology *see* Figure 1). The intracellular accumulation of Aβ peptides is similarly observed in transgenic mice expressing FAD mutations *(133–135).* It has been suggested that Aβ accumulates intracellularly in the brain in an

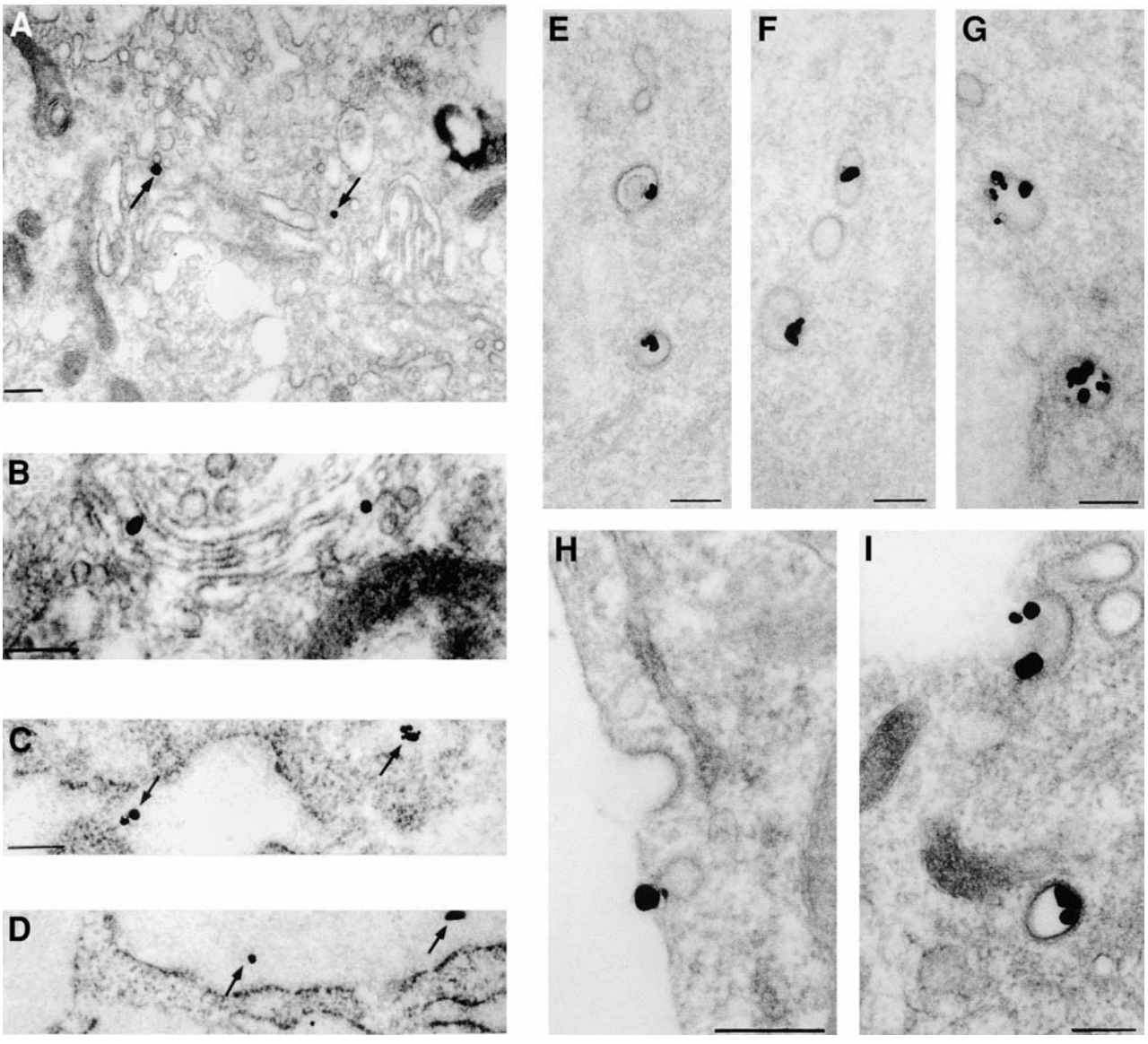

Fig. 2. Electron micrographs illustrating immunogold Aβ immunoreactive sites in subcellular compartments of neurally differentiated P19 cells, stably transfected to express the wild human APP 751 isoform. Aβ immunoreactivity was revealed applying McSA1, a highly specific monoclonal antibody to the human form of the Aβ fragment *(108).* The amyloidogenic Aβ peptides were observed in several intracellular compartments. Here their localization is illustrated in Golgi cisterns and in the transGolgi network (**A** and **B**); inside of dilated cisterns of the rough endoplasmic reticulum (**C** and **D**); within endosomes **(E–G);** as well as vesicular elements in contact or near the plasma membrane (**H** and **I**). Scale bars = 2 μm. Reprinted with permission from ref. *(108).*

aggregated detergent-insoluble pool *(123).* More significantly, this is also observed in AD pathology. Intraneuronal accumulation of Aβ is evident in AD-vulnerable regions such as the hippocampus/enthorrinal cortex, and it is speculated that it might precede plaque and NFT formation *(105).* Similarly, Aβ42 immunoreactivity in the cytoplasm and the cell processes of cortical neurons has been found by Mochizuki *(124).* Recently, the presence of intra-neuronal Aβ immunostaining in the hippocampus and cerebral cortex of very young Down syndrome patients (preceding the extracellular deposition of Aβ) has also been reported *(136).*

These findings beg the question as to whether the intracellular accumulation of Aβ plays an important pathological role in AD. Aβ amyloidogenesis could be initiated within living neurons rather than in the extracellular space. Because intraneuronal Aβ 42 accumulation occurs in early stages of AD pathology, Gouras and collaborators *(105)* have speculated that extracellular Aβ plaques develop from an initial intraneuronal pool of Aβ 1–42.

Intracellularly-accumulated Aβ peptides may originate within the cell or may in fact be material taken up from the extracellular milieu. In vitro evidence has shown that the amyloidogenic Aβ peptide can be taken up from the extracellular milieu. Aβ42 added to culture medium has been shown to be taken up by cells, and the amount of Aβ42 internalized is approx 5-fold higher than the amount of Aβ40, and shows a longer half-life *(132,137,138).* The Aβ42 contains non-enzymatic glycation modifications *(139),* racemic D-amino acids, and isopeptide bonds *(140),* usually present in long-lived polypeptides. It has also been reported that amyloid Aβ is also selectively internalized by hippocampal CA1 regions *(141).* Interestingly, the uptake of Aβ peptides, far from preventing amyloid deposition, relays Aβ peptides to lysosomes and endosomes where a low pH environment probably increases their rate of aggregation *(142).* Aβ42 accumulates as an insoluble residue in lysosomes where they might alter the normal catabolism of APP causing the accumulation of insoluble, amyloidogenic fragments *(143).* According to this hypothesis, intracellular Aβ accumulation induces cell lysis, and thus represents a mechanism for the genesis of amyloid deposits in AD (for review *see* refs. *42,106*). This possibility is supported by the fact that extracellular SPs are labeled with antibodies against representative lysosomal hydrolases *(144,145).* Immunohistochemical studies of the enthorhinal cortex and hippocampus of AD brains have revealed an inverse relationship between plaque size, pyramidal cell density, and intracellular Aβ accumulation—suggesting that plaques emerge as Aβ-loaded neurons *(105).* The mechanism for Aβ accumulation appears to be a self-propagating process where the addition of amyloidogenic fragments of APP in a nucleus of aggregated Aβ42 fragments generates the amyloid-protein aggregates *(42).*

There are an increasing number of reports from transgenic mice emphasizing the presence of Aβ plaque-independent cognitive decline. Thus, the loss of synaptophysin-immunoreactive (SYN-IR) presynaptic terminals in the frontal cortex has been shown to correlate with cognitive decline in AD *(146,147).* In this regard, it has been reported that a transgenic mouse over-expressing the human APP751 isoform, resulting in high CNS levels of soluble Aβ, develops severe age-dependent, spatial-learning deficits *(88).* Although a variety of behavioral changes occur in plaque-forming transgenic mice, severe to middle impairment results in this transgenic model long before plaque formation appears *(148).* In a different transgenic mouse model (one expressing PS1 mutations), Aβ42 accumulates within neurons, apparently inducing neurodegeneration without SP formation *(134,149).* In this model, neurodegeneration was accelerated in aged mice with increasing number of neurons containing intracellular Aβ42. Other cell abnormalities observed were: pyknosis of the nuclei, somatic cell shrinkage, and morphological irregularities of dendritic segments. In human AD brains, La Ferla and collaborators *(150)*

reported numerous Tunel-positive cells which coincided with intracellular Aβ immunoreactivity. In another study, it was shown that neurons simultaneously positive for intracellular Aβ42 and the Tunel assay, were significantly more abundant in AD brains than in controls *(134).* In yet another study, reports of deficits in synaptic transmission preceding amyloid deposition in transgenic mouse lines expressing the APP 'Indiana' and 'Swedish' FAD mutations were made *(151).* These studies collectively suggest that pathological changes begin before the onset of the classic amyloid plaque formation. Thus, the prevention of intracellular Aβ42 aggregation could be considered an interesting and, as yet uninvestigated, therapeutic target.

Amyloid Aβ Peptide Induces Tau Phosphorylation by Activating Tau Kinases

Abnormal tau phosphorylation makes an important contribution to AD pathology. Tau proteins belong to the microtubule-associated proteins (MAP) family. In the brain, they constitute a family of six proteins derived by alternative splicing from 352 to 441 amino acids *(152,153).* Tau is highly phosphorylated in fetus, but minimally phosphorylated in the normal adult brain *(153–155);* (for review *see* ref. *4*). Tau is abnormally phosphorylated in AD and is the main component of PHF and NFT *(156–158);* (for review *see* refs. *159,160*). In neurons displaying NFTs, the cytoskeleton is progressively replaced by bundles of PHFs *(161,162).* A correlation between the number of NFTs in the cortex and the degree and duration of dementia in AD has been found by several investigators *(163–165).* One of the most intriguing aspects of AD is the relationship between Aβ, and the abnormal hyperphosphorylation of tau and NFT formation. Numerous studies suggest that the extracellular Aβ aggregation precedes NFT formation. However NFTs in the absence of SP have also been reported *(80,166,167).* That Aβ accumulation occurs before NFT formation is supported by the fact that the progression in Down's syndrome progresses from intracellular accumulation of Aβ in neurons and astrocytes, to the development of neuritic plaques and neurofibrillary tangles with activation of microglial cells *(136).* Recently, it was reported that injection of exogenous Aβ into the CNS of a transgenic mice with a P301L tau mutation has led to the formation of NFT-like material in neurons of the amygdala projecting to the site of injection *(168).* Similar NFT-like formations were observed in neurons resulting from the crossing of APP tg line with a mice transgenic line bearing the P301L tau mutation *(169).* However, no NFT material has yet been obtained experimentally from animals expressing normal tau proteins.

The molecular pathway connecting an Aβ increase (intra or extracellular) to NFT formation is not yet known. At least 30 phosphorylation sites have been described for this protein (for review *see* ref. *4*). Hyperphosphorylation tends to weaken the tau-microtubule interaction. Most of these phosphorylation sites are on Ser-Pro and Thr-Pro motifs on the longest brain tau isoform (for review *see* ref. *170*), and the majority of the kinases involved in tau phosphorylation are part of the proline-directed protein kinases (PDPK). These include glycogen synthase kinase 3 (GSK3β) *(171,172),* tau-tubuline kinase *(173),* mitogen-activated protein kinase (MAPK) *(172,174),* and cyclin-dependent kinases including cdc2 and cdk5 *(172,175).* Non Ser/Thr-Pro sites can be phosphorylated by many other protein kinases, such as MARK *(176),* calcium/calmoduline-dependent protein kinase II (CaMPK II) *(177),* PKA *(178),* and casein kinase II *(179).* Stress activated protein kinases (SAP kinases) have also been proposed as tau phosphorylases *(180,181).* Several of these tau kinases have been described as phosphorylating tau in vitro; however it is less clear which enzymes abnormally phosphorylate tau in vivo rendering the AD pattern (for reviews *see* refs. *4,182*). Tau phosphorylation

in specific sites correlates with the severity of the neuropathology seen in AD cases and, as a result, the formation of NFTs has been proposed to occur in several steps:

1) Pretangle phospho-tau aggregates, which diffuse phospho-tau positive staining within the cytoplasm, with cell morphology normal;
2) Intraneuronal NFTs containing aggregated filamentous structures within the cytoplasm positive for phospho-tau, with phospho-tau present in proximal dendrites and axon hillocks; and
3) Extracellular NFTs, a densely immunoreactive extracellular NFT *(163,182).*

According to some reports, the more probable tau kinases in vivo in AD are: MAPK (ERK), CDK5 p38, JNK, and GSK3β *(77).* It is possible that the diverse tau kinases phosphorylate tau in a step-wise, time-dependent manner *(182).*

The authors have recently reported a rat transgenic model expressing mutated forms of APP 751 and PS1 displaying a phenotype of intracellular accumulation of Aβ peptides in neurons of the cerebral cortex and hippocampus, in the absence of extracellular amyloid plaques (Echeverria et al., submitted) (*see* Fig. 3). In these rats, starting from 6 mo old, the authors found ERK2 activation in the hippocampus concomitantly with an increase in tau phosphorylation, and in the absence of GSK3β or CDK5 activation (Echeverria et al., submitted). Previous studies have shown strong MAPK/ERK-P immunoreactivity in neurons containing tau deposits in AD *(184–186).* A similar activation of MAPK/ERK2 was observed in vitro after extracellular exposure to Aβ peptides *(187)* and after the intracellular increase of APP/Aβ content in stably wild, human-APP transfected cells *(188).* Interestingly, in post mortem brains with tauopathies, including AD, it has been shown that neurons and glial cells with early tau deposition display phospho-ERK immunoreactivity *(186).* This would suggest that MAPK/ERK participates in the early stages of tau phosphorylation in AD and other tauopathies. In line with this concept, our rat model found that intracellular Aβ accumulation activates protein kinases restricted to ERK2 at the initial phases of intracellular Aβ loading well before plaque formation (Echeverria et al., submitted), and prior to CDK5 or GSK3β activation. Therefore, it is plausible that tau phosphorylation is induced initially by kinases activated by intracellular Aβ accumulation, thus priming further, stepwise, phosphorylation by other kinases.

Concluding Remarks

In summary, besides the abundant evidence for toxic effects induced by the extracellular aggregates of Aβ amyloidgenic fragments, a case for a potential neurotoxic role for the excessive intracellular accumulation of Aβ fragments should be considered. The accumulation of intraneuronal Aβ fragments appears to be an early phase of the AD neuropathology. The excessive load of the amyloidgenic peptide appears to effect subcellular organelles and the level and quality of tau phosphorylation, apparently via the up-regulation of phospho-ERK2. The early intracellular accumulation of Aβ fragments might provoke cellular stress, thus making cortical and hippocampal regions vulnerable to further toxic attacks by extracellular amyloidgenic peptides. If these assumptions prove to be correct, the process of intracellular aggregation of amyloidgenic Aβ peptides could constitute a valuable target for early therapeutic intervention.

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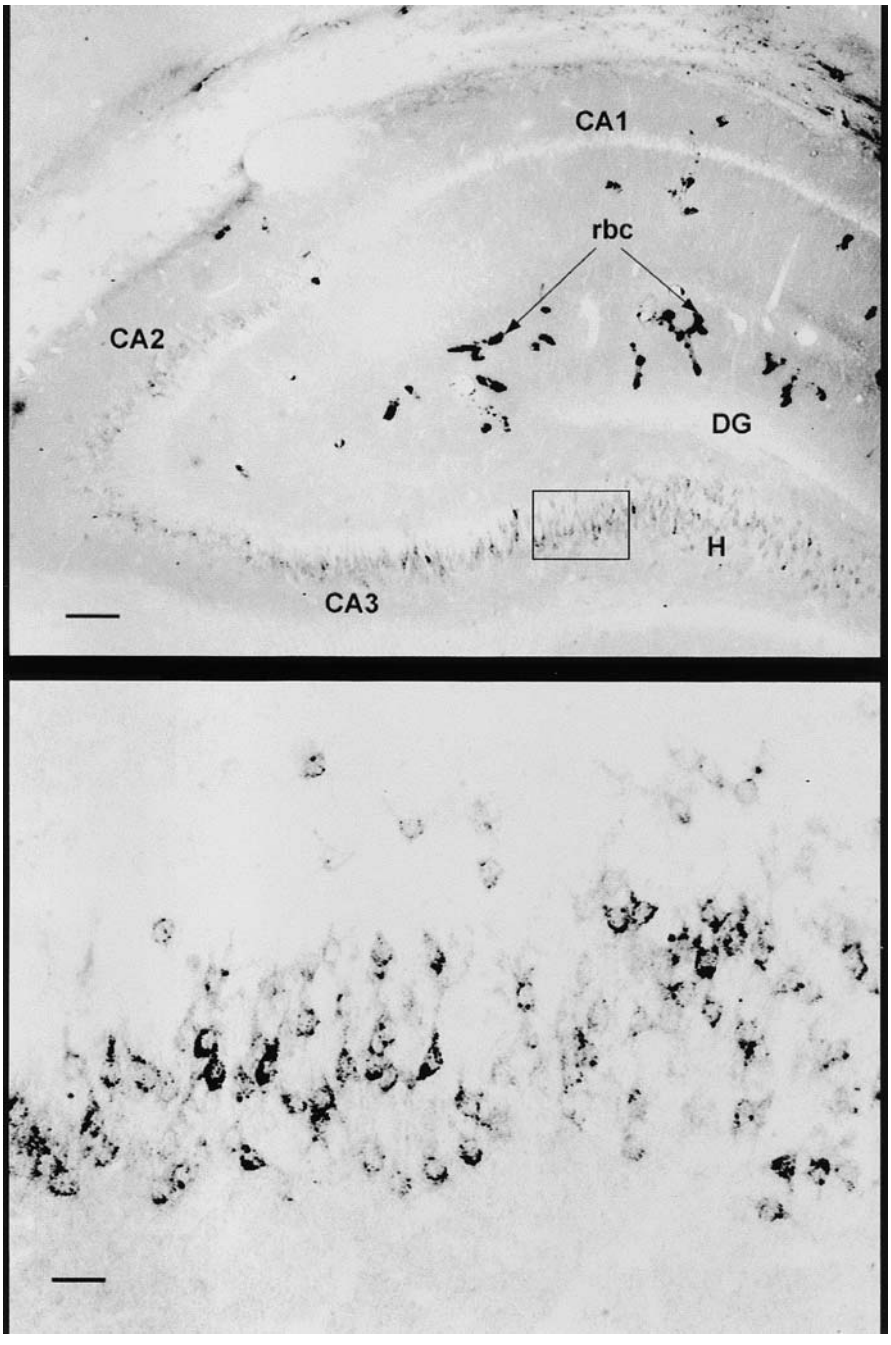

Fig. 3. Aβ immunoreactivity in the hippocampus of a rat transgenic model over expressing human amyloidgenic Aβ-peptide intraneuronarly. The Aβ immunoreactivity was detected with the McSA1 monoclonal antibody *(108).* (Upper panel) Low magnification micrograph of the rat hippocampus. Note intracellular accumulation of Aβ in neurons of the CA2, CA3, and hylus (H) regions. DG, dentate gyrus; rbc, denotes endogenous peroxide activity in red blood cells. Scale bar = 200 μm. (Lower panel) High magnification micrograph from boxed inset in upper panel showing the granular appearance of the intraneuronal aggregates immunoreactive material. Scale bar = 50 μm.

References

1. Sadovnick, D. and Lovestone, S. (2001) Genetic counselling, in *Clinical Diagnosis and Management of Alzheimer's Disease?* (Gauthier, S., ed.) Martin Dunitz, London, pp. 355–365.
2. Terry, R. D., Masliah, E., and Hansen, L. A. (1999) The neuropathology of Alzheimer's disease and the structural basis of its cognitive alterations, in *Alzheimer's Disease* (Terry, R. D., Katzman, R., and Bick, K. L., eds.) Raven Press, New York, pp. 187–206.
3. Goedert, M. (1998) Neurofibrillary pathology of Alzheimer's disease and other tauopathies *Prog. Brain Res.* **117,** 287–306.
4. Avila, J., Lim, F., Moreno, F., Belmonte, C., and Cuello, A. C. (2002) Tau function and dysfunction in neurons: its role in neurodegenerative disorders. *Mol. Neurobiol.* (In press.)
5. Haass, C., Schlossmacher, M. G., Hung, A. Y., et al. (1992) Amyloid b-peptide is produced by cultured cells during normal metabolism. *Nature* **359,** 322–325.
6. Shoji, M., Golde, T. E., Ghiso, J., et al. (1992) Production of the Alzheimer amyloid b protein by normal proteolytic processing. *Science* **258,** 126–129.
7. Walter, J., Kaether, C., Steiner, H., and Haass, C. (2001) The cell biology of Alzheimer's disease: uncovering the secrets of secretases. *Curr. Opin. Neurobiol.* **11,** 585–590.
8. Vassar, R. and Citron, M. (2000) Abeta-generating enzymes: recent advances in beta- and gamma-secretase research. *Neuron* **27,** 419–422.
9. Tamaoka, A., Sawamura, N., Odaka, A., Suzuki, N., Mizusawa, H., Shoji, S., and Mori, H. (1995) Amyloid beta protein 1–42/43 (A beta 1–42/43) in cerebellar diffuse plaques: enzymelinked immunosorbent assay and immunocytochemical study. *Brain Res.* **679,** 151–156.
10. Citron, M., Oltersdorf, T., Haass, C., et al. (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature* **360,** 672–674.
11. Hardy, J. (1999) The shorter amyloid cascade hypothesis. *Neurobiol. Aging* **20,** p. 85.
12. Selkoe, D. J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* **81,** 741–766.
13. Goate, A., Chartier-Harlin, M. C., Mullan, M., et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349,** 704–706.
14. Sherrington, R., Rogaev, E. I., Liang, Y., et al. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375,** 754–760.
15. Rogaev, E. I., Sherrington, R., Rogaeva, E. A., et al. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376,** 775–778.
16. Levy-Lahad, E., Wasco, W., Poorkaj, P., et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269,** 973–977.
17. Corder, E. H., Saunders, A. M., Strittmatter, W. J., et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261,** 921–923.
18. Strittmatter, W. J. (2000) Apolipoprotein E and Alzheimer's disease. *Ann. NY Acad. Sci.* **924,** 91–92.
19. Poirier, J. (1994) Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends Neurosci.* **17,** 525–530.
20. Bales, K. R., Verina, T., Dodel, R. C., et al. (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Genet.* **17,** 263–264.
21. Poirier. J. (2000) Apolipoprotein E and Alzheimer's disease. A role in amyloid catabolism. *Ann. NY Acad. Sci.* **924,** 81–90.
22. Blacker, D. and Tanzi, R. E. (1998) The genetics of Alzheimer disease: current status and future prospects. *Arch. Neurol.* **55,** 294–296.
23. Nicoll, J. A., Mrak, R. E., Graham, D. I., et al. (2000) Association of interleukin-1 gene polymorphisms with Alzheimer's disease. *Ann. Neurol.* **47,** 365–368.
24. Papassotiropoulos, A., Bagli, M., Feder, O., et al. (1999) Genetic polymorphism of cathepsin D is strongly associated with the risk for developing sporadic Alzheimer's disease. *Neurosci. Lett.* **262,** 171–174.
25. Kamboh, M. I., Ferrell, R. E., and DeKosky, S. T. (1998) Genetic association studies between Alzheimer's disease and two polymorphisms in the low density lipoprotein receptor-related protein gene. *Neurosci. Lett.* **244,** 65–68.
26. Yamanaka, H., Kamimura, K., Tanahashi, H., Takahashi, K., Asada, T., and Tabira, T. (1998) Genetic risk factors in Japanese Alzheimer's disease patients: alphal-ACT, VLDLR, and ApoE Neurobiol. *Aging* **19,** S43–S46.

27. Hu, Q., Kukull, W. A., Bressler, S. L., Gray, M. D., Cam, J. A., Larson, E. B., Martin, G. M., and Deeb, S. S. (1998) The human FE65 gene: genomic structure and an intronic biallelic polymorphism associated with sporadic dementia of the Alzheimer type. *Hum. Genet.* **103,** 295–303.
28. Montoya, S. E., Aston, C. E., DeKosky, S. T., Kamboh, M. I., Lazo, J. S., and Ferrell, R. E. (1998) Bleomycin hydrolase is associated with risk of sporadic Alzheimer's disease. *Nat. Genet.* **18,** 211–212.
29. Finckh, U., von der, K. H., Velden, J., et al. (2000) Genetic association of a cystatin C gene polymorphism with late-onset Alzheimer disease. *Arch. Neurol.* **57,** 1579–1583.
30. Jaffe, A. B., Toran-Allerand, C. D., Greengard, P., and Gandy, S. E. (1994) Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. *J. Biol. Chem.* **269,** 13,065–13,068.
31. Tang, M. X., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B., Andrews, H., and Mayeux, R. (1996) Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* **348,** 429–432.
32. Xu, H., Sweeney, D., Wang, R., Thinakaran, G., Lo, A. C., Sisodia, S. S., Greengard, P., and Gandy, S. (1997) Generation of Alzheimer b-amyloid protein in the trans-Golgi network in the apparent absence of vesicle formation. *Proc. Natl. Acad. Sci. USA* **94,** 3748–3752.
33. Zheng, H., Xu, H., Uljon, S. N., et al. (2002) Modulation of A(beta) peptides by estrogen in mouse models. *J. Neurochem.* **80,** 191–196.
34. Chang, D., Kwan, J. and Timiras, P. S. (1997) Estrogens influence growth, maturation, and amyloid beta-peptide production in neuroblastoma cells and in a beta-APP transfected kidney 293 cell line. *Adv. Exp. Med. Biol.* **429,** 261–271.
35. Mattson, M. P. and Chan, S. L. (2001) Dysregulation of cellular calcium homeostasis in Alzheimer's disease: bad genes and bad habits. *J. Mol. Neurosci.* **17,** 205–224.
36. Mattson, M. P. (2000) Neuroprotective signaling and the aging brain: take away my food and let me run. *Brain Res.* **886,** 47–53.
37. Borchelt, D. R., Thinakaran, G., Eckman, C. B., et al. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron* **17,** 1005–1013.
38. Fraser, P. E., Yang, D. S., Yu, G., et al. (2000) Presenilin structure, function and role in Alzheimer disease. *Biochim. Biophys. Acta.* **1502,** 1–15.
39. Chartier-Harlin, M. C., Crawford, F., Houlden, H., et al. (1991) Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature* **353,** 844–846.
40. Haass, C., Lemere, C. A., Capell, A., et al. (1995) The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway. *Nat. Med.* **1,** 1291–1296.
41. Citron, M., Westaway, D., Xia, W., et al. (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nature Med.* **3,** 67–72.
42. Glabe, C. (2001) Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *J. Mol. Neurosci.* **17,** 137–145.
43. Burger, P. C. and Vogel, F. S. (1973) The development of the pathologic changes of Alzheimer's disease and senile dementia in patients with Down's syndrome. *Am. J. Pathol.* **73,** 457–476.
44. Whalley, L. J. (1982) The dementia of Down's syndrome and its relevance to aetiological studies of Alzheimer's disease. *Ann. NY Acad. Sci.* **396,** 39–53.
45. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., and Beyreuther, K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. USA* **82,** 4245–4249.
46. Wisniewski, K. E., Wisniewski, H. M., and Wen, G. Y. (1985) Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann. Neurol.* **17,** 278–282.
47. Mann, D. M., Yates, P. O., Marcyniuk, B., and Ravindra, C. R. (1986) The topography of plaques and tangles in Down's syndrome patients of different ages. *Neuropathol. Appl. Neurobiol.* **12,** 447–457.
48. Cork, L. C. (1990) Neuropathology of Down syndrome and Alzheimer disease. *Am. J. Med. Genet.* **Suppl 7,** 282–286.
49. Fukuoka, Y., Fujita, T., and Ito, H. (1990) Histopathological studies on senile plaques in brains of patients with Down's syndrome Kobe. *J. Med. Sci.* **36,** 153–171.

50. Hof, P. R., Bouras, C., Perl, D. P., Sparks, D. L., Mehta, N., and Morrison, J. H. (1995) Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome. Quantitative regional analysis and comparison with Alzheimer's disease. *Arch. Neurol.* **52,** 379–391.
51. Hyman, B. T., West, H. L., Rebeck, G. W., Lai, F., and Mann, D. M. (1995) Neuropathological changes in Down's syndrome hippocampal formation. Effect of age and apolipoprotein E genotype. *Arch. Neurol.* **52,** 373–378.
52. Mann, D. M., Prinja, D., Davies, C. A., et al. (1989) Immunocytochemical profile of neurofibrillary tangles in Down's syndrome patients of different ages. *J. Neurol. Sci.* **92,** 247–260.
53. Alvarez, A., Toro, R., Caceres, A., and Maccioni, R. B. (1999) Inhibition of tau phosphorylating protein kinase cdk5 prevents beta-amyloid-induced neuronal death. *FEBS Lett.* **459,** 421–426.
54. Morishima, Y., Gotoh, Y., Zieg, J., et al. (2001) Beta-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. *J. Neurosci.* **21,** 7551–7560.
55. Sheng, J. G., Jones, R. A., Zhou, X. Q., McGinness, J. M., Van Eldik, L. J., Mrak, R. E., and Griffin, W. S. (2001) Interleukin-1 promotion of MAPK-p38 overexpression in experimental animals and in Alzheimer's disease: potential significance for tau protein phosphorylation. *Neurochem. Int.* **39,** 341–348.
56. Zhu, X., Castellani, R. J., Takeda, A., Nunomura, A., Atwood, C. S., Perry, G., and Smith, M. A. (2001) Differential activation of neuronal ERK, JNK/SAPK and p38 in Alzheimer disease: the 'two hit' hypothesis. *Mech. Ageing Dev.* **123,** 39–46.
57. Zhu, X., Rottkamp, C. A., Hartzler, A., et al. (2001) Activation of MKK6, an upstream activator of p38, in Alzheimer's disease. *J. Neurochem.* **79,** 311–318.
58. Checler, F., da Costa, C. A., Ancolio, K., Chevallier, N., Lopez-Perez, E., and Marambaud, P. (2000) Role of the proteasome in Alzheimer's disease. *Biochim. Biophys. Acta.* **1502,** 133–138.
59. McGeer, P. L. and McGeer, E. G. (1992) Complement proteins and complement inhibitors in Alzheimer's disease. *Res. Immunol.* **143,** 621–624.
60. Emmerling, M. R., Watson, M. D., Raby, C. A., and Spiegel, K. (2000) The role of complement in Alzheimer's disease pathology. *Biochim. Biophys. Acta.* **1502,** 158–171.
61. Smith, M. A., Rottkamp, C. A., Nunomura, A., Raina, A. K., and Perry, G. (2000) Oxidative stress in Alzheimer's disease. *Biochim. Biophys. Acta.* **1502,** 139–144.
62. Varadarajan, S., Yatin, S., Aksenova, M., and Butterfield, D. A. (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. *J. Struct. Biol.* **130,** 184–208.
63. Nunomura, A., Perry, G., Aliev, G., et al. (2001) Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **60,** 759–767.
64. Nixon, R. A., Mathews, P. M. and Ctaldo, A. M. (2001) The neuronal endosomal-lysosomal system in Alzheimer's disease. *J. Alz. Dis.* **3,** 97–107.
65. Casley, C. S., Canevari, L., Land, J. M., Clark, J. B., and Sharpe, M. A. (2002) Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J. Neurochem.* **80,** 91–100.
66. Grant, S. M., Morinville, A., Maysinger, D., Szyf, M., and Cuello, A. C. (1999) Phosphorylation of mitogen-activated protein kinase is altered in neuroectodermal cells overexpressing the human amyloid precursor protein 751 isoform. *Mol. Brain Res.* **72,** 115–120.
67. Canevari, L., Clark, J. B., and Bates, T. E. (1999) Beta-Amyloid fragment 25–35 selectively decreases complex IV activity in isolated mitochondria. *FEBS Lett.* **457,** 131–134.
68. Parks, J. K., Smith, T. S., Trimmer, P. A., Bennett, J. P., Jr., and Parker, W. D., Jr. (2001) Neurotoxic Abeta peptides increase oxidative stress in vivo through NMDA-receptor and nitric-oxide-synthase mechanisms, and inhibit complex IV activity and induce a mitochondrial permeability transition in vitro. *J. Neurochem.* **76,** 1050–1056.
69. Harkany, T., Hortobagyi, T., Sasvari, M., Konya, C., Penke, B., Luiten, P. G., and Nyakas, C. (1999) Neuroprotective approaches in experimental models of beta-amyloid neurotoxicity: relevance to Alzheimer's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **23,** 963–1008.
70. Auld, D. S., Kar, S., and Quirion, R. (1998) Beta-amyloid peptides as direct cholinergic neuromodulators: a missing link? *Trends Neurosci.* **21,** 43–49.

71. Kar, S., Seto, D., Gaudreau, P., and Quirion, R. (1996) Beta-amyloid-related peptides inhibit potassium-evoked acetylcholine release from rat hippocampal slices. *J. Neurosci.* **16,** 1034–1040.
72. Laskay, G., Zarandi, M., Varga, J., Jost, K., Fonagy, A., Torday, C., Latzkovits, L., and Penke, B. (1997) A putative tetrapeptide antagonist prevents beta-amyloid-induced long-term elevation of [$Ca2^+$]i in rat astrocytes. *Biochem. Biophys. Res. Commun.* **235,** 479–481.
73. Lin, H., Bhatia, R., and Lal, R. (2001) Amyloid beta protein forms ion channels: implications for Alzheimer's disease pathophysiology. *FASEB J.* **15,** 2433–2444.
74. Mattson, M. P., Barger, S. W., Cheng, B., Lieberburg, I., Smith-Swintosky, V. L., and Rydel, R. E. (1993) Beta-Amyloid precursor protein metabolites and loss of neuronal $Ca2^+$ homeostasis in Alzheimer's disease. *Trends Neurosci.* **16,** 409–414.
75. Mattson, M. P. and Pedersen, W. A. (1998) Effects of amyloid precursor protein derivatives and oxidative stress on basal forebrain cholinergic systems in Alzheimer's disease. *Int. J. Dev. Neurosci.* **16,** 737–753.
76. Tong, L., Thornton, P. L., Balazs, R., and Cotman, C. W. (2001) Beta-amyloid (1–42) impairs activity-dependent cAMP-response element-binding protein signaling in neurons at concentrations in which cell survival is not compromised. *J. Biol. Chem.* **276,** 17,301–17,306.
77. Ferrer, I., Blanco, R., Carmona, M., Puig, B., Dominguez, I., and Vinals, F. (2002) Active, phosphorylation-dependent MAP kinases, MAPK/ERK, SAPK/JNK and p38, and specific transcription factor substrates are differentially expressed following systemic administration of kainic acid to the adult rat. *Acta. Neuropathol. (Berl.)* **103,** 391–407.
78. Cummings, B. J., Pike, C. J., Shankle, R., and Cotman, C. W. (1996) Beta-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. *Neurobiol. Aging* **17,** 921–933.
79. Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274,** 99–102.
80. Naslund, J., Haroutunian, V., Mohs, R., Davis, K. L., Davies, P., Greengard, P., and Buxbaum, J. D. (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA* **283,** 1571–1577.
81. Chen, G., Chen, K. S., Knox, J., et al. (2000) A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408,** 975–979.
82. Gordon, M. N., King, D. L., Diamond, D. M., et al. (2001) Correlation between cognitive deficits and Aβ deposits in transgenic APP+PS1 mice. *Neurobiol. Aging* **22,** 377–385.
83. Frautschy, S. A., Baird, A., and Cole, G. M. (1991) Effects of injected Alzheimer b-amyloid cores in rat brain. *Proc. Natl. Acad. Sci. USA* **88,** 8362–8366.
84. Winkler, J., Connor, D. J., Frautschy, S. A., Behl, C., Waite, J. J., Cole, G. M., and Thal, L. J. (1994) Lack of long-term effects after beta-amyloid protein injections in rat brain. *Neurobiol. Aging* **15,** 601–607.
85. Giovannelli, L., Casamenti, F., Scali, C., Bartolini, L., and Pepeu, G. (1995) Differential effects of amyloid peptides beta-(1–40) and beta-(25–35) injections into the rat nucleus basalis. *Neuroscience* **66,** 781–792.
86. Giovannelli, L., Scali, C., Faussone-Pellegrini, M. S., Pepeu, G., and Casamenti, F. (1998) Long-term changes in the aggregation state and toxic effects of beta-amyloid injected into the rat brain. *Neuroscience* **87,** 349–357.
87. Rush, D. K., Aschmies, S., and Merriman, M. C. (1992) Intracerebral beta-amyloid (25–35) produces tissue damage: is it neurotoxic? *Neurobiol. Aging* **13,** 591–594.
88. Koistinaho, M., Ort, M., Cimadevilla, J. M., Vondrous, R., Cordell, B., Koistinaho, J., Bures, J., and Higgins, L. S. (2001) Specific spatial learning deficits become severe with age in beta-amyloid precursor protein transgenic mice that harbor diffuse beta-amyloid deposits but do not form plaques. *Proc. Natl. Acad. Sci. USA* **98,** 14,675–14,680.
89. Parvathy, S., Davies, P., Haroutunian, V., et al. (2001) Correlation between Abetax-40-, Abetax-42-, and Abetax-43-containing amyloid plaques and cognitive decline. *Arch. Neurol.* **58,** 2025–2032.
90. Stephan, A., Laroche, S., and Davis, S. (2001) Generation of aggregated beta-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. *J. Neurosci.* **21,** 5703–5714.

91. Janus, C., Chishti, M. A., and Westaway, D. (2000) Transgenic mouse models of Alzheimer's disease. *Biochim. Biophys. Acta.* **1502,** 63–75.
92. Morgan, D., Diamond, D. M., Gottschall, P. E., et al. (2000) A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408,** 982–985.
93. Mobley, W. C., Neve, R. L., Prusiner, S. B., and McKinley, M. P. (1988) Nerve growth factor increases mRNA levels for the prion protein and the beta-amyloid protein precursor in developing hamster brain. *Proc. Natl. Acad. Sci. USA* **85,** 9811–9815.
94. Rossner, S., Ueberham, U., Schliebs, R., Perez-Polo, J. R., and Bigl, V. (1998) The regulation of amyloid precursor protein metabolism by cholinergic mechanisms and neurotrophin receptor signaling. *Prog. Neurobiol.* **56,** 541–569.
95. Quon, D., Catalano, R., and Cordell, B. (1990) Fibroblast growth factor induces beta-amyloid precursor mRNA in ghal but not neuronal cultured cells. *Biochem. Biophys. Res. Commun.* **167,** 96–102.
96. Caporaso, G. L., Gandy, S. E., Buxbaum, J. D., Ramabhadran, T. V., and Greengard, P. (1992) Protein phosphorylation regulates secretion of Alzheimer beta/A4 amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* **89,** 3055–3059.
97. Hung, A. Y., Haass, C., Nitsch, R. M., Qiu, W. Q., Citron, M., Wurtman, R. J., Growdon, J. H., and Selkoe, D. J. (1993) Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *J. Biol. Chem.* **268,** 22,959–22,962.
98. Savage, M. J., Trusko, S. P., Howland, D. S., et al. (1998) Turnover of amyloid beta-protein in mouse brain and acute reduction of its level by phorbol ester. *J. Neurosci.* **18,** 1743–1752.
99. Haring, R., Fisher, A., Marciano, D., Pittel, Z., Kloog, Y., Zuckerman, A., Eshhar, N., and Heldman, E. (1998) Mitogen-activated protein kinase-dependent and protein kinase C-dependent pathways link the ml muscarinic receptor to beta-amyloid precursor protein secretion. *J. Neurochem.* **71,** 2094–2103.
100. Johnson-Wood, K., Lee, M., Motter, R., et al. (1997) Amyloid precursor protein processing and A beta42 deposition in a transgenic mouse model of Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **94,** 1550–1555.
101. Koo, E. H. and Squazzo, S. L. (1994) Evidence that production and release of amyloid b-protein involves the endocytic pathway. *J. Biol. Chem.* **269,** 17,386–17,389.
102. Perez, R. G., Squazzo, S. L., and Koo, E. H. (1996) Enhanced release of amyloid beta-protein from codon 670/671 "Swedish" mutant beta-amyloid precursor protein occurs in both secretory and endocytic pathways. *J. Biol. Chem.* **271,** 9100–9107.
103. Cataldo, A. M., Barnett, J. L., Pieroni, C., and Nixon, R. A. (1997) Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. *J. Neurosci.* **17,** 6142–6151.
104. Nixon, R. A., Cataldo, A. M., Paskevich, P. A., Hamilton, D. J., Wheelock, T. R., and Kanaley-Andrews, L. (1992) The lysosomal system in neurons. Involvement at multiple stages of Alzheimer's disease pathogenesis. *Ann. NY Acad. Sci.* **674,** 65–88.
105. Gouras, G. K., Tsai, J., Naslund, J., et al. (2000) Intraneuronal Ab42 accumulation in human brain. *Am. J. Pathol.* **156,** 15–20.
106. D'Andrea, M. R., Nagele, R. G., Wang, H. Y., Peterson, P. A., and Lee, D. H. (2001) Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease. *Histopathology* **38,** 120–134.
107. Soriano, S., Chyung, A. S., Chen, X., Stokin, G. B., Lee, V. M., and Koo, E. H. (1999) Expression of beta-amyloid precursor protein-CD3gamma chimeras to demonstrate the selective generation of amyloid beta(1–40) and amyloid beta(1–42) peptides within secretory and endocytic compartments. *J. Biol. Chem.* **274,** 32,295–32,300.
108. Grant, S. M., Ducatenzeiler, A., Szyf, M., and Cuello, A. C. (2000) Aβ immunoreactive material is present in several intracellular compartments in transfected, neuronally differentiated, P19 cells expressing the human amyloid β-protein precursor. *J. Alz. Dis.* **2,** 207–222.
109. Hartmann, T. (1999) Intracellular biology of Alzheimer's disease amyloid beta peptide. *Eur. Arch. Psychiatry Clin. Neurosci.* **249,** 291–298.
110. Cook, D. G., Forman, M. S., Sung, J. C., et al. (1997) Alzheimer's A beta(1–42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nature Med.* **3,** 1021–1023.
111. Greenfield, J. P., Tsai, J., Gouras, G. K., et al. (1999) Endoplasmic reticulum and trans-Golgi network generate distinct populations of

Alzheimer beta-amyloid peptides. *Proc. Natl. Acad. Sci. USA* **96,** 742–747.
112. Hartmann, T., Bieger, S. C., Bruhl, B., et al. (1997) Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. *Nature Med.* **3,** 1016–1020.
113. Petanceska, S. S., Seeger, M., Checler, F., and Gandy, S. (2000) Mutant presenilin 1 increases the levels of Alzheimer amyloid beta-peptide Abeta42 in late compartments of the constitutive secretory pathway. *J. Neurochem.* **74,** 1878–1884.
114. Martin, B. L., Schrader-Fischer, G., Busciglio, J., Duke, M., Paganetti, P., and Yankner, B. A. (1995) Intracellular accumulation of beta-amyloid in cells expressing the Swedish mutant amyloid precursor protein. *J. Biol. Chem.* **270,** 26,727–26,730.
115. Higaki, J., Quon, D., Zhong, Z., and Cordell, B. (1995) Inhibition of beta-amyloid formation identifies proteolytic precursors and subcellular site of catabolism. *Neuron* **14,** 651–659.
116. Yamazaki, T., Selkoe, D. J., and Koo, E. H. (1995) Trafficking of cell surface beta-amyloid precursor protein: retrograde and transcytotic transport in cultured neurons. *J. Cell Biol.* **129,** 431–442.
117. Tienari, P. J., Ida, N., Ikonen, E., et al. (1997) Intracellular and secreted Alzheimer b-amyloid species are generated by distinct mechanisms in cultured hippocampal neurons. *Proc. Natl. Acad. Sci. USA* **94,** 4125–4130.
118. Haass, C., Hung, A. Y., Schlossmacher, M. G., et al. (1993) Normal cellular processing of the beta-amyloid precursor protein results in the secretion of the amyloid beta peptide and related molecules. *Ann. NY Acad. Sci.* **695,** 109–116.
119. Pike, C. J., Burdick, D., Walencewicz, A. J., Glabe, C. G., and Cotman, C. W. (1993) Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J. Neurosci.* **13,** 1676–1687.
120. Lorenzo, A. and Yankner, B. A. (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc. Natl. Acad. Sci. USA* **91,** 12,243–12,247.
121. Roher, A. E., Baudry, J., Chaney, M. O., Kuo, Y. M., Stine, W. B., and Emmerling, M. R. (2000) Oligomerizaiton and fibril assembly of the mayloid-beta protein. *Biochim. Biophys. Acta.* **1502,** 31–43.
122. Skovronsky, D. M., Doms, R. W., and Lee, V. M. (1998) Detection of a novel intraneuronal pool of insoluble amyloid beta protein that accumulates with time in culture. *J. Cell Biol.* **141,** 1031–1039.
123. Lee, S. J., Liyanage, U., Bickel, P. E., Xia, W., Lansbury, P. T., Jr., and Kosik, K. S. (1998) A detergent-insoluble membrane compartment contains A beta in vivo. *Nat. Med.* **4,** 730–734.
124. Mochizuki, A., Tamaoka, A., Shimohata, A., Komatsuzaki, Y., and Shoji, S. (2000) Abeta42 positive non-pyramidal neurons around amyloid plaques in Alzheimer's disease. *Lancet* **355,** 42–43.
125. Walsh, D. M., Klyubin, I., Fadeeva, J. V., et al. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416,** 535–539.
126. Yankner, B. A., Dawes, L. R., Fisher, S., Villa-Komaroff, L., Oster-Granite, M. L., and Neve, R. L. (1989) Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science* **245,** 417–420.
127. Yankner, B. A., Caceres, A., and Duffy, L. K. (1990) Nerve growth factor potentiates the neurotoxicity of beta amyloid. *Proc. Natl. Acad. Sci. USA* **87,** 9020–9023.
128. Neve, R. L. and Robakis, N. K. (1998) Alzheimer's disease: a re-examination of the amyloid hypothesis. *Trends Neurosci.* **21,** 15–19.
129. Kowall, N. W., Beal, M. F., Busciglio, J., Duffy, L. K., and Yankner, B. A. (1991) An *in vivo* model for the neurodegenerative effects of b amyloid and protection by substance P. *Proc. Natl. Acad. Sci. USA* **88,** 7247–7251.
130. Harkany, T., O'Mahony, S., Kelly, J. P., et al. (1998) Beta-amyloid(Phe(SO3H)24)25–35 in rat nucleus basalis induces behavioral dysfunctions, impairs learning and memory and disrupts cortical cholinergic innervation. *Behav. Brain Res.* **90,** 133–145.
131. Geula, C., Wu, C. K., Saroff, D., Lorenzo, A., Yuan, M., and Yankner, B. A. (1998) Aging renders the brain vulnerable to amyloid beta-protein neurotoxicity. *Nat. Med.* **4,** 827–831.
132. Knauer, M. F., Soreghan, B., Burdick, D., Kosmoski, J., and Glabe, C. G. (1992) Intracellular accumulation and resistance to degradation of the Alzheimer amyloid A4/beta protein. *Proc. Natl. Acad. Sci. USA* **89,** 7437–7441.
133. Xia, W., Zhang, J., Ostaszewski, B. L., et al. (1998) Presenilin 1 regulates the processing of beta-amyloid precursor protein C-terminal fragments and the generation of amyloid beta-

protein in endoplasmic reticulum and Golgi. *Biochemistry* **37,** 16,465–16,471.
134. Chui, D. H., Tanahashi, H., Ozawa, K., et al. (1999) Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nat. Med.* **5,** 560–564.
135. Wirths, O., Multhaup, G., Czech, C., Blanchard, V., et al. (2001) Intraneuronal Abeta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. *Neurosci. Lett.* **306,** 116–120.
136. Gyure, K. A., Durham, R., Stewart, W. F., Smialek, J. E., and Troncoso, J. C. (2001) Intraneuronal abeta-amyloid precedes development of amyloid plaques in Down syndrome. *Arch. Pathol. Lab. Med.* **125,** 489–492.
137. Yang, A. J., Knauer, M., Burdick, D. A., and Glabe, C. (1995) Intracellular A beta 1–42 aggregates stimulate the accumulation of stable, insoluble amyloidogenic fragments of the amyloid precursor protein in transfected cells. *J. Biol. Chem.* **270,** 14,786–14,792.
138. Burdick, D., Kosmoski, J., Knauer, M. F., and Glabe, C. G. (1997) Preferential adsorption, internalization and resistance to degradation of the major isoform of the Alzheimer's amyloid peptide, A beta 1–42, in differentiated PC12 cells. *Brain Res.* **746,** 275–284.
139. Smith, M. A. and Perry, G. (1995) Free radical damage, iron, and Alzheimer's disease. *J. Neurol. Sci.* **134 Suppl,** 92–94.
140. Roher, A. E., Lowenson, J. D., Clarke, S., Woods, A. S., Cotter, R. J., Gowing, E., and Ball, M. J. (1993) Beta-Amyloid-(1–42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **90,** 10,836–10,840.
141. Bahr, B. A., Hoffman, K. B., Yang, A. J., Hess, U. S., Glabe, C. G., and lynch, G. (1998) Amyloid beta protein is internalized selectively by hippocampal field CA1 and causes neurons to accumulate amyloidogenic carboxyterminal fragments of the amyloid precursor protein. *J. Comp Neurol.* **397,** 139–147.
142. Burdick, D., Soreghan, B., Kwon, M., et al. (1992) Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *J. Biol. Chem.* **267,** 546–554.
143. Yang, A. J., Chandswangbhuvana, D., Shu, T., Henschen, A., and Glabe, C. G. (1999) Intracellular accumulation of insoluble, newly synthesized abetan-42 in amyloid precursor protein-transfected cells that have been treated with Abeta1–42. *J. Biol. Chem.* **274,** 20,650–20,656.
144. Cataldo, A. M., Thayer, C. Y., Bird, E. D., Wheelock, T. R., and Nixon, R. A. (1990) Lysosomal proteinase antigens are prominently localized within senile plaques of Alzheimer's disease: evidence for a neuronal origin. *Brain Res.* **513,** 181–192.
145. Omar, R., Pappolla, M., Argani, I., and Davis, K. (1993) Acid phosphatase activity in senile plaques and cerebrospinal fluid of patients with Alzheimer's disease. *Arch. Pathol. Lab. Med.* **117,** 166–169.
146. Sze, C. I., Troncoso, J. C., Kawas, C., Mouton, P., Price, D. L., and Martin, L. J. (1997) Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **56,** 933–944.
147. Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Alford, M., and Terry, R. (1994) Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci. Lett.* **174,** 67–72.
148. Koistinaho, M., Kettunen, M. I., Goldsteins, G., et al. (2002) Beta-amyloid precursor protein transgenic mice that harbor diffuse A beta deposits but do not form plaques show increased ischemic vulnerability: role of inflammation. *Proc. Natl. Acad. Sci. USA* **99,** 1610–1615.
149. Tabira, T., Chui, D. H., and Kuroda, S. (2002) Significance of intracellular Abeta42 accumulation in Alzheimer's disease. *Front Biosci.* **7,** a44–a49.
150. LaFerla, F. M., Troncoso, J. C., Strickland, D. K., Kawas, C. H., and Jay, G. (1997) Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Abeta stabilization. *J. Clin. Invest* **100,** 310–320.
151. Hsia, A. Y., Masliah, E., McConlogue, L., et al. (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. USA* **96,** 3228–3233.
152. Himmler, A., Drechsel, D., Kirschner, M. W., and Martin, D. W., 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.
153. Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., and Crowther, R. A. (1989) Multiple isoforms of human microtubule-associated protein tau: sequences and localization

in neurofibrillary tangles of Alzheimer's disease. *Neuron* **3,** 519–526.
154. Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., and Klug, A. (1988) Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proc. Natl. Acad. Sci. USA* **85,** 4051–4055.
155. Otvos, L., Jr., Feiner, L., Lang, E., Szendrei, G. I., Goedert, M., and Lee, V. M. (1994) Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. *J. Neurosci. Res.* **39,** 669–673.
156. Crowther, T., Goedert, M., and Wischik, C. M. (1989) The repeat region of microtubule-associated protein tau forms part of the core of the paired helical filament of Alzheimer's disease. *Ann. Med.* **21,** 127–132.
157. Crowther, R. A., Olesen, O. F., Jakes, R., and Goedert, M. (1992) The microtubule binding repeats of tau protein assemble into filaments like those found in Alzheimer's disease. *FEBS Lett.* **309,** 199–202.
158. Crowther, R. A., Olesen, O. F., Smith, M. J., Jakes, R., and Goedert, M. (1994) Assembly of Alzheimer-like filaments from full-length tau protein. *FEBS Lett.* **337,** 135–138.
159. Mandelkow, E. M. and Mandelkow, E. (1998) Tau in Alzheimer's disease. *Trends Cell Biol.* **8,** 425–427.
160. Iqbal, K., Alonso, A. D., Gondal, J. A., et al. (2000) Mechanism of neurofibrillary degeneration and pharmacologic therapeutic approach. *J. Neural Transm.* **59 Suppl,** 213–222.
161. Dustin, P. and Flament-Durand, J. (1982) Disturbances of axoplasmic transport in Alzheimer's disease, in *Axoplasmic Transport and Pathology.* (Weiss, D. G. and Gorio, A., eds.) Springer-Verlag, Berlin, pp. 131–136.
162. Grundke-Iqbal, I. and Iqbal, K. (1999) Tau pathology generated by overexpression of tau. *Am. J. Pathol.* **155,** 1781–1785.
163. Mena, R., Wischik, C. M., Novak, M., Milstein, C., and Cuello, A. C. (1991) A progressive deposition of paired helical filaments (PHF) in the brain characterizes the evolution of dementia in Alzheimer's disease. An immunocytochemical study with a monoclonal antibody against the PHF core. *J. Neuropathol. Exp. Neurol.* **50,** 474–490.
164. Wischik, C. M., Harrington, C. R., Mukaetova-Ladinska, E. B., Novak, M., Edwards, P. C., and McArthur, F. K. (1992) Molecular characterization and measurement of Alzheimer's disease pathology: implications for genetic and environmental aetiology. *Ciba Found. Symp.* **169,** 268–293.
165. Holzer, M., Holzapfel, H.-P., Zedlick, D., Brückner, M. K., and Arendt, T. (1994) Abnormally phosphorylated tau protein in Alzheimer's disease: Heterogeneity of individual regional distribution and relationship to clinical severity. *Neuroscience* **63,** 499–516.
166. Trojanowski, J. Q. and Lee, V. M. (1994) Paired helical filament tau in Alzheimer's disease. The kinase connection. *Am. J. Pathol.* **144,** 449–453.
167. Jellinger, K. A. and Bancher, C. (1998) Senile dementia with tangles (tangle predominant form of senile dementia). *Brain Pathol.* **8,** 367–376.
168. Gotz, J., Chen, F., van Dorpe, J., and Nitsch, R. M. (2001) Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Abeta 42 fibrils. *Science* **293,** 1491–1495.
169. Lewis, J., Dickson, D. W., Lin, W. L., et al. (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* **293,** 1487–1491.
170. Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A., and Hof, P. R. (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res. Brain Res. Rev.* **33,** 95–130.
171. Hanger, D. P., Hughes, K., Woodgett, J. R., Brion, J. P., and Anderton, B. H. (1992) Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci. Lett.* **147,** 58–62.
172. Tomidokoro, Y., Harigaya, Y., Matsubara, E., et al. (2001) Brain Abeta amyloidosis in APPsw mice induces accumulation of presenilin-1 and tau. *J. Pathol.* **194,** 500–506.
173. Takahashi, M., Tomizawa, K., Sato, K., Ohtake, A., and Omori, A. (1995) A novel tau-tubulin kinase from bovine brain. *FEBS Lett.* **372,** 59–64.
174. Drewes, G., Lichtenberg-Kraag, B., Doring, F., et al. (1992) Mitogen activated protein (MAP) kinase transforms tau protein into an Alzheimer-like state. *EMBO J.* **11,** 2131–2138.
175. Liu, W. K., Williams, R. T., Hall, F. L., Dickson, D. W., and Yen, S. H. (1995) Detection of a Cdc2-related kinase associated with

Alzheimer paired helical filaments. *Am. J. Pathol.* **146,** 228–238.
176. Drewes, G., Ebneth, A., Preuss, U., et al. (1997) A novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89,** 297–308.
177. Johnson, G. V. (1992) Differential phosphorylation of tau by cyclic AMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II: metabolic and functional consequences. *J. Neurochem.* **59,** 2056–2062.
178. Litersky, J. M. & Johnson, G. V. (1992) Phosphorylation by cAMP-dependent protein kinase inhibits the degradation of tau by calpain. *J. Biol. Chem.* **267,** 1563–1568.
179. Greenwood, J. A., Scott, C. W., Spreen, R. C., Caputo, C. B., and Johnson, G. V. (1994) Casein kinase II preferentially phosphorylates human tau isoforms containing an amino-terminal insert. Identification of threonine 39 as the primary phosphate acceptor. *J. Biol. Chem.* **269,** 4373–4380.
180. Goedert, M., Cuenda, A., Craxton, M., Jakes, R., and Cohen, P. (1997) Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases. *EMBO J.* **16,** 3563–3571.
181. Reynolds, C. H., Utton, M. A., Gibb, G. M., Yates, A., and Anderton, B. H. (1997) Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein *J. Neurochem.* **68,** 1736–1744.
182. Imahori, K., Hoshi, M., Ishiguro, K., et al. (1998) Possible role of tau protein kinases in pathogenesis of Alzheimer's disease. *Neurobiol. Aging* **19,** S93–S98.
183. Augustinack, J. C., Schneider, A., Mandelkow, E. M., and Hyman, B. T. (2002) Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta. Neuropathol. (Berl.)* **103,** 26–35.
184. Perry, G., Roder, H., Nunomura, A., et al. (1999) Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal phosphorylation. *NeuroReport* **10,** 2411–2415.
185. Knowles, R. B., Chin, J., Ruff, C. T., and Hyman, B. T. (1999) Demonstration by fluorescence resonance energy transfer of a close association between activated MAP kinase and neurofibrillary tangles: implications for MAP kinase activation in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **58,** 1090–1098.
186. Ferrer, I., Blanco, R., Carmona, M., and Puig, B. (2001) Phosphorylated mitogen-activated protein kinase (MAPK/ERK-P), protein kinase of 38 kDa (p38-P), stress-activated protein kinase (SAPK/JNK-P), and calcium/calmodulin-dependent kinase II (CaM kinase II) are differentially expressed in tau deposits in neurons and glial cells in tauopathies. *J. Neural Transm.* **108,** 1397–1415.
187. Greenberg, S. M., Koo, E. H., Selkoe, D. J., Qiu, W. Q., and Kosik, K. S. (1994) Secreted β-amyloid precursor protein stimulates mitogen-activated protein kinase and enhances tau phosphorylation. *Proc. Natl. Acad. Sci. USA* **91,** 7104–7108.
188. Grant, S. M., Shankar, S. L., Chalmers-Redman, R. M. E., Tatton, W. G., Szyf, M., and Cuello, A. C. (1999) Mitochondrial abnormalities in neuroectodermal cells stably expressing human amyloid precursor protein ($hAPP_{751}$). *NeuroReport* **10,** 41–46.