CMLS, Cell. Mol. Life Sci. 57 (2000) 705–715
1420-682X/00/050705-21 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Molecular basis of Alzheimer's disease

B. Drouet, M. Pinçon-Raymond, J. Chambaz* and T. Pillot

INSERM U-505, Institut des Cordeliers, 15 rue de l'Ecole de Médecine, 75006 Paris (France), Fax + 33 1 43 25 16 15, e-mail: jean.chambaz-u505@bhdc.jussieu.fr

Abstract. Despite an exponential production of data, Alzheimer's disease (AD) remains an enigma. Unresolved questions persist in the face of the heterogeneity of this neuropathology. Recent progress in understanding mechanisms for AD results from the study of amyloid precursor protein (APP) metabolism and the involvement of senile plaque-associated proteins. In addition to the amyloid cascade hypothesis, alternative schemes emerge, in which the amyloid peptide is not the primary effector of the disease. Perturbations of vesicular trafficking, the cytoskeletal network, and membrane cholesterol distribution could be central events. Furthermore, since the physiological role of APP, presenilins, and apolipoprotein E in the central nervous system are not completely understood, their involvement in AD etiology remains speculative. New actors have to be found to try to explain sporadic cases and non-elucidated familial cases.

Key words. Amyloid-β peptide; presenilin; Alzheimer's disease; apolipoprotein E; cholesterol homeostasis.

During the past 20 years, knowledge about the pathology of Alzheimer's disease (AD) has been accumulating. Despite an exponential production of data, which is often controversial, this neuropathology remains hazy and patchy. The absence of an early diagnosis and the lack of an animal model render it difficult to establish an order of events and to explain the molecular mechanisms leading to neuronal loss. In a first stage, hypotheses focused on the amyloid β peptide (Aβ) as the major actor in AD. But, the amyloid cascade hypothesis now appears to be sufficient and complementary hypotheses are required to fully explain the heterogeneous Alzheimer's pathology.

Why is AD still an enigma?

Identification of the actors

AD has been characterized by postmortem histopathological studies, genetic linkage, and epidemiological studies [1]. The three main characteristics of AD are amyloid deposits surrounding dying neurons (senile plaques), neurofibrillar degeneration with tangles, and cerebrovascular angiopathies. These events are associated with gliosis and neuronal loss, particularly of cholinergic neurons, and are localized in some specific brain areas. On the basis of epidemiological studies, AD cases have been classified according to age at onset (early: < 60 years; late: > 60 years) and mode of occurrence (familial transmission or sporadic cases).

Extracellular senile plaques contain numerous components, but much fibrillar Aβ. Aβ is the result of the cleavage of the amyloid precursor protein (APP) at three sites, α, β, and γ, yielding short peptides of 4 kDa, with heterogeneous amino- and carboxy-terminal ends. Amyloid plaques differ according to pathologies and topologies, either as congophilic fibrillar deposits made of Aβ(1–40) and (1–42) peptides in cerebral parenchymal areas or as amorphous deposits made of non-fibrillar Aβ(17–42) in the cerebellum. Mutations in genes of APP and presenilins (PSs) are linked to familial early-onset cases of AD, in which transmission is autosomal dominant [2, 3]. Mutations in the PS-1 gene, located on chromosome 14, are linked to more than 40% of early-onset cases, whereas APP (chromosome 21) and PS-2 (chromosome 1) mutations are far less common, as low as 1% [4].

* Corresponding author.

Apolipoprotein (apo) E and, recently, α2-macroglobulin (α2-MG) have been identified as risk factors [5]. Indeed, allele ε4 of apoE and deletion in exon 18 of α2-MG both confer an increased risk of sporadic AD. In addition, apoE allele 4 accelerates the age of onset. These two factors are localized in senile plaques along with other components such as apoA-I and apoJ, heparan sulfate proteoglycans (HSPGs), and elements of the immune system [6].

The neurofibrillary tangles are constituted mainly of paired helical filaments (PHFs). The formation unit of a PHF is the hyperphosphorylated and ubiquitinated form of tau, a microtubule-associated protein (MAP) [7, 8]. When hyperphosphorylated, tau loses its capacity to bind tubulin and stabilize microtubule assembly. Even if the degree of dementia correlates with the localization and severity of PHFs, the exact role of tangles in the development of AD remains unclear and controversial.

Open questions

Faced with these hallmarks of AD, a number of crucial questions arise. Is neurodegeneration induced by Aβ? How does Aβ act? What intracellular toxic mechanisms are induced by Aβ? Do the molecular factors binding to Aβ play a passive or active role? What are the effects of the accumulating intracellular pool of insoluble Aβ? How are glial cells involved in AD? Does apoE act as a protein chaperone and/or a neurotrophic factor in the homeostasis of cholesterol?

The amyloid cascade hypothesis

Under this hypothesis, the change of conformation and fibrillar aggregation of Aβ result in extracellular deposits, which interact with cells and might lead to neuronal death (fig. 1). This has been confirmed by many studies using cerebral cells in primary culture or neuronal cell lines. This model would target the over-

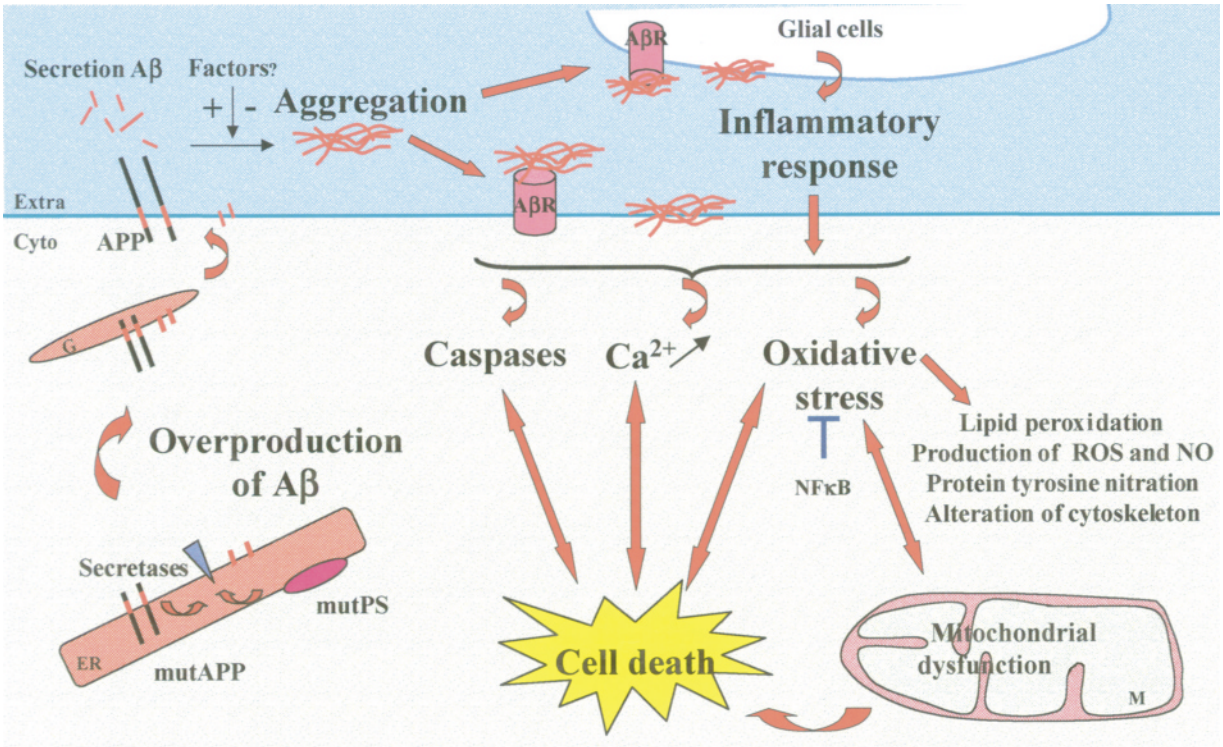

Figure 1. Amyloid cascade hypothesis. Mutated proteins, APP or PSs, increase membrane cholesterol content to alter APP processing, leading to an overproduction of Aβ. An increase in Aβ secretion may favor fibrillation and subsequent aggregation, which may be modulated by extracellular factors. Through receptor or direct interactions with neuronal membrane, or through the activation of glial cells, aggregated Aβ triggers a general toxic pathway. The identity of all effectors and the chronology of events are still unclear. It appears that Aβ-induced toxicity involves an oxidative stress, severe mitochondrial dysfunction, the activation of caspases, and an increase in cytosolic calcium. AβR, Aβ receptor; mutAPP and mutPSs, mutated APP and PS; G, Golgi apparatus; ER, endoplasmic reticulum; M, mitochondrion; extra and cyto, extracellular and cytoplasmic compartments; ROS reactive oxygen species; NO, nitric oxide.

production of Aβ and/or a deficiency in the clearance of the peptide as the main cause(s) of the disease.

The majority of studies conducted in this area have tried to determine the precise molecular mechanisms involved in the amyloid cascade hypothesis. These include characterization of Aβ-induced neurotoxicity, causes of peptide overproduction or accumulation in critical areas of the brain, molecular factors that could modify the accumulation, and aggregation and clearance of the Aβ peptide by neuronal and non-neuronal cells.

Overproduction, aggregation, and fibrillar-modulating factors

All events in favor of fibrillogenesis, namely fibrillation, overproduction of Aβ(1–42), decreased clearance of secreted Aβ (sAβ), and involvement of aggregating factors, might lead to neurodegeneration. Soluble Aβ peptides have been detected in cerebrospinal fluid (CSF) in normal and AD brains, mainly as Aβ(1–40). Biochemical studies have demonstrated the propensity of Aβ(1–42) to self-aggregate and facilitate nucleation-dependent polymerization of the shorter form, Aβ(1–40) [1]. Mutations in APP or PSs have been shown to modify the processing of APP, through modulation of secretase activities. This altered processing leads to an increase in Aβ, and particularly Aβ(1–42). It might trigger aggregation and induce the neurodegenerative process [9].

Numerous biochemical and cellular studies have shown that the aggregation might be modulated by some of the proteins present in senile plaques. apoE and apoJ, laminin, α2-MG, and α_1-antichymotrypsin (α1-ACT) inhibit fibrillogenesis [10]. Conversely, aggregation is accelerated by acetylcholinesterase (AChase) and perlecan [11, 12]. ApoE, α2MG, and α1-ACT which prevent fibrillation of Aβ, protect against neurotoxicity [13]. Indeed, the 'sticky' nature of the peptide Aβ results in the association of numerous proteins, eventually found in the senile plaques. Therefore, it precludes determining the chronology of their association and whether one protein or another plays an active role in the constitution of senile plaques. Factors responsible for or inducing aggregation of the peptide, as well as the cellular context in which it occurs, have yet to be established. A disequilibrium in the metabolism, not only of APP, but also of an extracellular factor(s), could prime aggregation and fibrillation of Aβ peptide. In hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D), patients develop cerebrovascular amyloidosis with non-fibrillar Aβ deposits. After injection in rat brain, soluble Aβ(1–40) formed fibrillar aggregates, whereas the Aβ(1–42) deposit contained non-fibrillar amorphous material [14]. These results strengthen the hypothesis of the intervention of more than one factor in the aggregation of Aβ.

Interaction with a specific receptor or with the plasma membrane?

Cross-linking, membrane binding, or double-hybrid studies have revealed a direct interaction of fibrillar Aβ with the $p75^{NGF}$ receptor, scavenger receptor and RAGE receptor [15–17]. The scavenger receptor of class A and RAGE receptor are expressed by glial cells and could mediate Aβ toxicity, leading to activation of microglia and production of reactive oxygen species (ROS) [18]. Binding of Aβ to $p75^{NGF}$ receptor has recently been reported to activate NF-κB in human neuroblastoma [19].

Another potential mechanism for inducing toxicity is by direct interaction with the membrane. In biochemical studies, soluble and fibrillar Aβ peptides have exhibited disrupting membrane properties, particularly at acidic pH [20–22]. Other studies have shown that Aβ is able to form ionic pores, perturbing membrane permeability. Thus, Aβ peptides could insert in plasma or intracellular membranes, inducing cell death and/or leading to peptide clearance.

Aβ toxicity pathway

The pathway by which Aβ induces neuronal cell death has been investigated and various mechanisms have been proposed, including intracellular calcium accumulation, nitric oxide (NO) and peroxide production, membrane lipid peroxidation, decreased membrane fluidity, and alteration of the cytoskeleton and nucleus. All of these intracellular events converge to the ubiquitous pathways of necrosis or apoptosis.

In neuronal cell lines, Aβ toxicity induces the production of ROS and NO. The production of these toxic compounds is accompanied by depletion in glutathione peroxidase (GSH), activation of inducible NO synthase (iNOS), accumulation of mitochondrial peroxynitrite, tyrosine nitration, and membrane lipid peroxidation. Bcl2 expression, sAPPα, tumor necrosis factor-α (TNF-α) and C2-ceramide pretreatment protect neurons against Aβ-induced oxidative stress, associated with increased expression of GSH and manganese superoxide dismutase (Mn-SOD) [23–25]. The protective mechanism involves the activation of NF-κB [26]. In some cases, however, Aβ treatment activates NF-κB, leading to an increased expression of iNOS in rat astrocytes [27], and to cell death in neuroblastoma [19]. Although several studies have reported the production of ROS by Aβ, antioxidant molecules do not appear to attenuate Aβ toxicity [28]. ROS production might be a secondary event in the Aβ-induced cell death.

A substantial cytosolic increase in calcium can induce cell death. Data on the calcium flux involved in Aβ-induced toxicity are contradictory. Two phases have been described upon Aβ exposure: an early Ca^{2+}-independent phase and a late Ca^{2+}-dependent phase involving the voltage-dependent calcium channel (VDCC), such as the L-type VDCC, and intracellular flux from the endoplasmic reticulum through the InsP3 receptor or ryanodine receptor [29]. In some studies, no variation in intracellular calcium leading to Aβ toxicity has been measured [30].

Necrosis or apoptosis?

Although the data remain controversial, evidence is accumulating for two types of cell death in AD: apoptosis and necrosis. Necrosis is reported to be induced by Aβ in PC12 cells [31], and by an inflammatory response of glial cells. Morphological changes, characteristics of apoptosis (membrane blebbing, nuclear chromatin condensation, DNA fragmentation), modulation of expression of apoptotic genes (decreased expression of Bcl2 and increased expression of Bax, cFos, cJun, p53, and Fas) are all induced by Aβ in neuronal primary culture and AD postmortem tissue [32–34].

However, determining whether Aβ directly triggers neuronal cell death or whether its effects are relayed by activated microglia is difficult. Furthermore, the properties of neurons and cell lines tested intervene in the type of cell death.

Several mechanisms for a multifactorial pathology?

This dramatic accumulation of data does not clarify the confusing picture of AD. The classical amyloid hypothesis cannot explain all the molecular and cellular events occurring in the different forms of AD. In the last few years, mechanisms have emerged in which the fibrillar amyloid deposit does not act as the primary effector of this neurodegeneration (fig. 2). There is no strong evidence pointing to toxicity of the Aβ deposit in vivo [for a review see ref. 35]. No correlation has been reported between the distribution and number of senile plaques and neurodegeneration or synaptic loss. Neither has any correlation been found between senile plaques and the degree of dementia, whereas neurofibrillary tangles were correlated with dementia. Moreover, amyloid deposits have been found in normal brain of non-demented elderly individuals.

Familial and sporadic cases require alternative hypotheses, as AD is clearly a heterogeneous disease. The mutations found in the three genes, APP, PS-1, and PS-2, lead to an overproduction of Aβ and are linked to around half of the familial cases. The etiology of the remaining familial cases and the sporadic cases has yet to be determined. All of the histologic studies have been conducted on postmortem slices from brain of individuals with AD. Thus, the impossibility of detecting early the neurological insults accentuates the difficulty in understanding the mechanisms of AD. In particular, trying to establish the chronology of events is hazardous, since distinguishing between early or secondary events with any degree of certainty is impossible.

Aβ deposit: a primary or secondary event in AD?

Most of the experiments conducted on Aβ toxicity have been performed with a suspension of fibrils, but deposits of Aβ fibrils are not always toxic per se. Indeed, a matrix of soluble or fibrillar Aβ has been reported to allow the adherence and development of cell line cultures or neuronal primary culture cells, which express integrins [36]. The fixation of Aβ peptide to integrins occurs through an Aβ sequence homologous to fibronectin [37]. Soluble Aβ is partially internalized and degraded via an $\alpha 5\beta 1$-integrin-mediated pathway, without inducing toxicity. Furthermore, immunohistochemistry studies of labeled Aβ deposits have established the existence of neuronal cell death prior to the deposition of fibrillar Aβ [38].

Accumulation of an intracellular insoluble pool has been reported, occurring over time or during internalization and degradation of Aβ(1–42). Soluble or fibrillar Aβ present membrane-perturbing properties, which could induce membrane damage and leakage of the intracellular Aβ pool. An amyloid deposit might be seeded with Aβ derived locally from disintegrating neuronal membrane or from the insoluble pool rather than from diffuse Aβ deposits or from secreted Aβ [39]. Thus, internalization could lead either to a protection of the cells against Aβ peptide by its total clearance, or to cell death through long-term intracellular aggregation.

Other factors in AD neuronal insults PSs, tau, APP, and others

PS-1 and PS-2 genes encode proteins that share 63% overall identity. Located mainly in the endoplasmic reticulum membrane, the PSs are membrane-bound proteins with six to eight transmembrane domains and are structurally homologous to G protein. PS-1 and PS-2 proteins undergo endoproteolytic processing to yield two stable fragments [40]. In recent years, a growing list of proteins interacting directly or indirectly with PSs have been established: tau and one of its kinases, glycogen synthase kinase 3β (GSK3β), filamin, an actin-binding protein, β-catenin, Go protein, calsenilin, and μ-calpain (fig. 3) [41–46]. From these results, two main aspects of PS function emerge: cell survival and

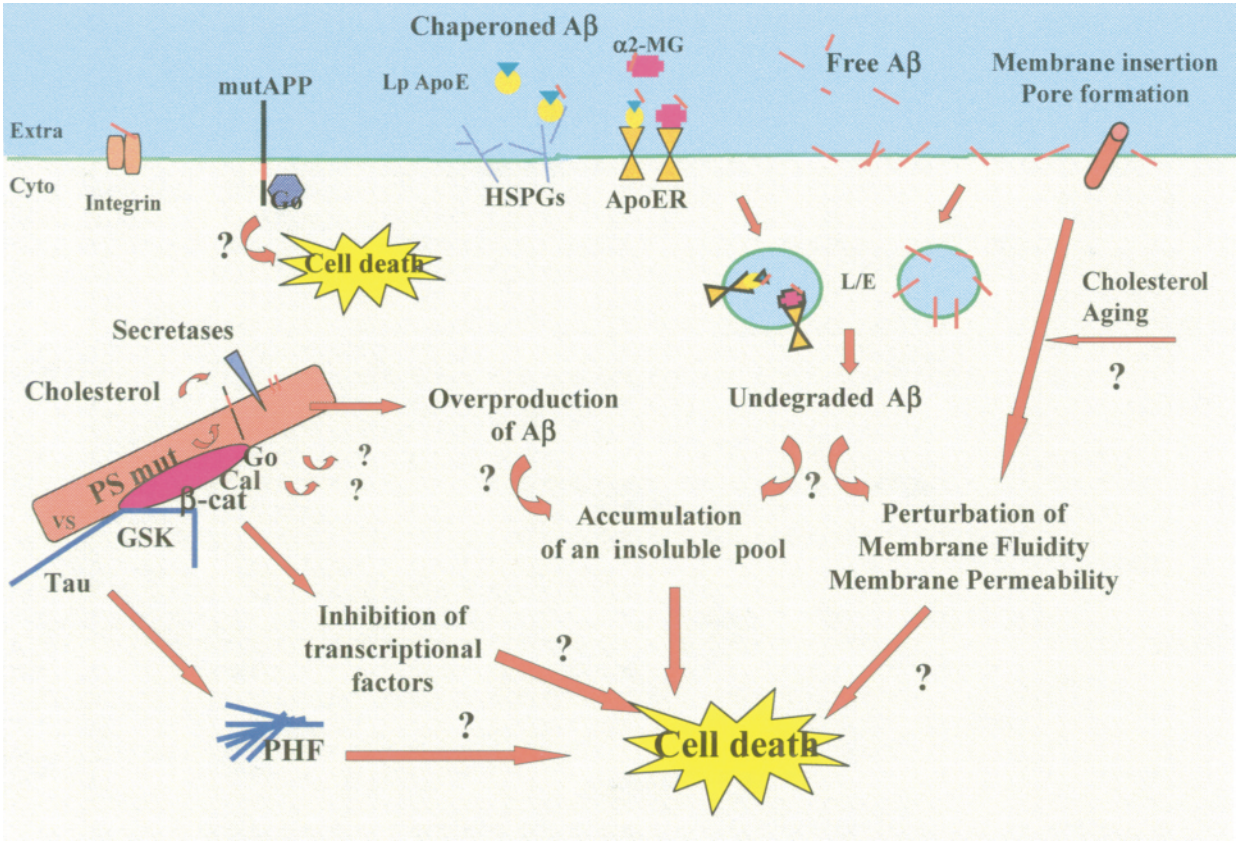

Figure 2. Alternative hypotheses. Different Aβ cytotoxic pathways could be elaborated from studies in vitro, in which deposition of fibrillar Aβ does not form the central event. The pathological role of Aβ might be caused via resistance to catabolism and accumulation of an insoluble intracellular Aβ pool, via perturbation of membrane properties, by insertion of peptide inside the lipid structure, or by ionic pore formation. Independently of the overproduction of Aβ, mutAPP and mutPSs may induce cell death through interaction with a Go protein and through phosphorylation of β-catenin and tau by activated glycogen synthase kinase (GSK). The involvement of the other proteins binding to PS, such as Go, calsenilin and filamin, has yet to be established in the pathological mechanism of AD. ApoER, apoE receptors; Lp ApoE, lipoprotein containing apoE; β-cat, β-catenin; Cal, calsenilin; VS, vesicular structures; L/E; lysosomal/endosomal compartments.

fate, notably through the Notch pathway, and vesicular trafficking.

Expression of mutated PS and overexpression of PSs in transfected cells have been reported to increase sensitivity to apoptosis [47, 48]. PS-2 appears to play a direct role in Fas-mediated apoptosis. PS-1 might regulate the JNK pathway, maybe by acting as an anchor protein that directs the activity of related kinases and phosphatases [47]. PS-1 and PS-2 contain a site recognized by caspase-3 close to the proteolytic site of cleavage [49]. This carboxy-terminal fragment resulting from the cleavage of pS-2 by caspase-3 inhibits apoptosis induced by Fas and TNF-α.

Mutated PSs destabilize calcium homeostasis, possibly through calsenilin, a calcium-binding protein [45, 50]. This neuronal protein interacts with the carboxy-terminal end of both PS-1 and PS-2.

Furthermore, neurons in AD brain and mutated PS-1-transfected cells overexpressed Par-4, a protein containing both a leucine zipper and a death domain implicated in apoptosis [51].

Destabilization of the cytoskeleton has been described as a critical step in apoptosis. In AD, this destabilization could occur through PS mutations in two ways: modulation of phosphorylation of tau leading to formation of PHFs, and/or overproduction of Aβ inducing apoptosis and alteration of the cytoskeleton as a final step. PS-1 and PS-2 immunostaining have been detected in neurofibrillary tangles from sporadic and familial cases of AD, suggesting a possible interaction between PS and tau [52]. These findings are inconsistent with another study which did not report any specific interaction of PS-1 with neurofibrillary tangles or senile plaques [53]. In vitro, PS has been reported to complex

both tau and GSK3β. Familial AD mutations increase the ability of PS-1 to bind GSK3β, leading to a modulation of tau phosphorylation, and may favor formation of PHFs [54].

Tau is reported to interact with other cytoskeletal proteins, such as actin and α-spectrin, and with phosphatases and kinases mediated by the anchorage of its N-terminal domain in the plasma membrane. Through all these interactions, tau is implicated in cytoskeletal maintenance and vesicular trafficking. Many studies have focused on the phosphorylation process of tau, but failed to establish the mechanism leading from tau hyperphosphorylation to formation of PHFs. Paradoxically, Canu et al. [55] have shown that induction of apoptosis by serum deprivation of cerebellar cells leads rapidly to the cleavage of dephosphorylated tau and finally microtubule destabilization. The induction of apoptosis by potassium serum deprivation activates caspase-3 and calpain. Calpain induces activation of calcineurin, a tau phosphatase. Dephosphorylation of tau provokes dual effects, increasing its ability to bind microtubules, but also increasing its vulnerability to proteolysis. However, recent studies have demonstrated that the stabilization of microtubules protects neuronal cells against Aβ-induced toxicity [56].

Thus, the mechanism of protection against apoptosis remains to be explained. Neurodegeneration in AD could involve different types of cytoskeletal damage, particularly in microtubule destabilization, an early pathway implicating cleavage of tau, and another driven by Aβ-induced toxicity.

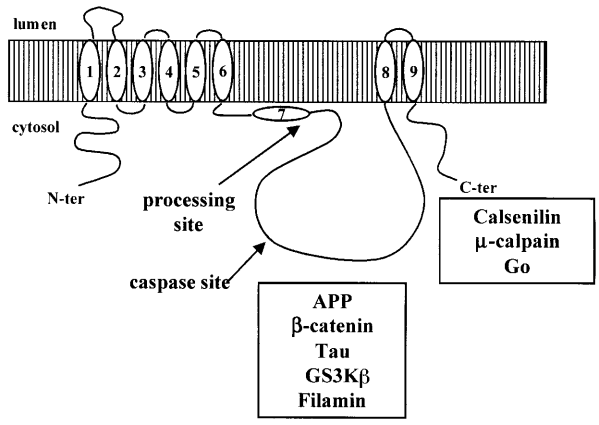

Figure 3. PSs have been localized in endoplasmic reticulum and Golgi membranes. These proteins are constituted of eight transmembrane domains. An enzymatic cleavage site yields two protein fragments, a caspase site being located near this processing site. The central and the carboxy-terminal cytosolic regions interact with APP, β-catenin, tau, GSK3β, filamin, calsenilin, and Go protein.

Mutations of PSs and APP have also been shown to deregulate vesicular trafficking and signal transduction through GSK3β, β-catenin, and Go protein. GSK3β modulates tau phosphorylation and also inactivates pyruvate dehydrogenase (PDH), which could lower acetyl-CoA and ATP levels. In hippocampal primary culture cells treated with Aβ, GSK3β is induced, and the acetylcholine level is reduced, whereas choline acetyl transferase and cholinesterase activity are unaffected by Aβ peptide. In a similar manner, muscarinic M1 receptor agonist enhances processing of APP in non-amyloidogene products and decreases phosphorylation of tau [7]. All these data correlate with results obtained in AD brain: a considerable decrease in the metabolic rate of glucose, a decrease in PDH activity, and predominant lesions in the cholinergic region. The primary effects of mutated PS may affect GSK3β, glucose turnover, and acetylcholine-derived products, leading to deregulation of the cholinergic innervated system and neuronal cell death. The overproduction of Aβ might be a secondary event in PS mutation.

Another intracellular disorder related to mutated PS is the instability of the β-catenin-PS complex. PS-1 may sequester cytoplasmic β-catenin in a membrane-associated complex [43, 53, 57]. This interaction has been demonstrated by immunoprecipitation in brain tissue and in neuroblastoma cells. A defect in PS-1 may induce the phosphorylation of β-catenin by GSK3β, inducing the catabolism of unstable β-catenin by proteasome. The cytoplasmic protein β-catenin is implicated in the Wg/Wnt signaling pathway through the Tcf/Lef family of transcription factors. This pathway is involved in the control of cell survival. The instability of the complex β-catenin/PS could explain the susceptibility of mutated PS neurons to apoptosis. A brain isoform of β-catenin, δ-catenin, has been reported, but its role in the normal and pathologic central nervous system (CNS) has not yet been determined [58].

APP has been implicated in cell adhesion, cell growth, neurite outgrowth, and neuroprotection. It has been suggested that a Go protein, interacting directly with APP, may mediate apoptosis in cells transfected with mutated APP.

Thus, involvement of APP and PS mutations may reflect an intracellular perturbation as the primary cause in AD, independently of Aβ overproduction.

Not forgetting glial cells

While neuronal loss is the main aspect of AD, the involvement of glial cells should not be forgotten. Neuritic plaques in AD are densely surrounded by reactive astrocytes and microglia. Microglial cells accumulate in dense Aβ plaques, rather than diffuse deposits, and act

as plaque-attacking scavenger cells. The majority of the internalized Aβ microaggregates are undegraded after uptake [59]. Microglial cells are activated by fibrillar Aβ, resulting in the production of reactive nitrogen species and TNF-α, and in a decrease in intracellular calcium. The reactive astrocytes participate in the inflammatory response in AD by secreting proinflammatory cytokines and by expressing iNOS. Fibrillar Aβ injected into the rat striatum is phagocytized by microglia, whereas nearby astrocytes form a virtual wall between fibrillar Aβ-containing microglia and neuropils. Astrocytes and microglia near the injected fibrillar Aβ site show a significant increase in iNOS [60]. Thus, Aβ neurotoxicity is mediated by factors released from activated microglia and astrocytes.

In addition to being involved in the digestion of plaques, glial cells in the CNS represent the major source of apoE, which modulates Aβ-induced ROS and is involved in cholesterol homeostasis and Aβ clearance [61, 62].

apoE: Aβ 'chaperone,' Aβ clearance effector and/or neurotrophic factor?

Genetic linkage and immunohistochemistry studies have suggested the involvement of apoE in the onset of AD. Nevertheless, the function of apoE in the normal and AD CNS is still unclear. The brain is the second major site of apoE expression [63]. Glial cells were previously thought to be the sole source of cerebral apoE, but a recent study has suggested that it is also expressed by human neuroblastoma [64]. ApoE acts as a lipid carrier protein and has been suggested to play a role in the transport of phospholipids and cholesterol in the CNS. In neuronal cultures, apoE increases neurite extension in an isoform-specific manner [65, 66]. According to the data, apoE4 has no effect or inhibits neurite outgrowth associated with depolymerization of microtubules [67]. Other studies have reported a toxic effect of unlipidated apoE4 on rat hippocampal neurons [68]. The uptake and internalization of lipidated apoE are mediated by interactions with HSPGs and with several apoE receptors. LDL-R and LRP, both members of the LDL-R family, are expressed in the brain [69]. Other receptors, apoE2 or LR7/8B, and LR11, are mainly expressed in brain, particularly by neurons [70]. LR7/8B binds apoE and α2-MG as substrates, as does LRP [71]. LR11 is expressed during development, depending on the neural cell type, and may play a role in neural organization and the pathogenesis of degenerative brain diseases.

Histopathological studies of AD brains have revealed apoE labeling in the amyloid deposits. The C-terminal end of truncated apoE appears to be the domain most frequently detected in deposits [72]. Biochemical tests have confirmed a direct interaction of apoE with Aβ, and with APP. Complex formation involves the carboxy-terminal domain of apoE and is apoE isoform specific. In vitro, apoE3 and E2 form complexes with Aβ, unlike apoE4 [73]. The association of apoE and Aβ inhibits fibril formation, the effect being more marked with free apoE than with lipidated apoE [74]. In neuronal cultures, apoE3 might protect neuronal cells against Aβ-induced toxicity through complexation and internalization of Aβ via apoE receptors [68, 75]. apoE has also been detected in neurofibrillary tangles [76]. The cytoplasmic localization of apoE is still unclear. However, biochemical studies have demonstrated an interaction between the isoforms E3 and E2 of apoE and dephosphorylated tau. Neither apoE4 nor the phosphorylated form of tau are involved in the complexes. Treatment of rat primary neuronal cultures by apoE peptides leads to dephosphorylation of tau by Ca^{2+}-dependent and G protein pathways [77].

In addition to these functions recent data have suggested the involvement of apoE in vasoconstriction and the activity of ciliary neurotrophin factor (CNTF). Vasoactive properties have been reported for both apoE and Aβ peptide [78]. The Aβ-apoE4 complex synergistically enhances vasoconstriction [79]. Furthermore, soluble Aβ-apoE complex has been found in the CSF of AD patients, and was not detected in normal CSF, probably because the levels were too low [80]. The precise effect of vasoactive properties in the development of AD has yet to be explored.

ApoE has recently been reported to bind to and potentiate the survival-promoting activity of CNTF on hippocampal cells. Both proteins are associated with the response to insults in the CNS. Binding of apoE could protect CNTF from oxidation or hydrolysis, and could stabilize complex binding to the receptor [81]. Potentiation of CNTF activity may strengthen the neuroprotective role of apoE in AD pathology.

Control of membrane properties by cholesterol

In AD, the isoform-specific protective role of apoE might occur at two levels. As a protein chaperone, apoE could prevent fibrillogenesis, clear Aβ peptide, and modulate tau phosphorylation. As a lipoprotein component, it could be involved in cholesterol homeostasis and neuronal development.

An age-related change has been detected in asymmetric membrane cholesterol distribution [82]. Similarly, a progressive increase in phospholipid metabolite formation has been observed during aging, which is accelerated in AD [83]. Elevation of membrane phospholipid metabolites may play a role in the deposition of Aβ, as some of these metabolites are known to increase the aggregation

of Aβ. Membrane fluidity is partially controlled by the ratio of cholesterol to phospholipids, and influences the activity of membrane proteins through the modulation of the structural order or degree of lipid packing.

Low concentrations of Aβ have been reported to slightly decrease the fluidity of brain membranes. However, important local effects of Aβ occurring in the membrane microdomains may be sufficient to alter the activity of receptors or ionic channels located in such microdomains. These perturbing effects have also been suggested by biochemical studies. Aβ peptide exhibited fusogenic properties [20]. At high concentrations, Aβ peptide has more pronounced effects on the membrane, probably by disrupting its structure [21, 22, 84].

The distribution of membrane cholesterol has another effect in AD, namely the modulation of APP processing. A rise in the level of membrane cholesterol decreases cleavage of APP by α-secretase [85], whereas cholesterol depletion leads to an inhibition of β-secretase cleavage and has no effect on α-secretase activity [86, 87]. Several hypotheses could explain this modulation of protease activity by cholesterol. Changes in cholesterol membrane concentration may modulate the vesicular traffic of APP so that the protein does not reach the cellular sites where β-cleavage takes place [88]. The α-secretase activity may depend on membrane fluidity [85]. Correspondingly, Aβ decreases cholesterol esterification and changes the distribution of free cholesterol in neurons [88].

Control of cholesterol homeostasis involves apoE and its receptors. Although the data are controversial, an LRP polymorphism seems likely to be genetically linked to AD. apoE, RAP and α2-MG are among the numerous ligands of LRP. α2-MG has recently been reported to be genetically associated with AD [5]. This major protease inhibitor with broad substrate specificity is detected in amyloid plaques. The association of α2-MG and Aβ prevents fibril formation and attenuates neurotoxicity of Aβ in cortical primary cultures [13, 89]. α2-MG has been suggested to mediate Aβ degradation and clearance via endocytosis through LRP or via a serine protease-α2-MG complex [90].

A conclusion is premature: hypotheses, only hypotheses

Progress in our understanding of AD requires an animal model faithfully reproducing the stigmata of AD, an earlier screening of AD patients, and a more precise delineation of pathological subtypes of AD. Most of the progress already achieved in AD research stems from studies on the role of plaque-associated proteins and APP. But if Aβ-induced toxicity has been clearly established in vitro, our understanding of the processes affecting the CNS in AD still remains speculative.

Milestones in future research are still the understanding of APP, PS, and apoE functions in normal brain and how these could be altered under pathological situations. Could the role of apoE in cholesterol homeostasis and neuronal development be maintained when apoE is associated with Aβ? How does PS act in the development of AD: as a factor promoting the overproduction of Aβ or as a protein managing the general intracellular pathway?

New actors and/or new hypotheses have to be found to try to explain sporadic cases and non-elucidated familial cases, and the pathways of selective neuronal death in this disease. Less than half familial AD cases are linked to identified loci and the sporadic cases are identified in terms of risk factors. Additional genomic loci and mitochondrial mutations remain to be explored [91]. Although considerable progress has been made in the study of AD, the molecular key(s) of AD is (are) still to be found.

Acknowledgements. B. Drouet was funded by an Avantis grant.

1 Wisniewski T., Ghiso J. and Fangione B. (1997) Biology of Aβ in Alzheimer's disease. Neurobiol. Dis. **4:** 313–328
2 Rogaev E. I., Sherrington R., Rogaeva E. A., Levesque G., Ikeda M., Liang Y. 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
3 Sherrington R., Rogaev E. I., Liang Y., Rogaeva E. A., Levesque G., Ikeda M. et al. (1995) Cloning of a gene bearing missense mutations in early-onset familiar Alzheimer's disease. Nature **375:** 754–759
4 Marx J. (1998) New gene tied to common form of Alzheimer's. Science **281:** 508–509
5 Blacker D., Wilcox M., Laird N. M., Rodes L., Horvath S. M., Go R. C. P. et al. (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer disease. Nat. Genet. **19:** 357–360
6 Kalaria R. J. (1993) The immunopathology of Alzheimer's disease and some related disorders. Brain Pathol. **3:** 333–347
7 Billlingsley M. L. and Kincaid R. (1997) Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. Biochem. J. **323:** 577–591
8 Imahori K., Hoshi M., Ishiguro K., Sato K., Takahashi M., Shiurba R. et al. (1998) Possible role of tau protein kinases in pathogenesis of Alzheimer's disease. Neurobiol. Aging **19:** S93–S98
9 Scheuner D., Eckman C., Jensen M., Song X., Citron M., Suzuki N. et al. (1996) Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat. Med. **2:** 864–868
10 Bronfman F. C., Garrido J., Alvarez A., Morgan C. and Inestrosa N. C. (1996) Laminin inhibits amyloid-β-peptide fibrillation. Neurosci. Lett. **218:** 201–203
11 Castillo G. M., Ngo C., Cummings J., Wight T. N. and Snow A. D. (1997) Perlecan binds to the β-amyloid proteins (Aβ) of Alzheimer's disease, accelerates Aβ fibril formation, and maintains Aβ fibril stability. J. Neurochem. **69:** 2452–2465
12 Alvarez A., Alarcon R., Opazo C., Campos E., Munoz F. J., Calderon F. et al. (1998) Stable complexes involving acetyl-

cholineterase and amyloid-β peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils. J. Neurosci. **18:** 3213–3223
13 Du Y., Bales K. R., Dodel R., Liu X., Glinn M. A., Horn J. et al. (1998) α2-Macroglobulin attenuates β-amyloid peptide 1–40 fibril formation and associated neurotoxicity of cultured fetal rat cortical neurons. J. Neurochem. **70:** 1182–1188
14 Shin R.-W., Ogino K., Kondo A., Saido T. C., Trojanowski J. W., Kitamoto T. et al. (1997) Amyloid β-protein (Aβ) 1–40 but not Aβ1–42 contributes to the experimental formation of Alzheimer disease amyloid fibrils in rat brain. J. Neurosci. **17:** 8187–8193
15 Yaar M., Zhai S., Pilch P. F., Doyle S. M., Eisenhauer P. B., Fine R. E. et al. (1997) Binding of β-amyloid to the p75 neurotrophin receptor induces apoptosis. Neurobiol. Dis. **100:** 2333–2340
16 Yan S., Chen X., Zhu H., Roher A., Slattery T., Zhao L. et al. (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature **382:** 685–691
17 El Khoury J., Hickman S., Thomas C., Cao L., Silverstein S. and Loike J. (1996) Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature **382:** 716–719
18 Liu Y., Dargusch R. and Schubert D. (1997) Beta amyloid toxicity does not require RAGE protein. Biochem. Biophys. Res. Commun. **237:** 37–40
19 Kuner P., Schubenel R. and Hertel C. (1998) Beta-amyloid binds to p75NTR and activates NfkappaB in human neuroblastoma cells. J. Neurosci. Res. **54:** 789–804
20 Pillot T., Goethals M., Vanloo B., Talussot C., Brasseur R., Rosseneu M. et al. (1996) Fusogenic properties of the C-terminal domain of the Alzheimer β-amyloid peptide. J. Biol. Chem. **271:** 13379–13382
21 McLaurin J. and Chakrabartty A. (1996) Membrane disruption by Alzheimer β-amyloid peptides mediated through specific binding to either phospholipids or gangliosides. J. Biol. Chem. **271:** 26482–26489
22 Muller W. E., Eckert G. P., Scheuer K., Cairns N. J., Maras A. and Gattaz W. F. (1998) Effects of beta-amyloid peptides on the fluidity of membranes from frontal and parietal lobes of human brain: high potencies of A-beta 1–43. J. Exp. Clin. Invest. **5:** 10–15
23 Keller J. N., Kindy M. S., Holtsberg F. W., St. Clair D. K., yen H.-C., Germeyer A. et al. (1998) Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J. Neurosci **18:** 687–697
24 Bruce-Keller A., Begley J. G., Fu W., Butterfield A. D., Bredesen D. E., Hutchins J. B. et al. (1998) Bcl-2 protects isolated plasma and mitochondrial membranes against lipid peroxidation induced by hydrogen peroxide and amyloid β-peptide. J. Neurochem. **70:** 31–39
25 Guo Q., Robinson N. and Mattson M. (1998) Secreted β-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-κB and stabilization of calcium homeostasis. J. Biol. Chem. **273:** 12341–12351
26 Mattson M. P., Goodman Y., Luo H., Fu W. and Furukawa K. (1997) Activation of NF-κB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration. J. Neurosci. Res. **49:** 681–697
27 Akama K., Albanese C., Pestall R. G. and Van Eldik L. J. (1998) Amyloid β-peptide stimulates nitric oxide production in astrocytes through an NFκB-dependent mechanism. Proc. Natl. Acad. Sci. USA **95:** 5795–5800
28 Pike C.J., Ramezan-Arab N. and Cotman C. W. (1997) β-amyloid neurotoxicity in vitro: evidence of oxidative stress but not protection by antioxidants. J. Neurochem. **69:** 1601–1611
29 Abe K. and Kimura H. (1996) Amyloid β toxicity consists of a Ca^{2+}-independent early phase and a Ca^{2+}-dependent late phase. J. Neurochem. **67:** 2074–2078
30 Gao Z., Collins H. W., Matschinsky F. M., Lee V. M.-Y. and Wolf B. A. (1998) Cytotoxic effect of β-amyloid on a human differentiated neuron is not mediated by cytoplasmic Ca^{2+} accumulation. J. Neurochem. **70:** 1394–1400
31 Behl C., Davis J. B., Klier G. F. and Schubert D. (1994) Amyloid β peptide induces necrosis rather than apoptosis. Brain Res. **645:** 253–264
32 Estus S., Tucker H. M., Rooyen C. van, Wright S., Brigham E., Wogulis M. et al. (1997) Aggregated amyloid-β protein induces cortical neuronal apoptosis and concomitant 'apoptotic' pattern of gene induction. J. Neurosci. **17:** 7736–7745
33 Paradis E., Douillard H., Koutroumanis M., Goodyer C. and LeBlanc A. (1996) Amyloid β peptide of Alzheimer's disease downregulates Bcl-2 and upregulates Bax expression in human neurons. J. Neurosci. **16:** 7533–7539
34 Monte S. M. de la, Sohn Y. K. and Wands J. R. (1997) Correlates of p53- and Fas (CD95)-mediated apoptosis in Alzheimer's disease. J. Neurol. Sci. **152:** 73–83
35 Neve R. L. and Robakis N. (1998) Alzheimer's disease: a re-examination of the amyloid hypothesis. Trends Neurosci. **21:** 15–19
36 Wujek J. R., Dority M. D., Frederickson R. C. A. and Brunden K. (1996) Deposits of Aβ fibrils are not toxic to cortical and hippocampal neurons in vitro. Neurobiol. Aging **17:** 107–113
37 Matter M. L., Zhang Z., Nordsedt C. and Ruolahti E. (1998) The $\alpha 5\beta 1$ integrin mediates elimination of amyloid-β peptide and protects against apoptosis. J. Cell Biol. **141:** 119–1030
38 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
39 Knauer M., Soreghan B., Burdick D., Kosmoki J. and Glabe C. (1992) Intracellular accumulation and resistance to degradation of the Alzheimer amyloid A4/β protein. Proc. Natl. Acad. Sci. USA **89:** 7437–7441
40 Tomita T., Tokuhiro S., Hashimoto T., Aiba K., Saido T. C., Maruyama K. et al. (1998) Molecular dissection of domains in mutant presenilin 2 that mediate overproduction of amyloidogenic forms of amyloid β peptides. J. Biol. Chem. **273:** 21153–21160
41 Takashima A., Muruyama M., Muruyama O., Khono T., Honda T., Yasutake K. et al. (1998) Presenilin 1 associates with glycogen synthase kinase-3β and its substrate tau. Proc. Natl. Acad. Sci. USA **95:** 9637–9641
42 Zhang W., Han S. W., McKeel D. W., Goate A. and Wu J. Y. (1998) Interaction of presenilins with the filamin family of actin-binding proteins. J. Neurosci. **18:** 914–922
43 Zhang Z., Hartmann H., Do V. M., Abramowski D., Sturchler-Pierrat C., Staufenbiel M. et al. (1998) Destabilization of β-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. Nature **395:** 698–702
44 Smine A., Xu X., Nishiyama K., Katada T., Gambetti P., Yadav S. P. et al. (1998) Regulation of brain G-protein go by Alzheimer's disease gene presenilin-1. J. Biol. Chem. **273:** 16281–16288
45 Buxbaum J. D., Choi E.-K., Luo Y., Lilliehook C., Crowley A. C., Merriam D. E. et al. (1998) Calsenilin: a calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment. Nat. Med. **4:** 1177–1181
46 Shinozaki K., Maruyama K., Kume H., Tomita T., Saido T., Iwatsubo T. et al. (1998) The presenilin 2 loop domain interacts with the mu-calpain C-terminal region. Int. J. Mol. Med. **1:** 797–799
47 Wolozin B., Alexander P. and Palacino J. (1998) Regulation of apoptosis by presenilin 1. Neurobiol. Aging **19:** S23–S27
48 Vito P., Wolozin B., Ganjei J. K., Iwasaki K., Lacana E. and D'Adamio L. (1996) Requirement of the familial Alzheimer's disease gene PS2 for apoptosis. J. Biol. Chem. **271:** 31025–31028
49 Kim T.-W., Pettingell W. H., Jung Y.-K., Kovacs D. and Tanzi R. E. (1997) Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease. Science **277:** 373–376

50 Keller J. N., Guo Q., Holtsberg F. W., Bruce-Keller A. J. and Mattson M. P. (1998) Increased sensitivity to mitochondrial toxin-induced apoptosis in neural cells expressing mutant presenilin-1. J. Neurosci. **18:** 4439–4450

51 Guo Q., Fu W., Xie J., Luo H., Sells S. F., Geddes J. et al. (1998) Par-4 is a mediator of neuronal degeneration associated with the pathogenesis of Alzheimer disease. Nat. Med. **4:** 957–962

52 Chui D. H., Shirotani K., Tanahashi H., Akiyama H., Ozawa K., Kunishi T. et al. (1998) Both N-terminal and C-terminal fragments of presenilin 1 colocalize with neurofibrillary tangles in neurons and dystrophic neurites of senile plaques in Alzheimer's disease. J. Neurosci. Res. **53:** 99–106

53 Xia W., Zhang J., Perez R., Koo E. H. and Selkoe D. J. (1997) Interaction between amyloid precursor protein and presenilins in mammalian cells: implications for the pathogenesis of Alzheimer disease. Proc. Natl. Acad. Sci. USA **94:** 8208–8213

54 Brownless J., Irving N.G., Brion J.P., Gibb B. J., Wagner U., Woodgett J. et al. (1997) Tau phosphorylation in transgenic mice expressing glycogen syntase kinase-3beta transgenes. Neuroreports **8:** 3251–3255

55 Canu N., Dus L., Barbato C., Ciotti M. T., Brancolini C., Rinaldi A. M. et al. (1988) Tau cleavage and dephosphorylation in cerebellar granule neurons undergoing apoptosis. J. Neurosci. **18:** 7061–7074

56 Michaelis M. L., Ranciat N., Chen Y., Betchel M., Ragan R., Hepperle M. et al. (1998) Protection against β-amyloid toxicity in primary neurons by paclitaxel (taxol). J. Neurochem. **70:** 1623–1627

57 Thinakaran G., Regard J. B., Bouton C. M., Harris C. L., Price D. L., Borchelt D. R. et al. (1998) Stable association of presenilin derivatives and absence of presenilin interactions with APP. Neurobiol. Dis. **4:** 438–453

58 Zhou J., Liyanage U., Medina M., Ho C., Simmons A., Lovett M. et al. (1997) Presenilin 1 interaction in the brain with a novel member of the armadillo family. Neuroreports **8:** 2085–2090

59 Paresce D., Chung H. and Maxfield F. R. (1997) Slow degradation of aggregates of the Alzheimer's disease amyloid β-protein by microglial cells. J. Biol. Chem. **272:** 29390–29397

60 Weldon P. T., Rogers S. D., Ghilardi J. R., Finke M. P., Cleary J. P., O'Hare E. et al. (1998) Fibrillar β-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo. J. Neurosci. **18:** 2161–2173

61 El Khoury J., Hickman S. E., Thomas C. A., Loike J. D. and Silverstein S. C. (1998) Microglia, scavenger receptors, and the pathogenesis of Alzheimer's disease. Neurobiol. Aging **19:** S81–S84

62 Vitek M. P., Snell J., Dawson H. and Colton C. A. (1997) Modulation of nitric oxide production in human macrophages by apolipoprotein-E and amyloid-beta peptide. Biochem. Biophys. Res. Commun. **240:** 391–394

63 Harr S. D., Uint L., Hollister R., Hyman B. and Mendez A. J. (1996) Brain expression of apolipoproteins E, J, and A-I in Alzheimer's disease. J. Neurochem. **66:** 2429–2435

64 Dupont-Wallois L., Soulié C., Sergeant N., Wavrant-de Wrieze N., Chartier-Harlin M.-C., Delacourte A. et al. (1997) ApoE synthesis in human neuroblastoma cells. Neurobiol. Dis. **4:** 256–364

65 Nathan B. P., Bellosta S., Sanan D. A., Wiesgraber K. H., Mahley R. W. and Pitas R. E. (1994) Differential effects of apolipoproteins E3 and E4 on neuronal outgrowth in vitro. Science **264:** 850–852

66 DeMattos R. B., Curtiss L. K. and Williams D. L. (1998) A minimally lipidated form of cell-derived apolipoprotein E exhibits isoform-specific stimulation of neurite outgrowth in the absence of exogenous lipids or lipoproteins. J. Biol. Chem. **273:** 4206–4212

67 Nathan B. P., Chang K.-C., Bellosta S., Brisch E., Ge N., Mahley R. W. et al. (1995) The inhibitory effect of apolioprotein E4 on neurite outgrowth is associated with microtubule depolymerization. J. Biol. Chem. **270:** 19791–19799

68 Jordan J., Galindo M. F., Miller R. J., Reardon C. A., Getz G. S. and LaDu M. J. (1998) Isoform-specific effect of apolipoprotein E on cell survival and β-amyloid-induced toxicity in rat hippocampal pyramidal neuronal cultures. J. Neurosci. **18:** 195–204

69 St Clair R. W. and Beisigel U. (1997) What do all the apolipoprotein E receptors do? Curr. Opin. Lipidol. **8:** 243–245

70 Schneider W., Nimpf J. and Bujo H. (1997) Novel members of the low density lipoprotein receptor superfamily and their potential roles in lipid metabolism. Curr. Opin. Lipidol. **8:** 315–319

71 Stockinger W., Hengstsschlager-Ottnad E., Novak S., Matus A., Höttinger M., Bauer J. et al. (1998) The low density lipoprotein receptor gene family: differential expression of two alpha2-macroglobulin receptors in the brain. J. Biol. Chem. **273:** 32213–32221

72 Aizawa Y., Fukatsu R., Takamuru Y., Tsuzuki K., Chiba H., Kobayashi K. et al. (1997) Amino-terminus truncated apolipoprotein E is the major species in amyloid deposits in Alzheimer's disease-affected brains: a possible role for apolipoprotein E in Alzheimer's disease. Brain Res. **768:** 208–214

73 Pillot T., Goethals M., Vanloo B., Lins L., Brasseur R., Vandekerchkhove J. et al. (1997) Specific modulation of the fusogenic properties of the Alzheimer (-amyloid peptide by apolipoprotein E isoforms. Eur. J. Biochem. **243:** 650–659

74 Beffert U. and Poirier J. (1998) ApoE associated with lipid has a reduced capacity to inhibit beta-amyloid fibril formation. Neuroreports **9:** 3321–3323

75 Williams K. R., Saunders A. M., Roses A. D. and Armati P. J. (1998) Uptake and internalization of exogenous apolipoprotein E3 by cultured human central nervous system neurons. Neurobiol. Dis. **5:** 271–279

76 Namba Y., Tomonaga M., Kawasaki H., Otomo E. and Ikeda K. (1991) Apolipoprotein E immunoreactivity in cerebral deposits and neurofibrillary tangles in Alzheimer's disease brain. Brain Res. **541:** 163–166

77 Wang X., Ciraolo G., Morris R. and Gruenstein E. (1997) Identification of a neuronal endocytic pathway activated by an apolipoprotein E (apoE) receptor binding peptide. Brain Res. **778:** 6–15

78 Suo Z., Humphrey J., Kundtz A., Sethi F., Placzek A., Crawford F. et al. (1998) Soluble Alzheimer's beta-amyloid constricts the cerebral vasculature in vivo. Neurosci. Lett. **257:** 77–80

79 Paris D., Town T., Parker T. A., Humphrey J. and Mullan M. (1998) Isoform-specific vasoconstriction induced by apolipoprotein e and modulation of this effect by Alzheimer's beta-amyloid peptide. Neurosci. Lett. **256:** 73–76

80 Permanne B., Perez C., Soto C., Frangione B. and Wisniewski T. (1997) Detection of apolipoprotein E/dimeric soluble amyloid (complexes in Alzheimer's disease brain supernatants. Biochem. Biophys. Res. Commun. **240:** 715–720

81 Gutman C. A., Strittmatter W., Weisgraber K. H. and Matthew W. D. (1997) Apolipoprotein E binds to and potentiates the biological activity of ciliary neurotrophic factor. J. Neurosci. **17:** 6114–6121

82 Igbavboa U., Avdulov N. A., Chochina S. V. and Wood W. G. (1997) Transbilayer distribution of cholesterol is modified in brain synaptic plasma membranes of knockout mice deficient in the low-density lipoprotein receptor, apolipoprotein E, or both proteins. J. Neurochem. **69:** 1661–1667

83 Klunk W. E., Xu C.-J., McClure R. J., Panchalingam K., Stanley J. A. and Pettergrew J. W. (1997) Aggregation of β-amyloid peptide is promoted by membrane phospholipid metabolites elevated in Alzheimer's disease brain. J. Neurochem. **69:** 266–272

84 Müller W. E., Koch S., Eckert A., Hartmann H. and Scheuer K. (1995) β-amyloid peptide decreases membrane fluidity. Brain Res. **674:** 133–136

85 Bodovitz S. and Klein W. L. (1996) Cholesterol modulates β-secretase cleavage of amyloid precursor protein. J. Biol. Chem. **271:** 4436–4440
86 Simons M., Keller P., De Strooper B., Beyreuther K., Dotti C. G. and Simons K. (1998) Cholesterol depletion inhibits the generation of β-amyloid in hippocampal neurons. Proc. Natl. Acad. Sci. USA **95:** 6460–6464
87 Racchi M., Baetta R., Salvietti N., Ianna P., Franceschini G., Paoletti R. et al. (1997) Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. Biochem. J. **322:** 893–898
88 Liu Y., Peterson D. A. and Schubert D. (1998) Amyloid beta peptide alters intracellular vesicle trafficking and cholesterol homeostasis. Proc. Natl. Acad. Sci. USA **95:** 13266–13271
89 Hughes S., Khorkova O., Goyal S., Knaeblein J., Heroux J., Riedel N. G. et al. (1998) α_2-Macroglobulin associates with (-amyloid peptide and prevents fibril formation. Proc. Natl. Acad. Sci. USA **95:** 3275–3280
90 Qiu W. Q., Borth W., Ye Z., Haas C., Teplow D. B. and Selkow D. (1996) Degradation of amyloid β-protein by a serine protease-α2-macroglobulin complex. J. Biol. Chem. **271:** 8443–8451
91 Pericak-Vance M. A., Bass M. P., Yamaoka L. H., Gaskell P. C, Scott W. K., Terwedow H. A. et al. (1997) Complete genomic screen in late-onset familial Alzheimer disease. Evidence for a new locus on chromosome 12. JAMA **278:** 1237–1241