Alzheimer's Disease: Genes, Proteins, and Therapy

DENNIS J. SELKOE

Department of Neurology and Program in Neuroscience, Harvard Medical School, and Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts

Selkoe, Dennis J. Alzheimer's Disease: Genes, Proteins, and Therapy. *Physiol Rev* 81: 741–766, 2001.—Rapid progress in deciphering the biological mechanism of Alzheimer's disease (AD) has arisen from the application of molecular and cell biology to this complex disorder of the limbic and association cortices. In turn, new insights into fundamental aspects of protein biology have resulted from research on the disease. This beneficial interplay between basic and applied cell biology is well illustrated by advances in understanding the genotype-to-phenotype relationships of familial Alzheimer's disease. All four genes definitively linked to inherited forms of the disease to date have been shown to increase the production and/or deposition of amyloid β-protein in the brain. In particular, evidence that the presenilin proteins, mutations in which cause the most aggressive form of inherited AD, lead to altered intramembranous cleavage of the β-amyloid precursor protein by the protease called γ-secretase has spurred progress toward novel therapeutics. The finding that presenilin itself may be the long-sought γ-secretase, coupled

with the recent identification of β-secretase, has provided discrete biochemical targets for drug screening and development. Alternate and novel strategies for inhibiting the early mechanism of the disease are also emerging. The progress reviewed here, coupled with better ability to diagnose the disease early, bode well for the successful development of therapeutic and preventative drugs for this major public health problem.

I. INTRODUCTION: AN HISTORICAL PERSPECTIVE

Few subjects in biomedicine have aroused the interest of the scientific and lay communities alike as has Alzheimer's disease (AD). The dramatic rise in life expectancy during the 20th century, from roughly 49 years to more than 76 years in the United States, has resulted in a burgeoning number of individuals achieving the age at which neurodegenerative disorders become common. Among these, AD has emerged as the most prevalent form of late-life mental failure in humans. It was not always so. When Alois Alzheimer, a Bavarian psychiatrist, first defined the clinicopathological syndrome that bears his name at a meeting in Munich in 1906, neither he nor his audience recognized that the disorder he described in a woman in her early 50s might ultimately turn out to be indistinguishable from common senile dementia. Indeed, it was not until the work of Blessed, Tomlinson, and Roth in the late 1960s that AD became generally accepted as the most common basis for senile dementia. We now recognize that a histopathological syndrome indistinguishable from that which Alzheimer originally described has an incidence which rises almost logarithmically with age. As a result, AD, originally believed to be a rare dementia occurring in the "presenile" period (that is, onset of symptoms under 65 years of age), is largely indistinguishable from senile dementia of the Alzheimer type, and the cases accrue at a linear rate rather than in a bimodal age distribution.

Alzheimer's original patient, a woman referred to as Auguste D. in his report, exemplified several cardinal features of the disorder that we still observe in most patients nowadays: progressive memory impairment; disordered cognitive function; altered behavior including paranoia, delusions, and loss of social appropriateness; and a progressive decline in language function. During the early and middle phases of this slow, inexorable process, the patient's alertness is well preserved, and motoric and sensory functions are essentially intact. However, as subjects continue to lose ground cognitively, slowing of motor functions such as gait and coordination often lead to a picture resembling extrapyramidal motor disorders such as parkinsonism.

For many decades after Alzheimer's original description, little progress in defining the pathogenesis of AD occurred. Although neuropathological studies led to growing recognition of the commonness of the syndrome, the study of AD and other idiopathic neurodegenerative disorders was marked by mechanistic ignorance and therapeutic nihilism. This situation began changing in the 1960s, when the advent of electron microscopy allowed Michael Kidd in England and Robert Terry in the United States to describe the striking ultrastructural changes underlying the two classical lesions which Alzheimer had linked: senile (neuritic) plaques and neurofibrillary tangles. In the mid 1970s, the first clear neurochemical clue as to what might underlie the dementing symptoms came from the observation that neurons synthesizing and releasing acetylcholine underwent variable but usually severe degeneration. This was observed as a decrease in the amounts and activities of the synthetic and degradative enzymes, choline acetyltransferase and acetylcholinesterase, in the limbic and cerebral cortices and an associated loss of cholinergic cell bodies in the subcortical nuclei that project to these regions, namely, the septal nuclei and the basal forebrain cholinergic system. As a result, substantial pharmacological research focused on attempting to enhance acetylcholine levels in the synaptic cleft, primarily by inhibiting the degradative enzyme. These efforts ultimately led to the only two drugs specifically approved to date for treating Alzheimer's disease in the United States: tetrahydroaminoacridine and donezepil.

In the late 1970s and early 1980s, variable deficits of other neurotransmitter systems were identified in AD brain tissue. It became increasingly clear that AD, unlike Parkinson's disease, did not involve degeneration of a single transmitter class of neurons but was highly heterogeneous. This realization appeared to explain the lack of robust clinical benefit in most patients treated with cholinergic drugs. Attention increasingly focused on attempting to identify the underlying mechanisms for the synpatic dysfunction and perikaryal degeneration that affected multiple classes of neurons in the limbic system and association cortices. In this context, investigators increasingly trained their sights on the two classical neuropathological lesions to which Alzheimer had called attention.

As neurochemists began attempting to identify the composition and molecular origin of the amyloid plaques and neurofibrillary tangles, they were reminded by their neuropathological colleagues that these lesions observed in the postmortem brain could be considered tombstones of the process that occurred late in disease and were thus unlikely to provide major insights into etiology and early pathogenesis. Thus one observed not only an ongoing debate about which of the two lesions might precede the other but a general sense that both lesions were likely to be preceded by some or many biochemical steps that might not become apparent simply by identifying the principal proteins composing these lesions. However, in-

creasingly rapid scientific progress since the mid 1980s has proven these concerns ill-founded.

Advances in biochemical pathology, that is, the use of compositional analyses and immunocytochemistry to define the subunit composition of the plaques and tangles, were followed by signal advances in the molecular genetics of AD that have validated the critical role of the subunit proteins in the fundamental mechanisms of AD as well as certain other degenerative dementias. The elucidation of the genotype-to-phenotype relationships for each genetic alteration linked to familial forms of AD (a process which is still very active) has led to a growing consensus about how at least the familial forms of the disorder may begin. The result of this continuing work is that a rough temporal outline of the disease cascade has begun to emerge. This process and the closely related effort to identify points for therapeutic intervention have been markedly assisted by the development of imperfect but nonetheless highly useful cellular and animal models of the presumed early features of the disease mechanism.

In this article, I review the extensive neuropathological, biochemical, genetic, cell biological, and transgenic modeling studies that have contributed to our growing understanding of the etiopathogenesis of AD. Given the enormous amount of scientific activity directed toward this problem and to related basic biological questions, the review will perforce be selective, and the reader will be referred to primary literature and review articles that cover particular features of this complex topic. At the end, we consider the imminent initiation of therapeutic trials directed at certain key features of the disease cascade. If these "rational" treatment approaches engender some success, AD may emerge as a triumph of reductionist biology applied to a disorder of the most complex of physiological systems, the human cerebral cortex.

II. DECIPHERING THE NEUROPATHOLOGICAL PHENOTYPE OF ALZHEIMER'S DISEASE

A. Neuritic Plaques

Neuritic plaques, one of the two diagnostic brain lesions observed in Alzheimer's original patient, are microscopic foci of extracellular amyloid deposition and associated axonal and dendritic injury, generally found in large numbers in the limbic and association cortices (24). Such plaques contain extracellular deposits of amyloid β-protein (Aβ) that occur principally in a filamentous form, i.e., as star-shaped masses of amyloid fibrils. Dystrophic neurites occur both within this amyloid deposit and immediately surrounding it. These neurites are often dilated and tortuous and are marked by ultrastructural abnormalities that include enlarged lysosomes, numerous mitochondria, and paired helical filaments, the latter generally indistinguishable from those that comprise the neu-

rofibrillary tangles (see below). Such plaques are also intimately associated with microglia expressing surface antigens associated with activation, such as CD45 and HLA-DR, and they are surrounded by reactive astrocytes displaying abundant glial filaments. The microglia are usually within and adjacent to the central amyloid core of the neuritic plaque, whereas the astrocytes often ring the outside of the plaque, with some of their processes extending centripetally toward the amyloid core. The time that it takes to develop such a neuritic plaque is unknown, but these lesions probably evolve very gradually over a substantial period of time, perhaps many months or years. The surrounding neurites that contribute to any one plaque can emanate from local neurons of diverse neurotransmitter classes. Much of the fibrillar Aβ found in the neuritic plaques is the species ending at amino acid 42 (Aβ_{42}), the slightly longer, more hydrophobic form that is particularly prone to aggregation (70). However, the Aβ species ending at amino acid 40 (Aβ_{40}), which is normally more abundantly produced by cells than Aβ_{42} (see below), is usually colocalized with Aβ_{42} in the plaque. The cross-sectional diameter of neuritic plaques in microscopic brain sections varies widely from 10 to >120 μm, and the density and degree of compaction of the amyloid fibrils which comprise the extracellular core also shows great variation among plaques.

B. The Nature of Diffuse ("Preamyloid") Plaques

When the Aβ peptide that Glenner originally identified in meningovascular amyloid deposits from Alzheimer brains (35) was recognized as the subunit of the plaque amyloid (38, 96, 144), many laboratories developed sensitive antibodies to endogenous or synthetic Aβ. Immunohistochemical staining with such antibodies revealed a far more extensive number of Aβ deposits than had been appreciated by the use of classical silver impregnation methods, such as the Bielschowsky and Bodian stains. In retrospect, it became apparent that the most sensitive silver staining methods (e.g., the modified Bielschowsky stain and the Gallya's silver stain) could also recognize many Aβ deposits that lacked the compacted, fibrillar appearance of the classical neuritic plaques. Many of the plaques found in limbic and association cortices, and virtually all of those in brain regions not clearly implicated in the typical symptomatology of AD (e.g., thalamus, caudate, putamen, cerebellum), showed relatively light, amorphous Aβ immunoreactivity that occurred in a finely granular pattern, without a clearly fibrillar, compacted center. Moreover, staining with silver stains highly capable of recognizing dystrophic neurites (e.g., the Bodian method) as well as immunohistochemistry for various neuronal/neuritic cytoskeletal proteins indicated that there was very little or no detectable neuritic dystrophy in most of these amorphous-appearing, nonfibrillar plaques.

The recognition of these amorphous plaques in the late 1980s (71, 166, 199) and their detection in regions that also contained many neuritic plaques (i.e., limbic and association cortices) led to the concept that they might represent precursor lesions of neuritic plaques. These lesions were thus referred to as "diffuse" plaques or "preamyloid deposits." When it later was determined that the Aβ peptides deposited in Alzheimer brain principally ended at either Aβ_{40} or Aβ_{42}, it became apparent that peptides ending at Aβ_{42} were the subunits of the material comprising the diffuse plaques, with little or no Aβ_{40} immunoreactivity, in contrast to the mixed (Aβ42 plus Aβ40) deposits that generally were found in the fibril-rich neuritic plaques (68, 69, 85, 128a). The hypothesis that diffuse plaques represent immature lesions that are precursors to the plaques with surrounding cytopathology arose from two lines of evidence. First, diffuse plaques were the sole form found in those brain regions that largely or entirely lacked neuritic dystrophy, glial changes, and neurofibrillary tangles and were not clearly implicated in the typical clinical symptoms of AD, e.g., cerebellum, striatum, and thalamus. Second, healthy aged humans free of AD or other dementing processes often showed solely diffuse plaques in limbic and association cortices, i.e., in the same regions as Alzheimer patients showed mixtures of diffuse and neuritic plaques. The notion that diffuse plaques could be earlier lesions was later supported by studies of transgenic mice expressing mutant human APP. These mice usually showed diffuse deposits before developing fibrillar, thioflavin S-positive, and Congo red-positive neuritic/glial plaques. Indeed, this hypothesis was particularly well supported by immunohistochemical studies of patients with Down's syndrome (85). Such individuals often display diffuse deposits as early as their teenage years but do not show neuritic/glial plaques until some two decades later, a time at which they first display abundant neurofibrillary tangles in limbic and association cortices.

C. Neurofibrillary Tangles Are Composed of Hyperphosphorylated Tau Proteins

Many neurons in the brain regions typically affected in AD (entorhinal cortex, hippocampus, parahippocampal gyrus, amygdala, frontal, temporal, parietal and occipital association cortices, and certain subcortical nuclei projecting to these regions) contain large, nonmembrane-bound bundles of abnormal fibers that occupy much of the perinuclear cytoplasm. Electron microscopy reveals that most of these fibers consist of pairs of ~10-nm filaments wound into helices (paired helical filaments or PHF), with a helical period of ~160 nm. Some tangle-bearing neurons also contain skeins of straight, 10- to 15-nm filaments interspersed with the PHF. Beginning in 1985, immunocytochemical and biochemical analyses of neurofibrillary tangles suggested that they were composed of the microtubule-associated protein tau (12, 43, 79, 107, 191). This was later confirmed by isolation of a subset of PHF that could be partially solubilized in strong solvents such as SDS (84) or digested with harsh proteases (75, 185), releasing tau proteins which migrated electrophoretically at a higher molecular weight than did normal tau prepared from tangle-free human or animal brains. This slower migration was shown to result from increased phosphorylation of tau; in vitro dephosphorylation with alkaline phosphatase returned this PHF-derived tau to essentially normal migration. Although some PHF can be solubilized by boiling in SDS (84), much of the tau in tangles is present in highly insoluble filaments (PHF) that are resistant to detergents such as SDS and chaotrophic solvents such as guanidine hydrochloride (145). Extensive analysis of the nature of hyperphosphorylated tau using antibodies specific for various phospho-tau epitopes has helped clarify which residues are phosphorylated in PHF tau (37, 80, 84, 97) A variety of kinases have been shown to be capable of phosphorylating tau in vitro at various sites (e.g., Refs. 37, 65). Nevertheless, it has not become clear whether one or more kinases are principally responsible for initiating the hyperphosphorylation of tau in vivo that leads to its apparent dissociation from microtubules and aggregation into insoluble paired helical filaments. In this regard, a recent study provides evidence that a dysregulation of cyclin-dependent kinase 5 (cdk5), as a result of proteolytic cleavage of its regulatory subunit p35 to yield a fragment (p25) which allows constitutive activity of the kinase, could play a major role in the hyperphosphorylation of tau that appears to underlie tangle formation in AD (111). It has been reported that calpain is responsible for cleavage of p35 and that treating cells with Aβ aggregates can trigger p35 activation and the subsequent cdk5-mediated phosphorylation of tau and perhaps other cytoplasmic substrates (83a).

The two classical lesions of AD, neuritic plaques and neurofibrillary tangles, can occur independently of each other. Tangles composed of tau aggregates that are biochemically similar to or, in some cases, indistinguishable from those in AD have been described in more than a dozen less common neurodegenerative diseases, in almost all of which one finds no Aβ deposits and neuritic plaques. Conversely, Aβ deposits can be seen in the brains of cognitively normal-aged humans in the virtual absence of tangles. There are also infrequent cases of AD itself which are "tangle poor," i.e., only a few neurofibrillary tangles are found in the neocortex despite abundant Aβ plaques (168). It appears that in quite a few such cases, an alternate form of neuronal cytoplasmic inclusion, the Lewy body (composed principally of α-synuclein protein), is found in cortical pyramidal neurons. In other words, the Lewy body variant of AD (not to be confused with diffuse Lewy body disease, which largely lacks Aβ plaques) may represent a tangle-poor form of AD that still has the usual

amount of Aβ plaque formation (51). The fact that neurofibrillary tangles composed of altered, aggregated tau proteins occur in disorders (e.g., subacute sclerosing panencephallitis, Kuf's disease, progressive supranuclear palsy, etc.) in the absence of Aβ deposition suggests that tangles can arise secondarily during the course of a variety of etiologically distinct neuronal insults. As we shall discuss, there is growing evidence that the formation of tangles in AD represents one of several cytological responses by neurons to the gradual accumulation of Aβ and Aβ-associated molecules.

D. Dystrophic Cortical Neurites Within and Outside Neuritic Plaques

Many of the dilated and tortuous neurites found within and immediately surrounding amyloid plaques contain PHF that are structurally, biochemically, and immunocytochemically indistinguishable from those that comprise the neurofibrillary tangles. In addition, plaques often contain numerous dystrophic neurites that are not immunoreactive for PHF tau. Tau-positive dystrophic neurites are also present in a more widespread distribution in the cortical neuropil outside of the neuritic plaques. The prevalence and density of dystrophic cortical neurites that contain altered forms of tau varies substantially among Alzheimer cases. There is evidence that cases that are particularly rich in neurofibrillary tangles are also those that show widespread tau-immunoreactive dystrophic cortical neurites (120). Some of the intraplaque and extraplaque dystrophic neurites are immunoreactive for phosphorylated forms of the neurofilament subunit proteins, and the latter can thus coexist with phosphotau reactivity. This finding suggests that there may be several substrates for the altered kinase and phosphatase (176) activities that occur in tangle-bearing neurons and dystrophic neurites.

E. Amyloid Microangiopathy

Aβ was originally isolated from amyloid-laden meningeal arterioles and venules that are often found just outside of the brains of patients with AD or Down's syndrome (34, 35). Similarly, small arterioles, venules, and capillaries within cerebral cortex also frequently bear amyloid deposits. This microvascular angiopathy is characterized at the ultrastructural level by amyloid fibrils found in the abluminal basement membrane of the vessels, sometimes with apparent extension or "spillover" of the fibrils into the surrounding perivascular neuropil (a lesion referred to as dyshorric angiopathy) (178). The Aβ peptides that occur as filaments in the microvessel basement membranes appear, on the basis of immunoreactivity, to be principally Aβ_{40} species, although evidence has been presented that the initially deposited species in ves-

sels destined to develop amyloid angiopathy may be Aβ_{42} (165). It is intriguing that meningeal arterioles that penetrate and traverse the cerebral cortex can have amyloid deposits in their walls that abruptly stop as the vessel enters the subcortical white matter. Only rare microvessels within the white matter show Aβ deposits. The extent of amyloid angiopathy varies widely among AD brains that have relatively similar burdens of parenchymal (i.e., plaque associated) Aβ. As a result, the contribution of this microvascular amyloidosis to the cortical dysfunction that occurs in AD and the mechanism by which amyloid alters microvascular function remain matters of active study (see for example Refs. 110, 172). Amyloid-bearing vessels composed of Aβ deposits essentially indistinguishable from those of AD can occur in the virtual absence of parenchymal Aβ deposits in the brains of elderly subjects without AD (178). Such amyloid-bearing vessels in this condition [referred to as congophilic amyloid angiopathy (CAA)], as well as those in AD can occasionally rupture, apparently due to hyaline necrosis surrounding the amyloid deposit in the vessel wall, leading to one or multiple cerebral hemorrhages (178). Nevertheless, the large majority of AD subjects do not experience cerebral hemorrhages, despite the presence of some or many microvascular amyloid deposits.

III. ORIGIN OF AMYLOID β-PROTEIN: CELL BIOLOGY OF β-AMYLOID PRECURSOR PROTEIN

A. Expression and Heterogeneity of APP

The purification and partial sequencing of the Aβ protein from meningovascular amyloid deposits in AD and Down's syndrome (34, 35) and the subsequent observation that Aβ was also the subunit of the plaque amyloid (38, 96, 144) led to the cloning of the gene encoding the β-APP (72). Aβ is derived from its large precursor protein by sequential proteolytic cleavages (see sect. III*B*). APP comprises a heterogeneous group of ubiquitously expressed polypeptides migrating between 110 and 140 kDa on electrophoretic gels (146). This heterogeneity arises both from alternative splicing (yielding 3 major isoforms of 695, 751, and 770 residues) as well as by a variety of posttranslational modifications, including the addition of *N*- and *O*-linked sugars, sulfation, and phosphorylation (62, 108, 181, 183). The APP splice forms containing 751 or 770 amino acids are widely expressed in nonneuronal cells throughout the body and also occur in neurons. However, neurons express even higher levels of the 695-residue isoform, which occurs at very low abundance in nonneuronal cells (45). The difference between the 751/770- and 695-residue forms is the presence in the former of an exon that codes for a 56-amino acid motif that is homologous to the Kunitz-type of serine protease inhibi-
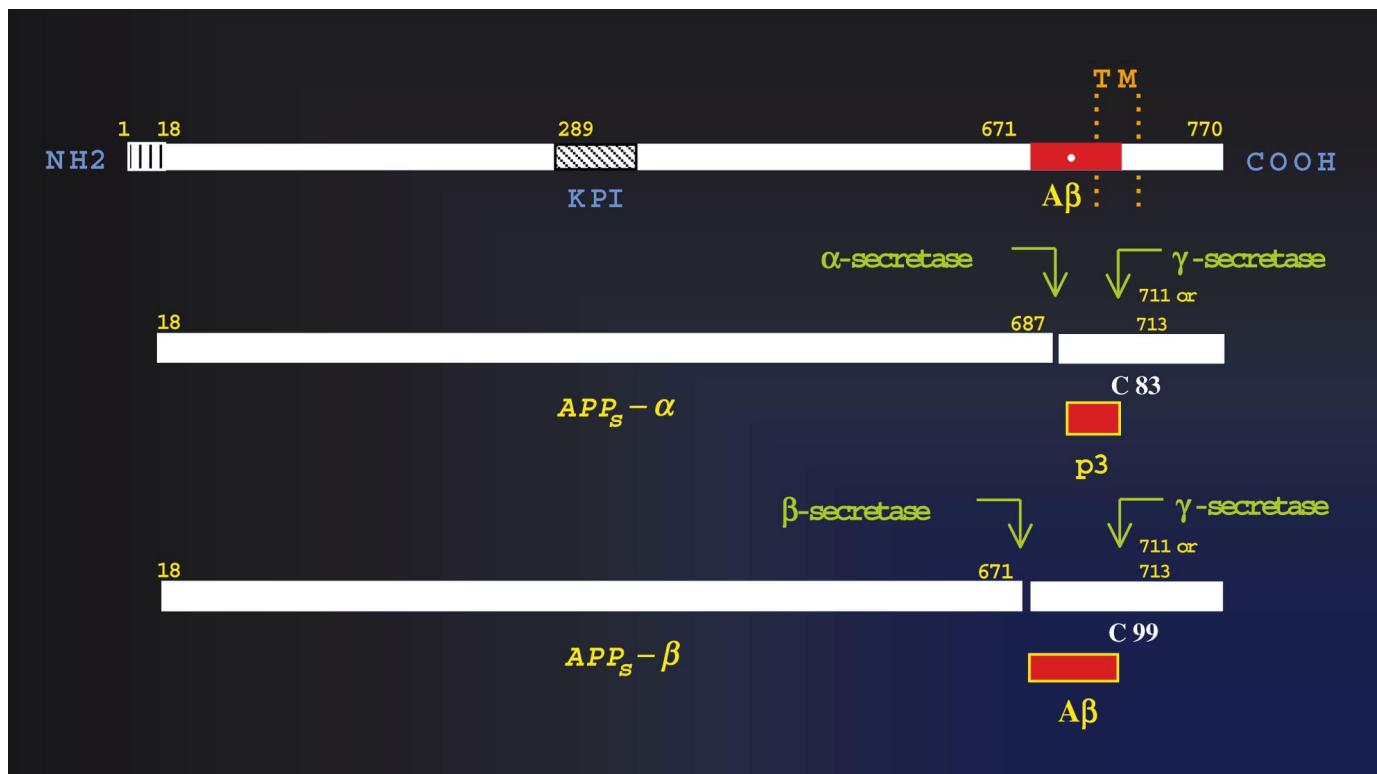
FIG. 1. Schematic diagrams of the β-amyloid precursor protein (APP) and its principal metabolic derivatives. Top diagram depicts the largest of the known APP alternate splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct relative positions. A 17-residue signal peptide occurs at the NH_2 terminus (box with vertical lines). Two alternatively spliced exons of 56 and 19 amino acids are inserted at residue 289; the first contains a serine protease inhibitor domain of the Kunitz type (KPI). A single membrane-spanning domain (TM) at amino acids 700–723 is indicated by the vertical dotted lines. The amyloid ß-protein (Aβ) fragment includes 28 residues just outside the membrane plus the first 12–14 residues of the transmembrane domain. In the middle diagram, the arrow indicates the site (after residue 687; same site as the white dot in the Aβ region of APP in the upper diagram) of a constitutive proteolytic cleavage made by protease(s) designated α-secretase that enables secretion of the large, soluble ectodomain of APP (APP$_s$-α) into the medium and retention of the 83-residue COOH-terminal fragment in the membrane. The C83 fragment can undergo cleavage by a protease(s) called γ-secretase at residue 711 or residue 713 to release the p3 peptides. The bottom diagram depicts the alternative proteolytic cleavage after residue 671 by a protease(s) called β-secretase that results in the secretion of the slightly truncated APP$_s$-β molecule and the retention of a 99-residue COOH-terminal fragment. The C99 fragment can also undergo cleavage by γ-secretase to release the Aβ peptides.

tors (KPI), indicating one potential function of these longer APP isoforms. Indeed, the KPI-containing forms of APP found in human platelets serve as inhibitors of factor XIa, which is a serine protease in the coagulation cascade (158). APP is highly conserved in evolution and expressed in all mammals in which it has been sought. A partial homolog of APP is found in *Drosophila* (referred to as APPL) (130). Indeed, APP is a member of a larger gene family, the amyloid precursor-like proteins (APLPs) (157, 182), which have substantial homology, both within the large ectodomain and particularly within the cytoplasmic tail, but are largely divergent in the Aβ region.

B. Trafficking and Proteolytic Processing of APP

APP is a single transmembrane polypeptide that is cotranslationally translocated into the endoplasmic reticulum via its signal peptide and then posttranslationally

modified ("matured") through the secretory pathway. Its acquisition of *N*- and *O*-linked sugars occurs rapidly after biosynthesis, and its half-life is relatively brief (~45–60 min in most cell types tested) (183). Both during and after the trafficking of APP through the secretory pathway, it can undergo a variety of proteolytic cleavages to release secreted derivatives into vesicle lumens and the extracellular space (Fig. 1). The first proteolytic cleavage identified, that made by an activity designated α-secretase, occurs 12 amino acids NH_2-terminal to the single transmembrane domain of APP (28, 156). This processing results in the release of the large soluble ectodomain fragment (α-APP$_s$) into the lumen/extracellular space and retention of an 83-residue COOH-terminal fragment (CTF) in the membrane. Alternatively, some APP molecules not subjected to α-secretase cleavage can be cleaved by an activity designated β-secretase, which principally cuts 16 residues NH_2-terminal to the α-cleavage site, generating a

slightly smaller ectodomain derivative (β-APP$_s$) (147) and retaining a 99-residue CTF (C99) in the membrane that begins at residue 1 of the Aβ region (reviewed in Ref. 143).

Until 1992, it was assumed that Aβ generation was a pathological event, because the cleavage of the C99 fragment resulting from the so-called γ-secretase activity appeared to occur in the middle of the transmembrane domain. It was assumed that this would require the release of C99 from the membrane, for example, as a result of some preexisting membrane injury that allowed access to a soluble protease. However, the use of sensitive Aβ antibodies to probe the conditioned media of APP-expressing cells revealed secreted Aβ that was constitutively released from cells under entirely normal cellular conditions (13, 50, 148, 151). This result suggested that the β-secretase cleavage could be followed by a constitutive cleavage at the COOH terminus of the Aβ region, made by an activity dubbed γ-secretase. At the same time, a peptide fragment designated p3 was discovered to be produced by the sequential actions of the α- and γ-secretases (44, 50). These unexpected findings indicated that Aβ production was a normal metabolic event, and indeed, the peptide was detected in both cerebrospinal fluid and plasma in healthy subjects throughout life (148, 151). Precisely where during its complex intracellular trafficking APP can undergo the α-, β-, and γ-secretase cleavages is not settled. Clearly, a substantial portion of α-APP$_s$ is generated by α-secretase acting on plasma membrane inserted APP (155). On the other hand, α-APP$_s$ can also be generated during the secretory intracellular trafficking of APP (23, 133). With regard to the β-secretase cleavage, this can occur in part late in the secretory trafficking of APP (49). The recent identification and cloning of β-secretase by several laboratories (63, 153, 177, 201) will now enable a precise localization of this novel membrane-anchored aspartyl protease. The sites of cleavage of the C99 and C83 fragments by γ-secretase and the nature of that enzyme are also under active study. It appears that Aβ_{40} and Aβ_{42} can be made in considerable part during the internalization and endosomal processing of APP (77, 112). There are conflicting data about whether much of Aβ_{42} is generated early in the secretory trafficking of APP (i.e., in endoplasmic reticulum, intermediate compartment, and early Golgi) or principally after APP reaches the cell surface. Some evidence suggests that Aβ peptides generated very early in the secretory pathway (i.e., in endoplasmic reticulum) may not be destined for secretion and are retained and catabolized inside cells (16). However, it is likely that the majority of Aβ generated within cells is destined for secretion. Steady-state levels of Aβ in human cerebrospinal fluid are in the range of 3–8 nM (101), whereas the level in plasma is generally under 500 pM (137). Aβ_{40} and Aβ_{42} species can both be detected in these extracellular fluids.

Pulse-chase experiments have demonstrated that most C83 and C99 fragments (the immediate substrates of γ-secretase) are generated from APP molecules that have undergone full *N*- plus *O*-linked glycosylation (i.e., within or after the Golgi) (49, 183). These results support the concept that the α-, β-, and γ-secretase cleavages of APP occur primarily at or near the cell surface, perhaps in substantial part in recycling endosomes (112).

In polarized epithelial cells such as Madin-Darby canine kidney (MDCK) cells, APP is principally targeted to the basolateral membrane, where it can undergo α-secretase cleavage to release α-APP$_s$ basolaterally, although a small fraction is targeted and processed apically (47, 48). In neurons, which are one of the cells that express the highest levels of APP in the body (particularly APP$_{695}$), APP can be anterogradely transported in the fast component of axonal transport (76). APP is present in vesicles in axonal terminals, although not specifically in synaptic vesicles. Cell biological studies demonstrate that APP in the axonal terminals can be retrogradely transported up the axon to the cell body, and some molecules are then fully translocated to the somatodendritic surface (200). During its retrograde axonal trafficking, some APP molecules can apparently recycle to the axolemmal surface (200). Although it has been assumed that APP axonal terminals might be a principal site for the generation of Aβ, this has not been definitively determined, and APP that recycles in endosomes at various neuronal subsites may be capable of undergoing the sequential β- and γ-secretase cleavages to release the peptide. Indeed, although APP is particularly abundantly expressed in neurons and they have been directly shown to secrete substantial amounts of Aβ peptides (50), other brain cells also express APP and release variable amounts of Aβ, including astrocytes, microglia, and endothelial and smooth muscle cells, and these could all contribute to the secreted pool of Aβ that eventually leads to extracellular deposition. Moreover, the fact that virtually all peripheral cells also express APP and generate Aβ and that Aβ is present in plasma raises the possibility that circulating Aβ could cross the blood-brain barrier and contribute to cerebral Aβ accumulation. Direct evidence that Aβ can cross the blood-brain barrier in small amounts using a mechanism consistent with receptor-mediated endocytosis has been reported (116, 117, 209).

C. Inferred Functions of APP and Its Derivatives

A number of possible functions have been ascribed to APP holoproteins and/or their major secreted derivative (α-APP$_s$) based on cell culture studies. Soluble α-APPs appear to be capable of acting as an autocrine factor (132) and a neuroprotective and perhaps neuritotrophic factor (98). The fact that the alternatively spliced forms containing 751 and 770 residues contain a 56-residue insert in the middle of the ectodomain encoding a KPI motif (167) has

led to in vitro studies that confirm an ability of these isoforms to inhibit serine proteases such as trypsin and chymotrypsin (154). As mentioned previously, the KPI-containing isoforms also function as an inhibitor of factor XIa (a serine protease) in the clotting cascade (158). The secreted APP isoforms can confer cell-cell and cell-substrate adhesive properties in culture (e.g., Ref. 140). The APP holoprotein has also been suggested to function in cell-cell interactions when inserted at the plasma membrane, based on in vitro studies (122). All of these imputed functions have not yet been clearly confirmed in vivo. Deletion of the APP gene in mice results in neither early mortality nor appreciable morbidity; cerebral gliosis and changes in locomotor behavior occur later in adult life (207), and neurons cultured at birth have diminished viability and retarded neurite outgrowth (113). This lack of a vital consequence of APP deletion in vivo may result from the fact that mammals express proteins closely homologous to APP, the APLPs (157, 182). Delineation of the precise functions of APP and its homologs in vivo awaits further study. No evidence has emerged that a fundamental cellular function of APP is lost in AD patients. Instead, APP mutations seem to act by a toxic gain-of-function mechanism, namely, by increasing production of the potentially cytotoxic $A\beta$ fragment (see below).

IV. GENETICS OF FAMILIAL ALZHEIMER'S DISEASE

A. Familial Forms of AD Closely Resemble the Common "Sporadic" Form

It has been known for at least several decades that clinically typical AD can cluster in families and can specifically be inherited in an autosomal dominant fashion. Estimates of the prevalence of inherited forms of AD have varied widely from as little as 5–10% to as high as 50% or more. Some investigators believe that in the fullness of time, a large majority of AD cases will be shown to have underlying genetic determinants, many of which may appear as polymorphic alleles that predispose to the disease but do not invariably cause it. Determining how frequently genetic factors underlie the disease is difficult in a late-onset disorder such as AD, particularly one that was not specifically diagnosed and recorded before the last two decades. Moreover, the recognition that polymorphic alleles of apolipoprotein E can predispose strongly to the development of AD in the 60s and 70s suggests that other polymorphic genes could predispose to the disorder but would be difficult to detect in genetic epidemiological studies, because they do not always produce the disease and will thus not show high penetrance.

Despite the uncertainty about the degree to which AD is accounted for by genetic factors, it has become clear from phenotypic analyses of familial versus apparently non-familial ("sporadic") cases that these two forms are phenotypically highly similar or often indistinguishable, save for the earlier age of onset of the known autosomal dominant forms. When the age of the patient is not known to the neuropathologist, the histological phenotype of the early-onset cases is very difficult to distinguish from those of common late-onset patients. Similarly, the clinical manifestations of familial (autosomal dominant) AD are generally quite similar or almost indistinguishable from those of the sporadic cases, although some families may show distinctive clinical signs (e.g., myoclonus, seizures, early and prominent extrapyramidal signs, etc.). This general phenotypic similarity strongly suggests that information about the mechanism of the autosomal dominant forms caused by mutations in the APP and presenilin genes is likely to be directly relevant to the pathogenesis of the common, apparently nonfamilial forms.

B. Missense Mutations in APP: A Very Rare Cause of Familial AD

The first specific genetic cause of AD to be identified was the occurrence of missense mutations in APP (36) (Table 1). Despite extensive genetic surveying, such mutations have only been confirmed in some two dozen or so families worldwide. Nevertheless, the location of the mutations (Fig. 2) and the subsequent delineation of their genotype-to-phenotype relationships have provided critical insights into the mechanism of AD. The mutations are strategically located either immediately before the β-secretase cleavage site, shortly after the α-secretase site, or shortly COOH-terminal to the γ-secretase cleavage site. The fact that, despite substantial investigation, no other mutations in the large APP protein that cause AD have been discovered strongly suggests that these missense mutations lead to AD by altering proteolytic processing at the three secretase sites in subtly different ways. This hypothesis has been confirmed by analysis of each of the mutations, initially in transfected cells or primary cells from patients and then in transgenic mouse models (reviewed in Ref. 142). Families harboring APP missense mutations that cause AD generally have the onset of the disorder before age 65, often in their 50s.

There is another way that alterations in the APP gene can predispose to the development of AD. The overexpression of structurally normal APP owing to elevated gene dosage in trisomy 21 (Down's syndrome) almost invariably leads to the premature occurrence of classical AD neuropathology (neuritic plaques and neurofibrillary tangles) during middle adult years. A life-long increase in APP expression due to duplication of all of chromosome 21 or, in the case of translocation Down's syndrome, that portion of 21q containing the APP gene results in overproduction of $A\beta_{40}$ and $A\beta_{42}$ peptides dating from birth

TABLE 1. *Confirmed genetic factors predisposing to Alzheimer's disease: relationships to the β-amyloid phenotype*

Chromosome	Gene Defect	Phenotype
21	β-APP mutations	↑ Production of all Aβ peptides or Aβ$_{40}$ peptides
19	ApoE4 polymorphism	↑ Density of Aβ plaques and vascular deposits
14	Presenilin 1 mutations	↑ Production of Aβ$_{42}$ peptides
1	Presenilin 2 mutation	↑ Production of Aβ$_{42}$ peptides

β-APP, β-amyloid precursor protein; Aβ, amyloid β-protein.

(173). This is assumed to be responsible for the strikingly early appearance of many Aβ$_{42}$ diffuse plaques, which can occur as soon as age 12 yr (85). Down's subjects often display diffuse plaques composed solely of Aβ$_{42}$ in their teens and 20s, with accrual of Aβ$_{40}$ peptides onto these plaques and the appearance of associated microgliosis, astrocytosis, and surrounding neuritic dystrophy usually beginning in their late 20s or 30s (85, 94). This observation underscores the importance of Aβ$_{42}$ accumulation as a seminal event in the development of AD-type brain pathology. The appearance of neurofibrillary tangles is also delayed until the late 20s, 30s, or beyond in most Down's patients. The gradual accrual of AD-type brain lesions in these individuals, who are retarded from birth for other reasons, appears to be associated in many cases with progressive loss of cognitive and behavioral functions after the age of 35 or so.

Because the entire chromosome 21 is duplicated in the vast majority of cases of Down's syndrome, it is difficult to attribute the Alzheimer syndrome that they develop directly to APP gene dosage. However, this issue has been essentially resolved by the recent evaluation of a patient with translocation Down's syndrome in which the obligate Down's region in the distal portion of chromosome 21 was duplicated, but the break point was telomeric to the APP gene. The subject bearing this particular translocation had typical phenotypic features of Down's syndrome but did not develop clear-cut evidence of behavioral deterioration during middle age. At autopsy, no significant Aβ deposition or other Alzheimer-type neuropathology was observed (119). This absence of amyloid deposition and attendant cytopathological changes is highly unusual in Down's subjects, and this case suggests that when this occurs, it is because the APP gene is not duplicated. The careful clinicopathological analysis of this unusual case provides further strong support for the primacy of Aβ deposition in producing classical AD neuropathology.
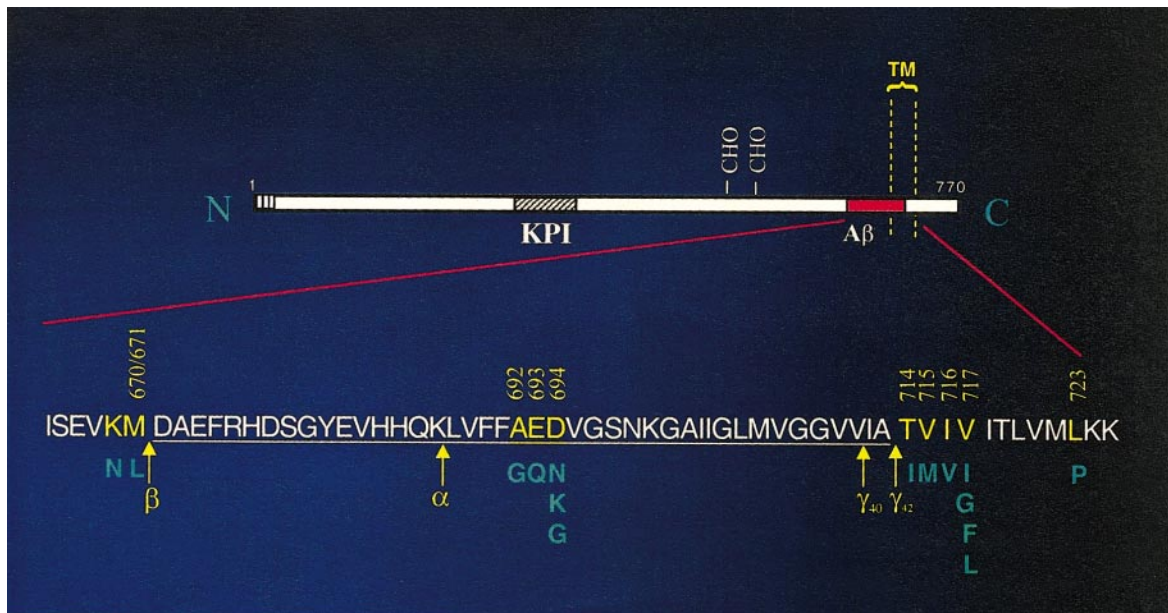
FIG. 2. β-APP mutations genetically linked to familial Alzheimer's disease or related disorders. The sequence within APP that contains the Aβ and transmembrane region is expanded and shown by the single-letter amino acid code. The underlined residues represent the Aβ1–42 peptide. The vertical broken lines indicate the location of the transmembrane domain. The bold letters below the line indicate the currently known missense mutations identified in certain patients with familial Alzheimer's disease and/or hereditary cerebral hemorrhage with amyloidosis. Three-digit numbers refer to the residue number according to the β-APP770 isoform.

C. Missense Mutations in the Presenilins: The Most Common Cause of Autosomal Dominant AD to Date

The realization that autosomal dominant AD is genetically heterogeneous led to intensive searches for loci in the genome besides APP that could explain the many families that did not link to chromosome 21. Establishment of a linkage of some of these families to chromosome 14 (135) led ultimately to further linkage analysis and positional cloning that identified a novel gene on chromosome 14q which came to be known as presenilin 1 (PS1) (150). Missense mutations were found that appeared to be causative of AD in certain families with clinical onset in their 40s and 50s, sometimes as early as the 30s. Shortly thereafter, an homologous gene was discovered on chromosome 1, mutations in which explain the early-onset kindreds referred to as the Volga German families, as well as AD in an Italian family (90). This gene was ultimately designated presenilin 2 (PS2). Further intensive genetic surveys have identified as many as 75 missense mutations in presenilin 1 and three in presenilin 2 as molecular causes of early-onset AD in several hundred families worldwide (reviewed in Ref. 52). Presenilin 1 missense mutations cause the earliest and most aggressive form of AD, commonly leading to onset of symptoms before the age of 50 and demise of the patient in his/her 60s. We discuss below how instructive these mutations have been for understanding both the role of presenilin in AD and gaining insight into the normal functions of these interesting polytopic membrane proteins.

D. The Apolipoprotein E4 Allele is a Major Genetic Risk Factor for Late-Onset AD

Whereas the autosomal dominant mutations in APP or the presenilins are quite infrequent causes of AD, the discovery that the $\epsilon 4$ allele of apolipoprotein E (ApoE) predisposes to AD provided a major genetic risk factor for the disorder in the typical late-onset period (163). Studies initiated by searching for proteins in human cerebrospinal fluid that could bind immobilized Aβ peptides on a filter led to the identification of ApoE as such a protein and the recognition that its gene localized to chromosome 19q, in a region previously found to show genetic linkage to AD in some late-onset families (163). Further genetic analyses indicated that the $\epsilon 4$ allele of ApoE is overrepresented in subjects with AD compared with the general population and that inheritance of one or two $\epsilon 4$ alleles heightens the likelihood of developing AD and makes its mean age of onset earlier than in subjects harboring $\epsilon 2$ and or $\epsilon 3$ alleles (18, 134). Thus the ApoE4 protein helps precipitate the disorder primarily in subjects in their 60s and 70s. There is also evidence that inheritance of the $\epsilon 2$ allele may

confer protection against the development of AD (17). Although inheritance of a single $\epsilon 4$ allele may increase the likelihood of developing AD in the 60s and 70s, some two- to fivefold and two $\epsilon 4$ alleles may increase the risk well above fivefold, it should be emphasized that ApoE4 is a risk factor for, not an invariant cause of, AD. Some humans homozygous for the $\epsilon 4$ isoform still show no Alzheimer symptoms in their ninth decade of life and beyond. Conversely, a great many humans develop AD without harboring $\epsilon 4$ alleles. The recognition that inheritance of $\epsilon 4$ predisposes humans to AD provided one of the first genetic risk factors for a common late-onset disease.

E. Other Genetic Alterations Predisposing to AD Are Likely

Whereas there is universal agreement that alterations in the four aforementioned genes can cause familial forms of AD, various methods of genetic analysis indicate that additional genes predisposing to AD exist. In this regard, an AD-linked locus on chromosome 12 in certain pedigrees appears to represent alterations in or near the gene encoding α_2-macroglobulin (α_2M) (10). A polymorphism in an intronic region of the α_2M gene segregates with the AD phenotype in some late-onset subjects (10). Additional studies confirming this association have appeared (2, 25, 102, 129), and work to determine whether the enhanced genetic risk is attributable directly to α_2M or to a nearby gene is underway.

The fact that numerous families exist whose AD phenotype does not link to any of the five genes implicated to date indicates that additional genetic risk factors and perhaps even dominantly transmitted causative genes will be found. Indeed, recent studies have revealed an apparent major locus for late-onset familial AD on chromosome 10q (8a). It is likely that over the next one to two decades, a much larger portion of AD will be shown to have genetic determinants than is currently believed. Indeed, clinical surveys already indicate that, upon careful questioning, a family history of first degree relatives with a dementing syndrome resembling AD is obtained in as many as one-half to two-thirds of patients presenting with clinically probable AD.

V. GENOTYPE-TO-PHENOTYPE CONVERSIONS IN FAMILIAL ALZHEIMER'S DISEASE

The rapid accrual of information about the proteolytic processing of APP and the aggregational properties of its Aβ derivatives coincided with the identification of gene defects that cause or predispose to AD. The systematic correlation of these two distinct bodies of knowledge during the last few years has led to an emerging understanding of the fundamental pathogenetic mechanism of

AD. Experiments to decipher the genotype-to-phenotype relationships have been conducted in cell culture, in transgenic mice and, most importantly, in patients who actually harbor the relevant genetic mutations. For all four genes unequivocally confirmed to date (APP, ApoE4, PS1, and PS2), inherited alterations in the gene products have been credibly linked to increases in the production and/or the cerebral deposition of the $A\beta$ peptides (142). Such studies have provided the strongest support for the hypothesis that cerebral accumulation of $A\beta$ is an early, invariant, and necessary event in the genesis of AD.

A. APP Mutations Increase the Production of $A\beta_{42}$ Peptides

The nine known missense mutations in APP currently linked to familial AD (Fig. 2) have been found to increase $A\beta$ production by subtly different mechanisms. A double mutation in the two amino acids immediately preceding the β-secretase cleavage site (often referred to as the "Swedish" APP mutation based on the ethnic origin of the family in which it occurs) induces increased cleavage by β-secretase to generate more $A\beta_{40}$ and $A\beta_{42}$. The five mutations occurring just COOH-terminal to the γ-secretase cleavage sites appear in slightly different ways to selectively enhance the production of $A\beta$ species ending at residue 42. The two remaining mutations that are located internally in $A\beta$ could be expected to enhance the aggregational properties of all $A\beta$ species, although this has only been shown for the E693Q mutation that causes hereditary cerebral hemorrahge with amyloidosis of the Dutch type (89). The other, immediately adjacent internal mutation (A692G) leads to a mixed phenotype of *1*) AD-type plaque and tangle formation associated with dementia and *2*) severe microvascular β-amyloidosis with occasional cerebral hemorrhages (56). This mutation has been shown in transfected cells to lead to changes in the heterogeneous NH_2-terminal β-secretase cleavages that have the overall effect of favoring production of the full-length peptide beginning at $A\beta$ Asp1(46). The recent cloning of β-secretase, a novel membrane-anchored aspartyl protease with its active site in its ectodomain and the identification of a close homolog thereof (BACE-2) (63, 153, 177, 201), has led to studies in transfected cells suggesting that the A692G APP mutation specifically enhances the proportion of APP that is cleaved by BACE-2 and shifts cleavage by the latter toward the Asp1 NH_2 terminus (M. Farzan, personal communication). These interesting early data suggest that the identification of the actual α-, β-, and γ-secretases will provide much clearer mechanistic insights into exactly how missense mutations in APP lead to heightened production of various $A\beta$ species, in each case inducing an amyloidogenic phenotype that produces AD.

B. Presenilin Mutations Increase the Production of $A\beta_{42}$ Peptides

Perhaps the most intriguing genotype-to-phenotype relationships in AD involve the presenilin mutations. When presenilin 1 and 2 were first cloned, the mechanism by which mutations in them produced the AD phenotype was an open matter and was not necessarily expected to involve enhanced $A\beta$ production. However, direct assays of $A\beta_{40}$ and $A\beta_{42}$ in the plasma and the cultured skin fibroblast media of humans harboring these mutations soon revealed a selective approximately twofold elevation of $A\beta_{42}$ levels (137). Extensive modeling of these mutations in cultured cells and transgenic mice has confirmed this finding (e.g., Refs. 11, 15, 26, 174, 195). A particularly important observation has been the finding that crossing mice transgenic for human APP with mice expressing a PS1 missense mutation leads to a substantially accelerated AD-like phenotype in the offspring, with $A\beta_{42}$ plaques (first diffuse and then mature) occurring as early as 3–4 mo of age (57). But even before confirmation of the $A\beta_{42}$-elevating effect of presenilin mutations was obtained in transfected cells and transgenic mice, quantitative image analysis of the brain amyloid deposits of patients who had these mutations using $A\beta_{42}$- and $A\beta_{40}$-specific antibodies demonstrated directly that inheritance of presenilin mutations leads to a 1.5- to 3-fold increase in the relative abundance of plaques containing $A\beta_{42}$ peptides, compared with the levels observed in sporadic cases of AD (86, 95). The molecular mechanism by which missense mutations in the presenilins selectively increase the γ-secretase cleavage of C99 (and also C83) to yield more peptides ending at $A\beta_{42}$ compared with $A\beta_{40}$ will be discussed after reviewing current knowledge about the complex biology of the presenilins (see sect. VI).

C. Inheritance of ApoE4 Alleles Increases Steady-State Levels of $A\beta$ Peptides in the Brain

Even before ApoE4 was recognized as a genetic risk factor for late-onset disease, immunohistochemistry had demonstrated the presence of ApoE protein in a high percentage of $A\beta$ deposits in AD brain tissue (103). Once the genetic connection between AD and ApoE4 inheritance was made, further immunohistochemical studies of brains of patients lacking or expressing the ApoE4 protein showed that inheritance of ApoE4 was associated with a significantly higher $A\beta$ plaque burden than was observed in patients lacking ApoE4 (31, 126, 138). Although some brains of ApoE4 allele carriers showed higher neurofibrillary tangle densities, overall this change did not usually reach the statistically significant levels of elevation observed for $A\beta$ deposits. Importantly, studies in nonoge-

narians who died without showing clear-cut clinical symptoms of AD demonstrated that ApoE4 genotype was again linked to enhanced amounts of diffuse $A\beta_{42}$ plaques in the brain, suggesting that the $A\beta$-elevating effects associated with ApoE4 inheritance could be observed presymptomatically or in hosts who would not necessarily develop AD (118).

The mechanism by which ApoE4 protein leads to increased $A\beta$ deposition has been difficult to pinpoint. No evidence has emerged that $A\beta$ production is significantly elevated in cells that coexpress APP with the ApoE4 protein versus with the ApoE2 or ApoE3 proteins (9). Rather, ApoE4 seems to enhance the steady-state levels of $A\beta$ peptides, $A\beta_{40}$ in particular (31), presumably by decreasing its clearance from the brain tissue in some way. In vitro studies quantifying the degree of $A\beta$ fibrillogenesis using synthetic peptides suggest that the presence of the ApoE4 protein results in increased numbers of fibrils, compared with levels obtained in the presence of ApoE3 (29, 93), although the way in which ApoE proteins cause these effects, e.g., by ApoE4 serving as a less effective inhibitor of $A\beta$ fibrillogenesis or rather as a more potent stimulator, is not settled. An alternative mechanism for the AD-promoting effect of ApoE4 inheritance emerges from evidence in transgenic mice expressing either the E4 or E3 human protein. Mice expressing E4 appear to have decreased neuritic outgrowth of cultured neurons and decreased maintenance of established neurites (105). These studies suggest that ApoE4 protein is less supportive of normal neuronal form and function than are ApoE3 or E2 proteins. However, such a neuronal vulnerability in ApoE4 gene carriers may not be the actual mechanism of the ApoE4 effect on the AD phenotype, given the fact that deposition of $A\beta$ into cerebral and meningeal vessels to produce the syndrome of congophilic amyloid angiopathy is also enhanced by the gene dosage of ApoE4, even in the absence of Alzheimer-type neuropathology (40). In other words, the fact that ApoE4 alleles have clearly been found to enhance $A\beta$ deposition not only in parenchymal plaque deposits but also in microvessels outside of the brain parenchyma (and in the absence of AD) potentially separates the ApoE4 effect in promoting the AD cerebral phenotype (i.e., neuritic plaques) from any deleterious effects ApoE4 may have on neuronal/neuritic function in general. Thus the most parsimonious explanation for ApoE4 effects vis á vis AD is that this isoform somehow enhances the deposition or decreases the clearance of $A\beta$ peptides, particularly $A\beta_{40}$, in both the cerebral cortex and its microvasculature.

Such an amyloid-enhancing mechanism is supported by studies in which mice transgenic for mutant human APP are crossed with mice in which the endogenous mouse ApoE gene is deleted. The resultant offspring show substantially decreased $A\beta$ plaque burden compared with that seen in the parental APP transgenic line, suggesting that the absence of ApoE significantly decreases the tendency of $A\beta$ to accrue

as diffuse and mature plaques (5). Moreover, mice lacking endogenous ApoE that express human ApoE3 or E4 plus mutant human APP develop less $A\beta$ deposits than similar mice expressing no ApoE at all (58).

An important caveat about in vitro studies attempting to elucidate the mechanisms by which the ApoE proteins induce such effects is that they need always to be conducted in the presence of lipid, i.e., where ApoE is assembled into lipoprotein particles. There is currently no evidence that any significant portion of ApoE proteins occurs as free polypeptides in brain or other tissues. As a result, early studies examining the effects of pure ApoE on $A\beta$ in vitro are difficult to interpret. Carefully designed in vitro and in vivo experiments should ultimately clarify whether ApoE4 increases $A\beta$ steady-state levels in brain by less efficiently preventing its aggregation, by inhibiting its degradation or its reuptake into cells, or by other effects on its clearance.

VI. FUNCTION OF PRESENILINS: A CENTRAL ROLE IN INTRAMEMBRANOUS PROTEOLYSIS

A. Cell Biology of the Presenilins

Shortly after the PS1 and PS2 genes were cloned and missense mutations within them shown to cause autosomal dominant AD, two important observations about their biology were made. First, the presenilin holoproteins (~44 kDa) were found to undergo constitutive endoproteolysis in many cell types and in the brain and thus exist in major part as stable heterodimers composed of the NH_2-terminal fragment (NTF) and COOH-terminal fragment (CTF) (11, 115, 123, 169). The very low levels of holoprotein in cells and tissue, together with the evidence from pulse-chase experiments that the holoprotein is rapidly converted into fragments (115), probably by endoproteolysis occurring within endoplasmic reticulum vesicles and subsequent stabilization of the fragments in the Golgi (206), suggests that the fragments are the principal biologically functional form of presenilins. The constitutive proteolytic cleavage site (115, 162) occurs within a hydrophobic portion of the cytoplasmic loop between the sixth and seventh of the eight putative transmembrane domains (91). The steady-state levels of presenilin NTFs and CTFs seem to be tightly regulated, as overexpression of PS1 in transfected cells or transgenic mice generally does not increase the overall level of PS fragments (11, 170). Excess PS holoproteins are rapidly degraded, mainly by the proteasome (73, 161). Once formed, PS fragments can associate into higher molecular mass (~100–200 kDa) complexes that may represent the principal form in which presenilin functions in cells (14, 205).

The second major observation was the identification of the homolog in *Caenorhabditis elegans* of the mam-

malian presenilins, a gene designated *sel-12* (88). *Sel-12* was identified in genetic screens as a facilitator of the worm homolog of Notch, *lin-12*. The existence of mutations in *sel-12* that decrease or eliminate its function has enabled the use of the nematode as a model system for studying the function of the human presenilins (6, 87). For example, a loss-of-function mutation in *sel-12* can produce a lethal defect in egg-laying in the worm that is due to a defect in lin-12 (i.e., Notch) signaling during differentiation of the vulva (88). Other proteins that interact genetically with sel-12 have been identified in *C. elegans* (192). In addition, the use of the yeast two-hybrid system has led to identification of several novel or known mammalian proteins that appear to interact with presenilin. Prominent among these are members of the Armadillo family called the catenins, including an apparent neuron-specific member of this family, designated δ-catenin (205, 208). Both δ- and β-catenins coimmunoprecipitate with presenilin 1. The catenin binding site appears to be in the distal portion of the large cytoplasmic loop between transmembrane (TM) domains 6 and 7. It has been shown that this region is dispensable for the function of presenilin in the γ-secretase mechanism (i.e., in Aβ generation), and therefore, the interaction with the catenins may not turn out to have pathogenic relevance in AD. Presenilins have recently been shown to participate in multi-protein complexes near and at the cell surface that include the cadherins, important molecules mediating cell-cell adhesion (33). Furthermore, the fact that mutations of conserved residues in PS1 as well as PS2 can elevate $A\beta_{42}$ production and are linked to familial AD suggests that sequences that diverge between the two homologs (such as the region of the PS1 loop which binds the catenins) are less likely to be required for the critical stabilization of the presenilin heterodimers and for their AD-promoting activity than are highly conserved sequences, such as their COOH termini (175). Indeed, the latter site is a good candidate for the binding of the currently unknown cellular factors that regulate presenilin endoproteolysis and stabilize the heterodimers (198).

The loss of function of presenilin produced by gene deletion in mice leads to a profound phenotype that includes markedly abnormal somitogenesis and axial skeletal development with shortened body length, as well as cerebral hemorrhages (149, 190). In addition, these mice, which die just before or at birth, show abnormal embryonic neurodevelopment in the forebrain marked by premature loss of neuronal precursors (149). Deletion of just one PS1 gene in the mouse has not been associated with any major phenotypic abnormalities to date. An important functional insight has been gained by complementation studies in presenilin homozygous knockout mice. Crossing presenilin heterozygous knockout mice with mice transgenic for AD-causing mutant PS leads to some offspring that have no endogenous (mouse) presenilin but express the human mutant form. Such mice survive and do not have the devastating phenotype found in presenilin homozygous mice, although they may have subtle alterations (20, 121). Therefore, missense mutations in the human presenilins that cause early-onset AD appear to act as gain rather than loss of function mutations.

B. Presenilin and the γ-Secretase Cleavage of APP

Presenilin knock-out mice have also proven to be critical for deciphering the role of presenilins in APP metabolism. Such mice show normal levels of APP holoproteins as well as normal secretory derivatives from the α- and β-secretase cleavages but grossly abnormal γ-secretase function (22). Neurons cultured from these mice (22) and the brain tissue itself (196) accumulate high levels of the γ-secretase APP substrates C83 and C99. There is a corresponding substantial (~70%) decrease in the production of both $A\beta_{40}$ and $A\beta_{42}$ (22). This evidence that presenilin plays a required role in the γ-secretase mechanism has received substantial support from several types of experiments. Even before the realization that presenilin is necessary for proper γ-secretase cleavage of APP, it was shown that presenilin could bind to and immunoprecipitate with full-length APP molecules in several cell types (184, 197). This interaction was shown not to require the cytoplasmic tail of APP (197). Because the presenilins have very small ectodomain loops, it was unlikely that presenilin and APP would interact via their respective ectodomains. This left the transmembrane domains as the likely site of interaction. However, this evidence for coimmunoprecipitation of presenilin and APP was sharply challenged by investigators who could show no such interaction (171). From this controversy arose two broad hypotheses for the mechanism of the presenilins in γ-secretase-mediated APP processing. The first, based on the ability to coprecipitate the proteins, suggested that presenilin participates directly in the γ-secretase mechanism, i.e., is part of the catalytic complex, presumably as a cofactor (197). The alternate hypothesis argued that presenilin and APP do not physically interact; rather, presenilin regulates the membrane trafficking of certain proteins, presumably including the components of the γ-secretase reaction (the protease and APP) in a way that allows them to come together (104, 171). In the author's laboratory, confirmation of the presenilin-APP interactions in multiple experiments and evidence that the two fractionate to the same enriched vesicular fractions on gradients (196, 206) and that the subcellular distribution of C83 and C99 (the immediate substrates of the γ-secretase reaction) was not altered in mice bearing or lacking PS1 suggested that presenilin was inseparable from the γ-secretase cleavage event, i.e., that it is a physical participant rather than having an indirect role via membrane trafficking.

In a separate line of work, Wolfe and colleagues (186,

187) designed peptidomimetic transition state analogs to attempt to inhibit γ-secretase and showed that certain difluoroalcohol and difluoroketone inhibitors mimicking the $A\beta_{40-45}$ region could indeed decrease Aβ secretion and raise cellular levels of C83 and C99. The chemical nature of these inhibitors strongly suggested that the unknown γ-secretase had the properties of an aspartyl protease (187). This concept also fit with evidence from cell biological experiments that the generation of Aβ appeared to require a mildly acidic pH (e.g., Ref. 44). Moreover, cathepsin D, a well-characterized soluble aspartyl protease, was considered as a potential candidate for γ-secretase, until it was shown that deletion of the cathepsin D gene in mice did not obviate Aβ production (131).

Accumulating data suggested that presenilin was physically inseparable from the γ-secretase reaction (197), including the finding of presenilin heterodimers within isolated vesicles that can undergo Aβ generation (196), and there was evidence that γ-secretase had the properties of an aspartyl protease (187). These considerations led Wolfe et al. (188) to identify and mutate two unusual intramembranous aspartates found near the middle of the predicted TM6 and TM7 domains of all presenilins. Mutation of either of these evolutionarily conserved aspartates to alanine or glutamate and subsequent cellular expression showed that the mutant holoprotein could no longer undergo endoproteolysis, signifying an essential role for both intramembranous aspartates in this reaction. Furthermore, mutation of either TM aspartate markedly reduced $A\beta_{40}$ and $A\beta_{42}$ production and elevated the C83 and C99 substrates, in a fashion essentially indistinguishable from the earlier effects noted for PS1 gene deletion (22). When these two phenomena (inhibition of presenilin endoproteolysis and of γ-secretase cleavage) were examined together by placing an aspartate-to-alanine mutation in the natural variant of presenilin that lacks exon 9 (and therefore the site for PS1 endoproteolytic cleavage), this mutant holoprotein still abrogated γ-secretase cleavage of APP (188). The latter result indicates that even in a presenilin isoform that cannot and does not undergo endoproteolysis, the TM aspartates are still required for proper γ-secretase processing of C83 and C99. An additional experimental approach was to express either wild-type or aspartate-mutant PS1 in microsomes and demonstrate that the former allowed de novo Aβ generation from recombinantly expressed C99, whereas the latter did not, and that Aβ generation in the presence of the wild-type presenilin occurred at mildly acidic, not neutral, pH (188).

The interpretation of the results just summarized has been a matter of some controversy. One interpretation is that presenilin is required as a "diaspartyl" cofactor for γ-secretase and that mutation of either aspartate prevents that function. The alternate interpretation is that presenilin actually represents γ-secretase, a novel intramembra-

nous protease activated by autoproteolysis (188). Recent evidence consistent with either hypothesis has come from subcellular factionation experiments in which it was shown that C83 and C99, the substrates of γ-secretase, can be coprecipitated with presenilin heterodimers in Golgi- and *trans*-Golgi network-like membrane vesicles, whereas the APP holoprotein coprecipitates with presenilin holoprotein in an earlier, endoplasmic reticulum-rich vesicular compartment (193, 194). These data confirm a direct interaction of the APP γ-secretase substrates with presenilin. Furthermore, the vesicles containing such complexes have substantial steady-state levels of Aβ when presenilin is wild type but not when it contains the aspartate mutations, and new Aβ can be generated in a cell-free reaction from the former but not the latter vesicles (193). Interestingly, stable expression of the TM aspartate to alanine mutations in both PS1 and PS2 in the same cell decreases Aβ production to undetectable levels, suggesting an absolute requirement for functional presenilins (and their TM aspartates in particular) to generate any Aβ (74).

At this writing, absolute resolution of whether presenilin serves either as γ-secretase or as a necessary cofactor has not been achieved. Two types of experiments could resolve this issue. First, one could demonstrate the generation of Aβ in artificial phospholipid vesicles (liposomes) expressing only presenilin and its substrates, C99 or C83. However, it appears highly likely that presenilin and APP exist in multiprotein complexes, including the presence of the limiting cellular factor(s) that allow endoproteolysis and stabilize the fragments (169), and these other members of the reaction have not been identified. Therefore, the reconstitution experiments face two major obstacles: the potential need for one or several unknown cofactor proteins to allow the reaction to proceed, and the need to allow presenilin to fold properly into artificial membranes in a way that allows its proteolytic activity. Given the difficulty and length of time that may be needed to achieve this goal, a second and more attractive experimental approach has been undertaken. The facts that widespread screening of compounds on Aβ-secreting cells has yielded inhibitors with high potency and with all of the properties of bona fide γ-secretase inhibitors and that other such inhibitors have been rationally designed (187) have allowed identification of the cognate targets of these inhibitors. It has been shown that such compounds bind specifically and selectively to presenilin heterodimers (28a, 91a). This result is tantamount to proving that presenilin and γ-secretase are one and the same. Although it remains formally possible that an unknown protease could be so intimately associated with presenilin that the inhibitors bind to presenilin while also binding to the active site, the use of compounds that are transition state mimics and have a sequence closely resembling the substrate (the Aβ region of APP) (28a) would make it highly likely that the protein to which the compound

bound (i.e., PS) was the actual aspartyl protease. Such evidence goes a long way toward resolving the question of whether presenilin is the long-sought γ-secretase and is thus a direct target for inhibition to chronically lower brain Aβ levels. In this regard, phase I clinical trials of a γ-secretase inhibitor emerging from broad-scale screening have recently been initiated (29a).

C. Presenilin as a Key Mediator of Notch Signaling

As the work summarized in the preceding section was unfolding, further analyses of presenilin/sel-12 function in simpler organisms (e.g., *Drosophila* and *C. elegans*) led to the important insight that presenilin was required for proper Notch signaling (21, 164, 204). Signal transduction mediated by the cell surface receptors Notch in *Drosophila* and lin-12 and glp-1 in *C. elegans* has been shown to be essential for a large variety of cell fate decisions during development (for reviews, see Refs. 4, 41). The vital importance of cell-cell interactions controlled by the lin-12/Notch pathway for proper development of vertebrates and invertebrates is clear from many genetic analyses, but the biochemical mechanism by which these receptors transmit cell surface signals to the nucleus to alter expression of a variety of downstream genes in the Notch pathway has been poorly understood. It has appeared increasingly probable that signal transduction by ligand-activated Notch receptors might involve proteolytic processing of the receptor to release the intracellular domain to the nucleus (e.g., Ref. 78). Mutation of an amino acid at the putative cleavage site within or just cytoplasmic to the single TM domain of Notch markedly decreased Notch signaling in mammalian cells, thus linking intramembranous proteolysis of Notch with its function in activating transcription of nuclear genes (139). Very low concentrations of the intracellular domain of Notch (NICD) appear to reach the nucleus, making this fragment difficult to detect immunocytochemically (139). Either during or after nuclear entry of NICD, it can bind to and activate members of the CSL family of downstream Notch effectors such as CBF-1 and Su(H).

Compelling evidence that the presenilin proteins are essential participants in this cleavage event has arisen from studies of flies and mammalian cells (Fig. 3). Lethal loss of function mutations in the *Drosophila* presenilin abolish Notch signaling by preventing NICD from being released to the nucleus (164, 204). The presenililn null mutations produce a somatic and neural phenotype in the fly that is highly similar to that of flies lacking Notch. Moreover, mouse cells devoid of PS1 undergo markedly decreased proteolytic release of NICD from a Notch construct (21, 159). Peptidomimetic compounds designed to inhibit the γ-secretase processing of APP (187) show the same rank potency in inhibiting the intramembranous cleavage of Notch (21). It has recently been shown that the aspartate-mutant forms of

PS, which block γ-secretase proteolysis of C83 and C99 of APP (188), also inhibit the release of NICD and its translocation to the nucleus (8, 124). Therefore, it appears that presenilin can serve as an essential cofactor in the γ-secretase cleavage of Notch or, more likely, as the protease itself (Fig. 3). It should also be noted that complex formation between Notch and presenilin has been observed (125). It appears that Notch and presenilin can interact at or close to the cell surface, because biotinylation of each protein has been observed and biotinylated Notch can be recovered by immunoprecipitating presenilin heterodimers (124). Several other studies have also suggested a localization of at least some presenilin molecules at or close to the plasma membrane (33, 82, 141). This emerging evidence of a surface localization of mammalian presenilin fits with the fact that *Drosophila* presenilin has been reported to be detected in or very near the plasma membrane (203). Before publication of the various results just summarized, the majority of studies examining presenilin subcellular localization suggested that it resided primarily or solely in endoplasmic reticulum and early Golgi compartments (e.g., Refs. 3, 81).

D. Presenilin May Be Required for Proteolysis of Other Integral Membrane Proteins

The exciting parallels between Notch processing and APP processing with regard to the presenilins have raised the question of whether there may be other substrates that require presenilin for proteolysis and other enzymes that are intramembranous aspartyl proteases. In this regard, other members of the APP family, namely, APLP1 and APLP2, are very likely to undergo presenilin-mediated intramembranous proteolysis as part of their constitutive metabolism; CTFs of APLP2 are sharply increased in mice lacking presenilin 1 (104). One substrate possibly linked to presenilin function is Ire1, a protein that is a key sensor for the accumulation of unfolded proteins in the endoplasmic reticulum and thus initiates the unfolded protein response (UPR) pathway. Examination of Ire1 processing in cells derived from PS1 knockout mice suggested that presenilin is required for proper cleavage of Ire1, putatively within its single transmembrane domain (106). In separate experiments, AD-causing missense mutations in PS1 were reported to alter UPR signaling (103). However, a role for PS1 in Ire1 processing and the UPR has not yet been confirmed. It is likely that additional substrates will be identified, but we can already list at least five kinds of putative substrates that require presenilin for their cleavage: APP, Notch receptors, APLP1, APLP2, and perhaps presenilin itself. Various chimeric type 1 membrane proteins having heterologous transmembrane domains can undergo PS-mediated intramembranous cleavage in flies, confirming the relative lack of sequence specificity of presenilin/γ-secretase (163a). It therefore appears that presenilin is generally responsible for the proteolytic turnover of the
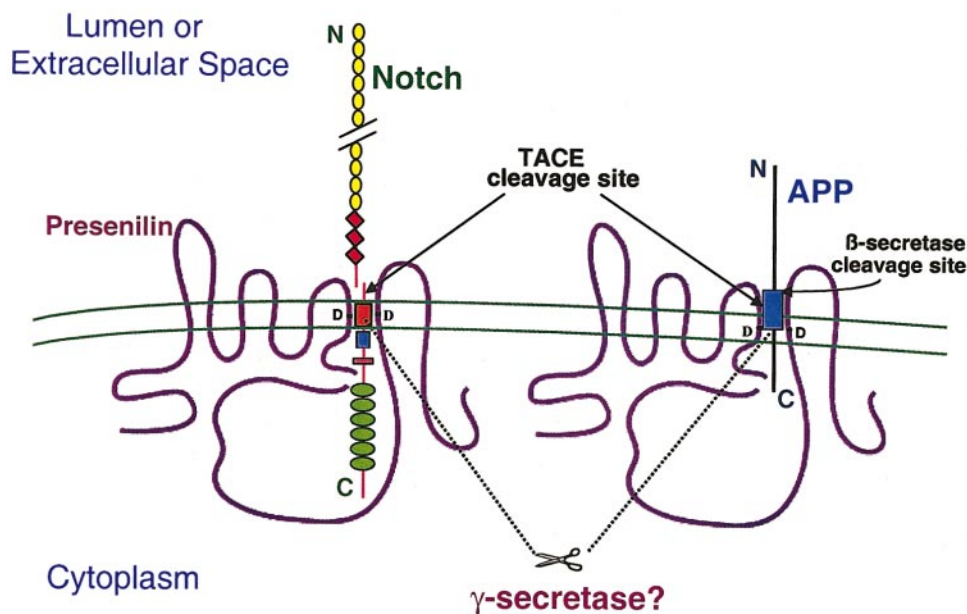
FIG. 3. Hypothetical model of the role of presenilin (PS) in Notch and APP processing based on current information. The diagram shows the predicted 8 TM domain topology of PS, which occurs principally as a cleaved heterodimer. Some Notch and APP molecules form complexes with PS. Two aspartates (D) in TM6 and TM7 of PS are required for the cleavages of Notch and APP within their TM domains, and these may align with the respective sites of cleavage in the two substrates. It is unknown whether PS directly effects these cleavages or whether a still unidentified aspartyl protease (γ-secretase) present in the complexes does so. PS-mediated proteolysis of both Notch and APP is preceded by ectodomain shedding due to tumor necrosis factor-α converting enzyme (TACE). Alternatively, APP can undergo ectodomain shedding by β-secretase. Several motifs are depicted in Notch: epidermal growth factor-like repeats (yellow circles), LNG repeats (orange diamonds), a single TM (white box), the RAM23 domain (blue square), a nuclear localization sequence (red rectangle), and 6 cdc10/ankyrin repeats (green ovals). After the putative intramembranous cleavage mediated by PS, the Notch intracellular domain is released to the nucleus to activate transcription of target genes. APP contains the Aβ region (light blue box), which is released into the lumen after sequential cleavages of APP by β-secretase and then γ-secretase/PS. The fate of the APP intracellular domain is unknown.

transmembrane domains of a variety of single-pass proteins that undergo ectodomain release.

The recent progress in Notch and presenilin biology allows one to place the emerging public health catastrophe of AD into a new perspective. It may turn out that the principal conserved function of the presenilins is to mediate the final proteolytic cleavage of the Notch receptors, thereby conferring great developmental advantages during evolution. However, the survival of large numbers of humans far beyond reproductive age due to advances such as antibiotics may have increasingly permitted a kinetically less favored substrate of this reaction (APP) to be converted to a highly stable, long-lived and self-aggregating product ($A\beta_{42}$) that can accumulate to produce progressive neurodegeneration. Quantitative biochemical comparisons of Notch and APP as binding partners and substrates of presenilin would help to support or deny this hypothesis.

I would even speculate that partial loss of function mutations in human PS1 could decrease the efficiency of APP processing to $A\beta_{42}$ (just as AD-causing gain of function mutations increase it), and that such mutations might be found in very old humans (centenarians) who show very little age-related $A\beta$ accumulation and thus have escaped AD. It would be particularly interesting to search for such "AD escapees" among the very old who carry one or two ApoE4 alleles and yet have failed to develop clinical or neuropathological evidence of AD.

VII. THE COMPLEX INFLAMMATORY AND NEUROTOXIC CASCADE OF ALZHEIMER'S DISEASE

Although many of the details of APP and presenilin biology reviewed above and the roles of these proteins in genetic forms of AD have been well confirmed, the subsequent events triggered by excessive $A\beta_{42}$ accumulation in brain regions important for memory and cognition remain the subject of intensive study and debate. A full description of the extensive information on the possible downstream events that follow $A\beta$ accumulation is beyond the scope of this review, which is focused instead on the key gene products that appear to initiate AD. However, I review here the current understanding of certain principal steps in the AD cascade that seem ultimately to lead to the loss of synapses and somata, the dendritic dystrophy, and the neurotransmitter deficits that are the proximate basis for the dementia (Fig. 4).

A. Clues to the Temporal Evolution of AD Emerge From Studies of Down's Syndrome and APP Transgenic Mice

Because brain tissue from patients with AD can only be studied at the end of the patient's life, it has been difficult to establish directly the sequence of pathogenic events in the disorder. However, powerful clues to this sequence have come from deciphering the highly similar, if not indistinguishable, neuropathological process that occurs in Down's syndrome. Because Down's patients develop their first diffuse plaques at the end of the first or the beginning of the second decade of life and yet do not show full-blown AD histopathology until the end of the third or fourth decade, young and middle-aged subjects dying with Down's syndrome can be carefully examined to attempt to establish a temporal sequence of changes (e.g., Ref. 85).

Another source of dynamic information about the disease cascade has been the study of mice transgenic for mutant human APP, either with or without coexpression of mutant presenilin. Although lesion formation occurs in a temporally compressed fashion in these mice (29b, 61), some features of the cellular and protein changes that precede and/or accompany neuritic/neuronal alteration can be deduced by careful analysis of such models. Based on studies of Down's syndrome and transgenic models as well as the age-related histopathological and biochemical changes observed in the brains of elderly humans dying without dementia, one can begin to construct a rough sequence of AD pathogenesis.

B. $A\beta_{42}$ Accumulation, Diffuse Plaques, and the Accrual of $A\beta_{40}$

The increased production of $A\beta_{42}$ documented in patients with APP and presenilin mutations (as well as in children with Down's syndrome) leads to rising levels of $A\beta_{42}$, both in interstitial fluid of the brain and probably intracellularly. With regard to the latter possibility, it has recently been shown that the neurons of young subjects with Down's syndrome as well as some neurons in aged normals and subjects with mild AD can show intraneuronal immunoreactivity for $A\beta_{42}$ (39). Indeed, stable dimers of $A\beta_{42}$ have been detected in nonneural and neural cells in culture, before their release into the medium (180). Because $A\beta_{42}$ is far more prone to aggregation into protofibrils and fibrils than $A\beta_{40}$ (53, 70, 179), $A\beta_{42}$ generally forms the earliest morphologically detectable deposits (diffuse plaques), well before the accrual of $A\beta_{40}$ on such lesions (68, 69, 85). A major unresolved question is whether activation of glial cells and injury to neurites and their cell bodies is mediated by or even requires $A\beta$ plaque formation or rather is initiated and propagated by
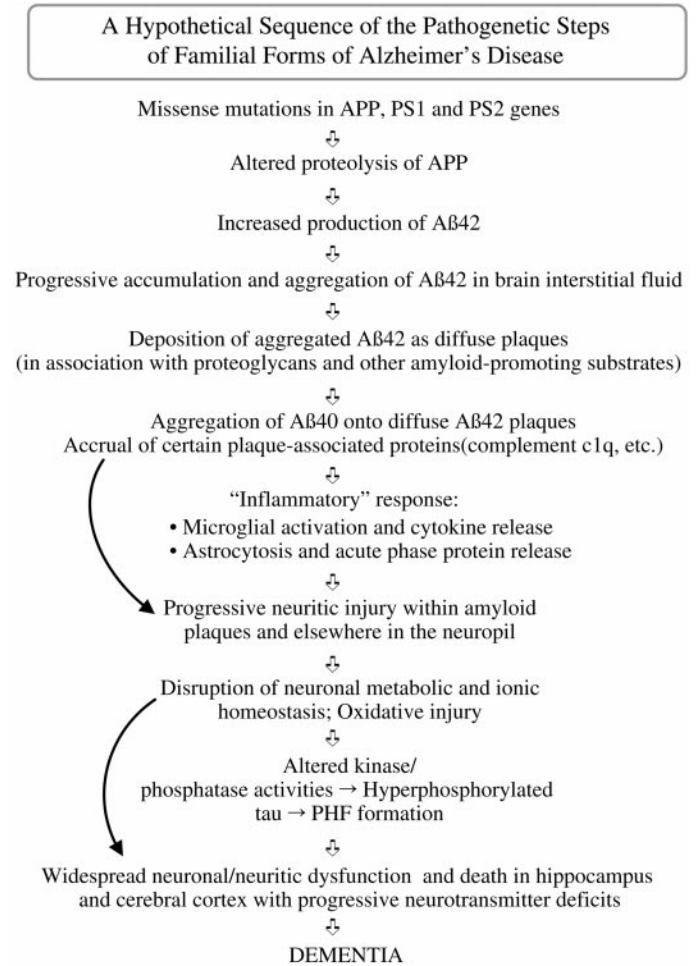
FIG. 4. A hypothetical sequence of the pathogenetic steps of familial forms of Alzheimer's disease.

small, relatively soluble and diffusible oligomeric species of $A\beta$, perhaps resembling the protofibrils found in synthetic peptide studies. There is evidence in transgenic mice expressing mutant human APP that structural changes of synapses as well as electorphysiological alterations can be detected in relatively young mice before the formation of any $A\beta_{42}$ deposits (diffuse plaques) (60). Such in vivo studies are mirrored in some respects by studies of toxicity in cultured neurons exposed to stable oligomeric species of synthetic $A\beta$ (e.g., Refs. 55, 83). One cannot yet conclude that in AD, oligomeric species of $A\beta_{42}$ (which have only been partially characterized in human brain) are able to initiate cellular dysfunction before any plaque formation; rather, it may turn out that some plaque formation is necessary to allow the cellular pathology to begin, but perhaps only because the plaques represent a substantial reservoir of aggregated $A\beta_{42}$ species that can continuously release diffusible oligomers, protofibrils, and the like to potentially activate and injure surrounding cells. Thought of in this way, plaque development may represent an invariant accompaniment of the

disease and even be necessary for its early clinical manifestations, but the plaques per se may not be the principal source of cellular injury. It would be interesting to be able to examine the progression of cytopathology in transgenic mice that develop stable $A\beta_{42}$ oligomers but never go on to form diffuse or fibrillar plaques, but this would be a difficult model to achieve.

With its substantially greater resistance to fibrillogenesis, $A\beta_{40}$ may only codeposit on preexisting $A\beta_{42}$ diffuse plaques after considerable time. In Down's syndrome, immunohistochemical studies clearly suggest that this interval may be years or perhaps even a decade or more (85). In the case of patients dying from the effects of presenilin missense mutations, the $A\beta_{42}$ plaque burden is very high, and there can sometimes be rather few $A\beta_{40}$ immunopositive plaques, raising the question of whether $A\beta_{42}$ deposition is by itself sufficient to initiate and propagate disease. Generally, however, $A\beta_{40}$ becomes another major component of senile plaques as they mature and become increasingly fibrillar.

C. The Inflammatory Process in AD

Like other aspects of research on the mechanism of AD, studies of brain inflammatory changes were at first unpopular and deemed to be unlikely to lead to an understanding of early pathogenesis. Nonetheless, the early efforts of a few investigators have been followed by a large number of studies that document a profound inflammatory disturbance in limbic and association cortices in AD. Activated microglia displaying a variety of cell surface markers that distinguish them from the resting microglia resident in the brain are often found within and immediately surrounding maturing amyloid plaques that contain at least some $A\beta$ fibrils. There is evidence that HLA-DR-immunoreactive microglia can appear in at least some diffuse plaques, presumably before the development of fibrillar, thioflavin-, and Congo red-positive mature plaques (e.g., Ref. 67). Microglia are reasonable candidates for early cellular respondents in the $A\beta$-mediated pathogenic cascade, because they represent monocyte/macrophage-derived cells in the nervous system that are capable of responding rapidly to the accrual of extracellular ("foreign") material. It is likely that microglia and astrocytes would perceive $A\beta$ oligomers and fibrils as a foreign material, because these kinds of $A\beta$ assemblies are apparently never observed during brain development and in the immature nervous system. With the assumption that microglia can become activated by $A\beta$ aggregates and/or proteins linked to them, it is likely that they participate in the triggering of the classical complement pathway. Indeed, an initial component of that pathway, C1q, has been shown to bind to $A\beta$ in vitro (127) and trigger the complement cascade, including the accrual of the membrane attack complex C5b-9 (66). Microglia could also be the source of interleukin-1β and a variety of other proinflammatory cytokines that have been detected immunohistochemicallly in AD brain sections (e.g., Refs. 42, 100). Cytokine release could in turn explain, at least in part, the prominent reactive astrocytosis that immediately surrounds many maturing, fibril-rich plaques. Such astrocytes are another likely source of a variety of inflammatory mediators, including additional cytokines and acute phase proteins such as α_1-antichymotrypsin, which is known to be intimately associated with fibrillar plaque cores (1).

The complex ways in which $A\beta$ accumulation might lead to microgliosis, astrocytosis, and the overproduction and release of various inflammatory mediators has been reviewed in detail (100, 128). Mounting evidence for early inflammatory changes seen in the brains of Down's syndrome and of APP transgenic mice (although generally less robustly than in humans) suggests that the inflammatory response may be an important mediator of subsequent neuritic/neuronal injury.

D. Free Radical Accumulation, Peroxidative Injury, and Altered Calcium Homeostasis May Mediate Neuritic/Neuronal Injury

Considerable experimental evidence suggests that the effects of $A\beta$ accumulation, including those arising from an $A\beta$-initiated inflammatory response, may include excessive generation of free radicals and peroxidative injury to proteins, lipids, and other macromolecules (e.g., Refs. 7, 54). Among the many possible metabolic consequences of progressive $A\beta$ accumulation and aggregation (whether occurring inside and/or outside neurons), altered ionic homeostasis, particularly excessive calcium entry into neurons, could well contribute to selective neuronal dysfunction and death, based on studies of the effects of aggregated $A\beta$ in culture (92, 99, 114). Two major questions about $A\beta$-mediated neuritic and neuronal injury are as follows: *1*) does the initial injury occur at the level of synapses or dendrites rather than in the cell body, and *2*) does cell injury require binding of $A\beta$ monomers or oligomers to specific cell-surface receptors or rather does it occur via a general perturbation of the plasma membrane implicating many cell surface proteins? Attempts to identify specific cell-surface molecules that could serve as bona fide $A\beta$ receptors have included implication of the receptor for advanced glycation end products (RAGE) (202), the scavenger receptors (27), and a novel $A\beta$-binding protein called BBP1 (109). However, it can be argued that neurons and other cells clearly did not evolve receptors for binding $A\beta$ oligomers/fibrils (since such are very unlikely to occur in normal biology during development), and yet it is such oligomeric or polymeric species that

appear to confer cell toxicity, not monomers. Therefore, it is reasonable to speculate that Aβ-mediated neuritic injury does not involve a specific ligand-receptor interaction in the classical sense but rather a perturbation of the plasma membrane by the hydrophobic aggregates that might alter the functional properties of a variety of cell-surface molecules. In any event, excess calcium accumulation seems one reasonable downstream mediator of Aβ-induced toxicity (99) and could explain, for example, the activation of certain kinases that could subsequently contribute to the hyperphosphorylation of tau polypeptides before their polymerization into paired helical filaments.

It is now clear from the discovery of human tau mutations that cause severe neurofibrillary degeneration (64, 160) that the accumulation of hyperphosphorylated forms of tau and the presumed dysfunction of such molecules in maintaining microtubule stabilization represent a major threat to neuronal form and function. As mentioned earlier, a particularly intriguing recent development in attempting to understand the link between Aβ accumulation, neuronal injury, and tangle formation has come from the recognition that AD neurons bearing neurofibrillary tangles accumulate a fragment of p35, the regulatory protein for the cyclin-dependent kinase 5 (cdk5), a kinase which is capable of phosphorylating tau and other proteins (111). According to these data, calpain may be activated in some neurons to cleave p35 and generate the p25 fragment that is mislocated within the cell and allows constitutive activity of cdk5 (83a). The resultant phosphorylation of various cellular substrates including tau could explain how abnormal tau accumulates and ultimately polymerizes into PHF. Whether extracellular Aβ oligomers are capable of triggering the conversion of p35 to p25 inside neurons to initiate this cascade remains to be seen. This or several other suggested mechanisms could explain the almost invariant accumulation of hyperphosphorylated tau and subsequent tangle formation that is a key cytopathological feature in affected brain regions in almost all AD subjects.

The ultimate effects of the complex inflammatory, ionic, and oxidative changes that occur in affected cortical regions is neuritic dystrophy, synaptic loss, shrinkage of neuronal perikarya, and selective neuronal loss. Presumably, these processes occur gradually over many years in the preclinical phase of AD and then continue during its clinical progression. An obvious result of such a synaptotoxic process would be the loss of cholinergic enzymes (choline acetyltransferase and acetylcholinesterase) as well as injury to a variety of other neurotransmitter and neuromodulator systems. Such losses would seriously compromise synaptic transmission and could explain the difficulty in storing new information and failure to retrieve recently stored information that characterizes the early stages of AD.

VIII. TREATING AND PREVENTING ALZHEIMER'S DISEASE

A. Remaining Questions Abound

Although the outlines of a pathogenic cascade (Fig. 4) that could explain cognitive dysfunction in AD patients are emerging, many important questions remain. One would like to know the relative contributions of extracellular and intraneuronal Aβ accumulation in potentially initiating neurotoxicity. Also, are Aβ fibrils the principal toxic moiety in the disease or, more likely, are smaller assemblies (stable but diffusable oligomers) the microglia-activating and neuron-injuring species? Is apoptosis of neurons an important part of the pathogenic cascade that, if inhibited, would slow or prevent brain dysfunction (19). Although both of the presenilins (particularly mutant presenilin 2) have been associated with enhanced apoptosis in cell culture studies (59, 189), expression of FAD mutations in presenilins by knock-in techniques in mice have not yet produced clear evidence that such mutations enhance neuronal apoptosis in vivo (152). How a putative proapoptotic function of presenilin relates to the possibility that presenilin is an aspartyl protease that processes APP, Notch, and other intramembranous substrates needs to be elucidated. The presence of innumerable tangle-bearing neurons in the AD brain that still show relatively well-preserved organelle structure by electron microscopy, coupled with evidence that mutations in tau can lead to accelerated tangle formation and ultimately neuronal death, may suggest that a gradual nonapoptotic neuronal dysfunction (i.e., the tau alteration/microtubule destabilization process) can precede neuronal death, which then might finally occur by apoptosis.

What about the selective vulnerability of neuronal populations to the AD process? Local and regional differences in the pathogenic process may arise on at least two broad levels. First, Aβ_{42} can accumulate chronically in some brain regions (e.g., cerebellum, striatum, and thalamus) with very little evolution to fibril-rich amyloid plaques and little associated neuritic and glial cytopathology. This finding suggests the possible existence of pro- or anti-aggregating factors that vary among brain regions and that enable Aβ_{42} to proceed into oligomeric forms or prevent it from doing so. Second, even in regions where abundant Aβ oligomerization/fibrillization can occur, some surrounding neurons and their processes appear to undergo few or no neurotoxic changes. This suggests some intrinsic ability on the part of certain neurons to resist Aβ-mediated cytotoxicity.

B. Potential Therapeutic Strategies

Despite these and other unresolved questions, sufficient progress in delineating the disease cascade has now

been achieved to envision several discrete targets for treatment. Inhibitors of Aβ production, that is, small compounds that cross the blood-brain barrier and decrease but do not eliminate either β- or γ-secretase activity, could be therapeutic in the early clinical phases of the disease, particularly in patients with minimal cognitive impairment, and in nondemented subjects. In the case of γ-secretase inhibitors, these could be designed to decrease Aβ production by some 30–40% or so, hopefully without interfering in a quantitatively meaningful way with Notch processing. The fact that very small amounts of the Notch intracellular fragment are apparently sufficient to activate signaling in cells (139) may mean that some decrease in Notch proteolysis can be tolerated, particularly in AD patients, in whom developmental issues are not at stake.

An alternate and attractive approach would be to use small molecules to bind Aβ monomers and prevent their assembly into potentially cytotoxic oligomers. However, if an anti-aggregating compound solely blocked amyloid fibril formation, this could actually allow increased accumulation of metastable intermediates such as oligomers and might theoretically aggravate the disease. One advantage of an anti-oligomerization strategy is that one would be targeting a purely pathological event in the disease, rather than interfering with normal metabolic reactions such as those of β- and γ-secretase.

A third general approach would be to administer anti-inflammatory drugs that could interfere with aspects of the microglial, astrocytic, and cytokine responses that occur in the AD brain. The epidemiological evidence that consumption of nonsteroidal anti-inflammatory drugs for other purposes may be associated with a somewhat lower likelihood of developing AD could potentially be explained on this basis. However, it would presumably be best to design novel compounds that interfere with one or more specific steps in the Aβ-induced inflammatory cascade in the brain, rather than relying on conventional anti-inflammatory drugs that have considerable potential toxicity, particularly in older patients.

Finally, one could use a variety of antioxidants, free radical scavengers, calcium channel blockers, and modulators of certain signal transduction pathways that might protect neurons from the downstream effects of the accumulation of Aβ intracellularly and/or extracellularly. The problem with this approach may turn out to be that there are multiple ways in which neurons respond to Aβ and the Aβ-associated inflammatory process, and blocking one or two of these might not significantly decrease overall neuronal dysfunction and loss. One could also envision the use of neurorestorative factors, e.g., neurotrophins and small compounds mimicking their actions, that might rescue synapses and cell bodies undergoing active injury. However, this approach would operate in the presence of ongoing new injury from the putative cytotoxic effects of Aβ.

An intriguing approach to lower the levels of Aβ and reduce Aβ deposits in the brain comes from a recent study in APP transgenic mice. Parenteral immunization with synthetic human Aβ peptide led to a strong humoral response and the apparent movement of some of the Aβ antibodies across the blood-brain barrier into the brain parenchyma (136). Although the mechanism remains unclear, the anti-Aβ antibody response led to enhanced clearing of Aβ deposits in mice that already had begun to develop plaques, possibly by the recruitment of local microglia. Moreover, immunization of young mice before the development of Alzheimer-type histopathology was associated with a marked inhibition of subsequent plaque formation and the associated gliosis and neuritic dystrophy. Presumably, the very high levels of Aβ antibodies induced peripherally in these mice led to a small fraction crossing the blood-brain barrier and acting centrally. No untoward antigen-antibody reaction ensued, i.e., the inflammatory cytopathology in the mouse was prevented rather than worsened. The recent initiation of human trials using this Aβ vaccination approach will be followed with great interest.

Because the success of any one of these strategies cannot be predicted and because two or more approaches might ultimately be combined, all such approaches and others not reviewed here need to be pursued. Current, largely symptomatic treatments aimed at enhancing the levels of depleted neurotransmitters, particularly acetylcholine, may continue to be useful, even if more specific treatments aimed at early steps in the disease are forthcoming.

IX. CONCLUSION

A new diagnostic and treatment paradigm is emerging from the very substantial progress in elucidating the functions and dysfunctions of gene products implicated in AD. In the future, it is likely that individuals reaching their 50s or beyond will be offered a specific risk-assessment profile to determine their likelihood of developing AD. Such an assessment, modeled on that now widely used to judge the risk of serious atherosclerotic disease, would include inquiry about a positive family history of AD or a related dementia, identification of specific predisposing genetic factors, structural and functional brain imaging to detect evidence of presymptomatic lesions, and measurement of Aβ_{42}, tau, and other markers of the neuropathology in cerebrospinal fluid and perhaps (in the case of Aβ) even in blood. On the basis of further epidemiological experience with such assessment measures in large populations of healthy elderly AD subjects, it should be possible to estimate, first crudely and later more accurately, the likelihood that an individual will develop AD. If this can be accomplished, then those at particularly high risk could be offered preventative treatments with one or

more of the agents contemplated in the previous section. Although the achievement of an integrated diagnostic and therapeutic approach to this complex and devastating disorder may seem remote, the current rate of scientific progress and the likelihood of novel clinical trials in the near future indicate that some level of practical success may come sooner than one might think.

Address for reprint requests and other correspondence: D. J. Selkoe, Center for Neurologic Diseases, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, HIM 730, Boston, MA 02115.

REFERENCES

1. ABRAHAM CR, SELKOE DJ, AND POTTER H. Immunochemical identification of the serine protease inhibitor, α_1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell* 52: 487–501, 1988.

2. ALVAREZ V, ALVAREZ R, LAHOZ CH, MARTINEZ C, PENA J, GUISASOLA M, SALAS-PUIG J, MORIS G, URIA D, MENES BB, RIBACOBA R, VIDAL JA, SANCHEZ JM, AND COTO E. Association between an alpha(2) macroglobulin DNA polymorphism and late-onset Alzheimer's disease. *Biochem Biophys Res Commun* 264: 48–50, 1999.

3. ANNAERT WG, LEVESQUE L, CRAESSAERTS K, DIERINCK I, SNELLINGS G, WESTAWAY D, ZHANG L, ST GEORGE-HYSLOP P, CORDELL B, FRASER P, AND DE STROOPER B. Presenilin 1 controls γ-secretase processing of amyloid precursor protein in pre-Golgi compartments of hippocampal neurons. *J Cell Biol* 147: 277–294, 1999.

4. ARTAVANIS-TSAKONAS S, RAND MD, AND LAKE RJ. Notch signaling: cell fate control and signal integration in development. *Science* 284: 770–776, 1999.

5. BALES KR, VERINA T, DODEL RC, DU Y, ALTSTIEL L, BENDER M, HYSLOP P, JOHNSTONE EM, LITTLE SP, CUMMINS DJ, PICCARDO P, GHETTI B, AND PAUL SM. Lack of apolipprotein E dramatically reduces amyloid β-peptide deposition. *Nature Genet* 17: 263–264, 1997.

6. BAUMEISTER R, LEIMER U, ZWECKBRONNER I, JAKUBEK C, GRUNBERG J, AND HAASS C. Human presenilin-1, but not familial Alzheimer's disease (FAD) mutants, facilitate *Caenorhabditis elegans* notch signalling independently of proteolytic processing. *Genes Function* 1: 149–159, 1997.

7. BEHL C, DAVIS JB, LESLEY R, AND SCHUBERT D. Hydrogen peroxide mediates amyloid β protein toxicity. *Cell* 77: 817–827, 1994.

8. BEREZOVSKA O, JACK AC, MCLEAN P, ASTER JC, HICKS C, XIA, W, WOLFE MS, KIMBERLY WT, WEINMASTER G, SELKOE DJ, AND HYMAN BT. Aspartate mutations in presenilin and γ-secretase inhibitors both impair Notch1 proteolysis and nuclear translocation with relative preservtion of Notch signaling. *J Neurochem* 75: 583–593, 2000.

8a. BERTRAM L, BLACKER D, MULLIN K, KEENEY D, JONES J, BASU S. YHU S, MCINNIS MG, GO RCP, VEKRELLIS K, SELKOE DJ, SAUNDERS AJ, AND TANZI RE. Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science*. In press.

9. BIERE AL, OSTASZEWSKI B, ZHAO H, GILLESPIE S, YOUNKIN SG, AND SELKOE DJ. Co-expression of β-amyloid precursor protein (βAPP) and apolipoprotein E in cell culture: analysis of βAPP processing. *Neurobiol Dis* 2: 177–187, 1995.

10. BLACKER D, WILCOX MA, LAIRD NM, RODES L, HORVATH SM, GO RC, PERRY R, WATSON B JR, BASSETT SS, MCINNIS MG, ALBERT MS, HYMAN BT, AND TANZI RE. Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nature Genet* 19: 357–360, 1998.

11. BORCHELT DR, THINAKARAN G, ECKMAN CB, LEE MK, DAVENPORT F, RATOVITSKY T, PRADA C-M, KIM G, SEEKINS S, YAGER D, SLUNT HH, WANT R, SEEGER M, LEVEY AI, GANDY SE, COPELAND NG, JENKINS NA, PRICE DL, YOUNKIN SG, AND SISODIA SS. Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1–42/1–40 ratio in vitro and in vivo. *Neuron* 17: 1005–1013, 1996.

12. BRION J, PASSAREIRO E, NUNEZ J, AND FLAMENT-DURAND J. Mise en evidence immunologique de la protein tau au niveau des lesions de degenerescence neurofibrillaire de la maladie D'Alzheimer. *Arch Biol* 95: 229–235, 1985.

13. BUSCIGLIO J, GABUZDA DH, MATSUDAIRA P, AND YANKNER BA. Generation of β-amyloid in the secretory pathway in neuronal and non-neuronal cells. *Proc Natl Acad Sci USA* 90: 2092–2096, 1993.

14. CAPELL A, GRUNBERG J, PESOLD B, DIEHLMANN A, CITRON M, NIXON R, BEYREUTHER K, SELKOE DJ, AND HAASS C. The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100- to 150-kDa molecular mass complex. *J Biol Chem* 273: 3205–3211, 1998.

15. CITRON M, WESTAWAY D, XIA W, CARLSON G, DIEHL T, LEVESQUE G, JOHNSON-WOOD K, LEE M, SEUBERT P, DAVIS A, KHOLODENKA D, MOTTER R, SHERRINGTON R, PERRY B, YAO H, STROME R, LIEBERBURG I, ROMMENS J, KIM S, SCHENK D, FRASER P, ST. GEORGE-HYSLOP P, AND SELKOE DJ. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. *Nature Med* 3: 67–72, 1997.

16. COOK DG, FORMAN MS, SUNG JC, LEIGHT S, KOLSON DL, IWATSUBO T, LEE VMY, AND DOMS RW. Alzheimer's Aβ(1—42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nature Med* 3: 1021–1023, 1997.

17. CORDER EH, SAUNDERS AM, RISCH NJ, STRITTMATTER WJ, SCHMECHEL DE, GASKELL PC JR, RIMMLER JB, LOCKE PA, CONNEALLY PM, SCHMADER KE, SMALL GW, ROSES AD, HAINES JL, AND PERICAK-VANCE MA. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer's disease. *Nature Genet* 7: 180–184, 1994.

18. CORDER EH, SAUNDERS AM, STRITTMATTER WJ, SCHMECHEL DE, GASKELL PC JR, SMALL GW, ROSES AD, HAINES JL, AND PERICAK-VANCE MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261: 921–923, 1993.

19. COTMAN CW. Apoptosis decision cascades and neuronal degeneration in Alzheimer's disease. *Neurobiol Aging* 19: S29–S32, 1998.

20. DAVIS JA, NARUSE S, CHEN H, ECKMAN C. YOUNKIN S, PRICE DL, BORCHELT DR, SISODIA SS, AND WONG PC. An Alzheimer's disease-linked PS1 variant rescues the developmental abnormalities of PS1-deficient embryos. *Neuron* 20: 603–609, 1998.

21. DE STROOPER B, ANNAERT W, CUPERS P, SAFTIG P, CRAESSAERTS K, MUMM JS, SCHROETER EH, SCHRIJVERS V, WOLFE MS, RAY WJ, GOATE A, AND KOPAN R. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 398: 518–522, 1999.

22. DE STROOPER B, SAFTIG P, CRAESSAERTS K, VANDERSTICHELE H, GUNDULA G, ANNAERT W, VON FIGURA K, AND VAN LEUVEN F. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391: 387–390, 1998.

23. DE STROOPER B, UMANS L, VAN LEUVEN F, AND VAN DEN BERGHE H. Study of the synthesis and secretion of normal and artificial mutants of murine amyloid precursor protein: cleavage of APP occurs in a late compartment of the default secretion pathway. *J Cell Biol* 121: 295–304, 1993.

24. DICKSON DW. The pathogenesis of senile plaques. *J Neuropathol Exp Neurol* 56: 321–339, 1997.

25. DODEL RC, DU Y, BALES KR, GAO F, EASTWOOD B, GLAZIER B, ZIMMER R, CORDELL B, HAKE A, EVANS R, GALLAGHER-TH0OMPSON D, THOMPSON LW, TINKLENBERG JR, PFEFFERBAUM A, SULLIVAN EV, YESAVAGE J, ALSTIEL L, GASSER T, FARLOW MR, MURPHY GM JR, AND PAUL SM. Alpha2 macroglobulin and the risk of Alzheimer's disease. *Neurology* 54: 438–442, 2000.

26. DUFF K, ECKMAN C, ZEHR C, YU X, PRADA C-M, PEREZ-TUR J, HUTTON M, BUEE L, HARIGAYA Y. YAGER D, MORGAN D, GORDON MN, HOLCOMB L, REFOLO L, ZENK B, HARDY J, AND YOUNKIN S. Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. *Nature* 383: 710–713, 1996.

27. EL KHOURY J, HICKMAN SE, THOMAS CA, CAO L, SILVERSTEIN SC, AND LOIKE JD. Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils. *Nature* 382: 716–719, 1996.

28. ESCH FS, KEIM PS, BEATTIE EC, BLACHER RW, CULWELL AR, OLTERSDORF T, MCCLURE D, AND WARD PJ. Cleavage of amyloid β-peptide during constitutive processing of its precursor. *Science* 248: 1122–1124, 1990.

28a. ESLER WP, KIMBERLY WT, OSTASZEWSKI BL, DIEHL TS, MOORE CL, TSAI J-Y, RAHMATI T, XIA W, SELKOE DJ, AND WOLFE MS. Transition-state analog inhibitors of γ-secretase bind directly to Presenilin-1. *Nat Cell Biol* 2: 428–434, 2000.

29. EVANS KC, BERGER EP, CHO C-G, WEISGRABER KH, AND LANSBURY PT

JR. Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. *Proc Natl Acad Sci USA* 92: 763–767, 1995.

29a. FELSENSTEIN KM. The next generation of AD therapeutics: the future is now (Abstract). *World Alzheimer Congress 2000: 7th Annual Conference on AD and Related Disorders,* p. 613.

29b. GAMES D, ADAMS D, ALESSANDRINI R, BARBOUR R, BERTHELETTE P, BLACKWELL C, CARR T, CLEMENS J, DONALDSON T, GILLESPIE F, GUIDO T, HAGOPIAN S, JOHNSON-WOOD K, KHAN K, LEE M, LEIBOWITZ P, LIEBERBURG I, LITTLE S, MASLIAH E, MCCONLOGUE L, MONTOYA-ZAVALA M, MUCKE L, PAGANINI L, PENNIMAN E, POWER M, SCHENK D, SEUBERT P, SNYDER B, SORIANO F, TAN H, VITALE J, WADSWORTH S, WOLOZIN B, AND ZHAO J. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* 373: 523–527, 1995.

30. GARCIA JV, FENTON BW, AND ROSNER MR. Isolation and characterization of an insulin-degrading enzyme from *Drosophila melanogaster. Biochemistry* 27: 4237–4244, 1988.

31. GEARING M, MORI H, AND MIRRA SS. Aβ-peptide length and apolipoprotein E genotype in Alzheimer's disease. *Ann Neurol* 39: 395–399, 1996.

32. GEARING M, WILSON RW, UNGER ER, SHELTON ER, CHAN HW, MASTERS CL, BEYREUTHER K, AND MIRRA SS. Amyloid precursor protein (APP) in the striatum in Alzheimer's disease: an immunohistochemical study. *J Neuropathol Exp Neurol* 52: 22–30, 1993.

33. GEORGAKOPOULOS A, MARAMBAUD P, EFTHIMIOPOULOS S, SHIOI J, CUI W, LI HC, SCHUTTE M, GORDON R, HOLSTEIN GR, MARTINELLI G, MEHTA P, FRIEDRICH VL JR, AND ROBAKIS NK. Presenilin-1 forms complexes with the cadherin/catenin cell-cell adhesion system and is recruited to intercellular and synaptic contacts. *Mol Cell* 4: 893–902, 1999.

34. GLENNER GG AND WONG CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem Biophys Res Commun* 122: 1131–1135, 1984.

35. GLENNER GG AND WONG CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* 120: 885–890, 1984.

36. GOATE A, CHARTIER-HARLIN MC, MULLAN M, BROWN J, CRAWFORD F, FIDANI L, GUIFFRA L, HAYNES A, IRVING N, JAMES L, MANT R, NEWTON P, ROOKE K, ROQUES P, TALBOT C, PERICAK-VANCE M, ROSES A, WILLIAMSON R, ROSSOR M, OWEN M, AND HARDY J. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349: 704–706, 1991.

37. GOEDERT M, TROJANOWSKI JQ, AND LEE VM-Y. The neurofibrillary pathology of Alzheimer's disease. In: *The Molecular and Genetic Basis of Neurological Disease* (2nd ed.), edited by Rosenberg RN, Prusiner SB, DiMauro S, and Barchi RL. Boston, MA: Butterworth-Heinemann, 1996, p. 613–627.

38. GOREVIC P, GONI F, PONS-ESTEL B, ALVAREZ F, PERESS R, AND FRANGIONE B. Isolation and partial characterization of neurofibrillary tangles and amyloid plaque cores in Alzheimer's disease: immunohistological studies. *J Neuropathol Exp Neurol* 45: 647–664, 1986.

39. GOURAS GK, TASI J, NASLUND J, VINCENT B, EDGAR M, GREENFIELD JP, HAROUTUNIAN V, BUXBAUM JD, XU H, GREENGARD P, AND RELKIN NR. Intraneuronal Aβ$_{42}$ accumulation in human brain. *Am J Pathol* 156: 15–20, 2000.

40. GREENBERG SM, REBECK GW, VONSATTEL JPG, GOMEZ-ISLA T, AND HYMAN BT. Apolipoprotein E ε4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann Neurol* 38: 254–259, 1995.

41. GREENWALD I. LIN-12/Notch signaling: lessons from worms and flies. *Genes Dev* 12: 1751–1762, 1998.

42. GRIFFIN WST, STANLEY LC, LING C, WHITE L, MACLEOD V, PERROT LJ, WHITE CL III, AND ARAOZ C. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci USA* 86: 7611–7615, 1989.

43. GRUNDKE-IQBAL I, IQBAL K, TUNG YC, QUINLAN M, WISNIEWSKI HM, AND BINDER LI. Abnormal phosphorylation of the microtubule-associated protein t (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA* 83: 4913–4917, 1986.

44. HAASS C, HUNG AY, SCHLOSSMACHER MG, TEPLOW DB, AND SELKOE DJ. β-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. *J Biol Chem* 268: 3021–3024, 1993.

45. HAASS C, HUNG AY, AND SELKOE DJ. Processing of β-amyloid precur-

sor protein in microglia and astrocytes favors a localization in internal vesicles over constitutive secretion. *J Neurosci* 11: 3783–3793, 1991.

46. HAASS C, HUNG AY, SELKOE DJ, AND TEPLOW DB. Mutations associated with a locus for familial Alzheimer's disease result in alternative processing of amyloid β-protein precursor. *J Biol Chem* 269: 17741–17748, 1994.

47. HAASS C, KOO EH, CAPELL A, TEPLOW DB, AND SELKOE DJ. Polarized sorting of β-amyloid precursor protein and its proteolytic products in MDCK cells is regulated by two independent signals. *J Cell Biol* 128: 537–547, 1995.

48. HAASS C, KOO EH, TEPLOW DB, AND SELKOE DJ. Polarized secretion of β-amyloid precursor protein and amyloid β-peptide in MDCK cells. *Proc Natl Acad Sci USA* 91: 1564–1568, 1994.

49. HAASS C, LEMERE CA, CAPELL A, CITRON M, SEUBERT P, SCHENK D, LANNFELT L, AND SELKOE DJ. The Swedish mutation causes early-onset Alzheimer's disease by β-secretase cleavage within the secretory pathway. *Nature Med* 1: 1291–1296, 1995.

50. HAASS C, SCHLOSSMACHER M, HUNG AY, VIGO-PELFREY C, MELLON A, OSTASZEWSKI B, LIEBERBURG I, KOO EH, SCHENK D, TEPLOW D, AND SELKOE DJ. Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature* 359: 322–325, 1992.

51. HANSEN LA, MASLIAH E, GALASKO D, AND TERRY RD. Plaque-only Alzheimer disease is usually the Lewy body variant, and vice versa. *J Neuropathol Exp Neurol* 52: 648–654, 1993.

52. HARDY J. The Alzheimer family of diseases: many etiologies, one pathogenesis? *Proc Natl Acad Sci USA* 94: 2095–2097, 1997.

53. HARPER JD, WONG SS, LIEBER CM, AND LANSBURY PT JR. Observation of metastable Aβ amyloid protofibrils by atomic force microscopy. *Chem Biol* 4: 119–125, 1997.

54. HARRIS ME, HENSLEY K, BUTTERFIELD DA, LEEDLE RA, AND CARNEY JM. Direct evidence of oxidative injury produced by the Alzheimer's beta-amyloid peptide (1—40) in cultured hippocampal neurons. *Exp Neurol* 131: 193–202, 1995.

55. HARTLEY D, WALSH DM, YE CP, DIEHL T, VASQUEZ S, VASSILEV PM, TEPLOW DB, AND SELKOE DJ. Protofibrillar intermediates of amyloid β-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J Neurosci* 19: 8876–8884, 1999.

56. HENDRIKS L, VAN DUIJN CM, CRAS P, CRUTS M, VAN HUL W, VAN HARSKAMP F, WARREN A, MCINNIS MG, ANTONARAKIS SE, MARTIN J-J, HOFMAN A, AND VAN BROECKHOVEN C. Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β-amyloid precursor protein gene. *Nature Genet* 1: 218–221, 1992.

57. HOLCOMB L, GORDON MN, MCGOWAN E, YU X, BENKOVIC S, JANTZEN P, WRIGHT K, SAAD I, MUELLER R, MORGAN D, SANDERS S, ZEHR C, O'CAMPO K, HARDY J, PRADA CM, ECKMAN C, YOUNKIN S, HSIAO K, AND DUFF K. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Med* 4: 97–100, 1998.

58. HOLTZMAN DM, BALES KR, WU S, BHAT P, PARSADANIAN M, FAGAN AM, CHANG LK, SUN Y, AND PAUL SM. Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J Clin Invest* 103: R15–R21, 1999.

59. HONG CS, CAROMILE L, NOMATA Y, MORI H, BREDESEN DE, AND KOO EH. Contrasting role of presenilin-1 and presenilin-2 in neuronal differentiation in vitro. *J Neurosci* 19: 637–643, 1999.

60. HSIA AY, MASLIAH E, MCCONLOGUE L, YU GQ, TATSUNO G, HU K, KHOLODENKO D, MALENKA RC, NICOLL RA, AND MUCKE L. Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc Natl Acad Sci USA* 96: 3228–3233, 1999.

61. HSIAO K, CHAPMAN P, NILSEN S, EKMAN C, HARIGAYA Y, YOUNKIN S, YANG F, AND COLE G. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274: 99–102, 1996.

62. HUNG AY AND SELKOE DJ. Selective ectodomain phosphorylation and regulated cleavage of β-amyloid precursor protein. *EMBO J* 13: 534–542, 1994.

63. HUSSAIN I, POWELL D, HOWLETT DR, TEW DG, MEEK TD, CHAPMAN C, GLOGER IS, MURPHY KE, SOUTHAN CD, RYAN DM, SMITH TS, SIMMONS DL, WALSH FS, DINGWALL C, AND CHRISTIE G. Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol Cell Neurosci* 14: 419–427, 1999.

64. HUTTON M, LENDON C, RIZZU P, BAKER M, FROELICH S, HOULDEN H, PICKERING-BROWN S, CHAKRAVERTY S, ISAACS A, GROVER A, HACKETT J,

ADAMSON J, LINCOLN S, DICKSON D, DAVIES P. PETERSON R, STEVENS M, DE GRAAFF E, WAUTERS E, VAN BAREN J, HILLEBRAND M, JOOSSE M, KWON J, NOWOTNY P, CHE L, NORTON J, MORRIS J, REE L, TROJANOWSKI J, BASUN H, LANNFELT L, NEYSTAT M, FAHN S, DARK F, TANNENBERG T, DODD P, HAYWARD N, KOWK J, SCHOFIELD P, ANDREADIS A, SNOWDEN J, CRAUFURD D, NEARY D, OWEN F, OOSTRA B, HARDY J, GOATE A, VAN SWIETEN, MANN D, LYNCH T, AND HUTINK P. Association of missense and 5′-splice-site mutations in tau with the inherited FTDP-17. *Nature* 393: 702–705, 1998.

65. ILLENBERGER S, ZHENG-FISCHHOFER Q, PREUSS U, STAMER K, BAUMANN K, TRINCZEK B, BIERNAT J, GODEMANN R, MANDELKOW EM, AND MANDELKOW E. The endogenous and cell cycle-dependent phosphorylation of tau protein in living cells: implications for Alzheimer's disease. *Mol Biol Cell* 9: 1495–1512, 1998.

66. ITAGAKI S, AKIYAMA H, SAITO H, AND MCGEER PL. Ultrastructural localization of complement membrane attack complex (MAC)-like immunoreactivity in brains of patients with Alzheimer's disease. *Brain Res* 645: 78–84, 1994.

67. ITAGAKI S, MCGEER PL, AKIYAMA H, ZHU S, AND SELKOE DJ. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J Neuroimmunol* 24: 173–182, 1989.

68. IWATSUBO T, MANN DM, ODAKA A, SUZUKI N, AND IHARA Y. Amyloid β protein (Aβ) deposition: Aβ42(43) precedes Aβ40 in Down syndrome. *Ann Neurol* 37: 294–299, 1995.

69. IWATSUBO T, ODAKA A, SUZUKI N, MIZUSAWA H, NUKINA H, AND IHARA Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron* 13: 45–53, 1994.

70. JARRETT JT, BERGER EP, AND LANSBURY PT JR. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 32: 4693–4697, 1993.

71. JOACHIM CL, MORRIS JH, AND SELKOE DJ. Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. *Am J Pathol* 135: 309–319, 1989.

72. KANG J, LEMAIRE HG, UNTERBECK A, SALBAUM JM, MSTERS CL, GRZESCHIK K-H, MULTHAUP G, BEYREUTHER K, AND MULLER-HILL B. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 325: 733–736, 1987.

73. KIM TW, PETTINGELL WH, HALLMARK OG, MOIR RD, WASCO W, AND TANZI RE. Endoproteolytic cleavage and proteasomal degradation of presenilin 2 in transfected cells. *J Biol Chem* 272: 11006–11010, 1997.

74. KIMBERLY WT, XIA W, RAHMATI R, WOLFE MS, AND SELKOE DJ. The transmembrane aspartates in presenilin 1 and 2 are obligatory for γ-secretase activity and amyloid ß-protein generation. *J Biol Chem* 275: 3173–3178, 2000.

75. KONDO J, HONDA T, MORI H, HAMADA Y, MIURA R, OGAWARA M, AND IHARA Y. The carboxyl third of tau is tightly bound to paired helical filaments. *Neuron* 1: 827–834, 1988.

76. KOO EH, SISODIA SS, ARCHER DA, MARTIN LJ, WEIDEMANN A, BEYREUTHER K, MASTERS CL, FISCHER P, AND PRICE DL. Precursor of amyloid protein in Alzheimer's disease undergoes fast anterograde axonal transport. *Proc Natl Acad Sci USA* 87: 1561–1565, 1990.

77. KOO EH AND SQUAZZO S. Evidence that production and release of amyloid β-protein involves the endocytic pathway. *J Biol Chem* 269: 17386–17389, 1994.

78. KOPAN R, SCHROETER EH, WEINTRAUB H, AND NYE JS. Signal transduction by activated Notch: importance of proteolytic processing and its regulation by the extracellular domain. *Proc Natl Acad Sci USA* 93: 1683–1688, 1996.

79. KOSIK KS, JOACHIM CL, AND SELKOE DJ. Microtubule-associated protein, tau, is a major antigenic component of paired helical filaments in Alzheimer's disease. *Proc Natl Acad Sci USA* 83: 4044–4048, 1986.

80. KOSIK KS, ORECCHIO LD, BINDER L, TROJANOWSKI JQ, LEE VM-Y, AND LEE G. Epitopes that span the tau molecule are shared with paired helical filaments. *Neuron* 1: 817–825, 1988.

81. KOVACS DM, FAUSETT HJ, PAGE KJ, KIM T-W, MORI RD, MERRIAM DE, HOLLISTER RD, HALLMARK OG, MANCINI R, FELSENSTEIN KM, HYMAN BT, TANZI RE, AND WASCO W. Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nature Med* 2: 224–229, 1996.

82. LAH JJ, HEILMAN CJ, NASH NR, REES HD, YI H, COUNTS SE, AND LEVEY AI. Light and electron microscopic localization of presenilin-1 in primate brain. *J Neurosci* 17: 1971–1980, 1997.

83. LAMBERT MP, BARLOW AK, CHROMY BA, EDWARDS C, FREED R, IOSATOS M, MORGAN TE, ROZOVSKY I, TROMMER B, VIOLA KL, WALS P, ZHANG C, FINCH CE, KRAFFT GA, AND KLEIN WL. Diffusible, nonfribrillar ligands derived from Aβ$_{1–42}$ are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA* 95: 6448–6453, 1998.

83a. LEE M-S, KWON YT, LI M, PENG J, FRIEDLANDER RM, AND TSAI L-H. Neurotoxicity induces cleavage of p35 to p25 by calpain. Nature 405: 360–364, 2000.

84. LEE VMY, BALIN BJ, OTVOS L, AND TROJANOWSKI JQ. A68: a major subunit of paired helical filaments and derivatized forms of normal tau. *Science* 251: 675–678, 1991.

85. LEMERE CA, BLUSTZJAN JK, YAMAGUCHI H, WISNIEWSKI T, SAIDO TC, AND SELKOE DJ. Sequence of deposition of heterogeneous amyloid β-peptides and Apo E in Down syndrome: implications for initial events in amyloid plaque formation. *Neurobiol Dis* 3: 16–32, 1996.

86. LEMERE CA, LOPERA F, KOSIK KS, LENDON CL, OSSA J, SAIDO TC, YAMAGUCHI H, RUIZ A, MARTINEZ A, MADRIGAL L, HINCAPIE L, ARANGO L JC, ANTHONY DC, KOO EH, GOATE AM, SELKOE DJ, AND ARANGO V JC. The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. *Nature Med* 2: 1146–1150, 1996.

87. LEVITAN D, DOYLE TG, BROUSSEAU D, LEE MK, THINAKARAN G, SLUNT HH, SISODIA SS, AND GREENWALD I. Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 93: 14940–14944, 1996.

88. LEVITAN D AND GREENWALD I. Facilitation of *lin-12*-mediated signalling by *sel-12*, a *Caenorhabditis elegans S182* Alzheimer's disease gene. *Nature* 377: 351–354, 1995.

89. LEVY E, CARMAN MD, FERNANDEZ-MADRID IJ, POWER MD< LIEBERBURG I, VAN DUINEN SG, BOTSGTAM, LUYENDIJK W, AND FRANGIONE B. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. *Science* 248: 1124–1126, 1990.

90. LEVY-LAHAD E, WASCO W, POORKAJ P, ROMANO DM, OSHIMA J, PETTINGELL H, YU C, JONDRO PD, SCHMIDT SD, WANG K, CROWLEY AC, FU Y-H, GUENTETTE SY, GALAS D, NEMENS E, WIJSMAN EM, BIRD TD, SCHELLENBERG GD, AND TANZI RE. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 269: 973–977, 1995.

91. LI X AND GREENWALD I. Additional evidence for an eight-transmembrane-domain topology for *Caenorhabditis elegans* and human presenilins. *Proc Natl Acad Sci USA* 95: 7109–7114, 1998.

91a. LI Y-M, XU M, AND LAI M-T. Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* 405: 689–694, 2000.

92. LORENZO A AND YANKNER B. β-Amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. *Proc Natl Acad Sci USA* 91: 12243–12247, 1994.

93. MA J, YEE A, BREWER HB JR, DAS S, AND POTTER H. The amyloid-associated proteins α$_1$-antichymotrypsin and apolipoprotein E promote the assembly of the Alzheimer β-protein into filaments. *Nature* 372: 92–94, 1994.

94. MANN DM, IWATSUBO T, FUKUMOTO H, IHARA Y, ODAKA A, AND SUZUKI N. Microglial cells and amyloid beta protein (A beta) deposition: association with A beta 40-containing plaques. *Acta Neuropathol* 90: 472–477, 1995.

95. MANN DMA, IWATSUBO T, CAIRNS NJ, LANTOS PL, NOCHLIN D, SUMI SM, BIRD TD, POORKAJ P, HARDY J, HUTTON M, PRIHAR G, CROOK R, ROSSOR MN, AND HALTIA M. Amyloid beta protein (A-beta) deposition in chromosome 14-linked Alzheimer's disease: predominance of A-beta [42(43)]. *Ann Neurol* 40: 149–156, 1996.

96. MASTERS CL, SIMMS G, WEINMAN NA, MULTHAUP G, MCDONALD BL, AND BEYREUTHER K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci USA* 82: 4245–4249, 1985.

97. MATSUO ES, SHIN RW, BILLINGSLEY ML, VAN DEVOORDE A, O'CONNOR M, TROJANOWSKI JQ, AND LEE VM. Biopsy-derived adult human brain tau is phosphorylated at many of the same sites as Alzheimer's disease paired helical filament tau. *Neuron* 13: 989–1002, 1994.

98. MATTSON M, CHENG B, CULWELL A, ESCH F, LIEBERBURG I, AND RYDEL R. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the β-amyloid precursor protein. *Neuron* 10: 243–254, 1993.

99. MATTSON MP, CHENG B, DAVIS D, BRYANT K, LIEBERBURG I, AND RYDEL RE. β-Amyloid peptides destabilize calcium homeostasis and ren-

der human cortical neurons vulnerable to excitotoxicity. *J Neurosci* 12: 379–389, 1992.

100. McGeer PL and McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res Rev* 21: 195–218, 1995.

101. Motter R, Vigo-Pelfrey C, Kholodenko D, Barbour R, Johnson-Wood K, Galasko D, Chang L, Miller B, Clark C, Green R, Olson D, Southwick P, Wolfert R, Munroe B, Lieberburg I, Seubert P, and Schenk D. Reduction of beta-amyloid peptide 42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 38: 643–648, 1995.

102. Myllykangas L, Polvikoski T, Sulkava R, Verkkoniemi A, Crook R, Tienari PJ, Pusa AK, Niinisto L, O'Brien P, Kontula K, Hardy J, Haltia M, and Perez-Tur J. Genetic association of alpha$_2$-macroglobulin with Alzheimer's disease in a Finnish elderly population. *Ann Neurol* 46: 382–390, 1999.

103. Namba Y, Tomonaga M, Kawasaki H, Otomo E, and Ikeda K. Apolipoprotein E immunoreactivity in cerebral deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jacob disease. *Brain Res* 541: 163–166, 1991.

104. Naruse S, Thinakaran G, Luo JJ, Kusiak W, Tomita T, Iwatsubo T, Qian X, Ginty DD, Price DL, Borchelt DR, Wong PC, and Sisodia SS. Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron* 21: 1213–1221, 1998.

105. Nathan BP, Bellosta S, Sanan DA, Weisgraber KH, Mahley RW, and Pitas RE. Differential effects of apolipoprotein E3 and E4 on neuronal growth in vitro. *Science* 264: 850–852, 1994.

106. Niwa M, Sidrauski C, Kaufman RJ, and Walter P. A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. *Cell* 99: 691–702, 1999.

107. Nukina N and Ihara Y. One of the antigenic determinants of paired helical filaments is related to tau protein. *J Biochem* 99: 1541–1544, 1986.

108. Oltersdorf T, Ward PJ, Henriksson T, Beattie EC, Neve R, Lieberburg I, and Fritz LC. The Alzheimer amyloid precursor protein. Identification of a stable intermediate in the biosynthetic/degradative pathway. *J Biol Chem* 265: 4492–4497, 1990.

109. Ozenberger BA, Lo CF, Kalkowski EM, Walker S, Smith SC, Wood A, Bard J, and Jacobsen JS. The β-amyloid binding protein BBP1 mediates cellular vulnerability to aβ by a G protein and caspase-dependent mechanism *Soc Neurosci Abstr* 1561, 1999.

110. Paris D, Town T, Parker TA, Tan J, Humphrey J, Crawford F, and Mullan M. Inhibition of Alzheimer's beta-amyloid induced vasoactivity and proinflammatory response in microglia by a cGMP-dependent mechanism. *Exp Neurol* 157: 211–221, 1999.

111. Patrick GN, Zukerberg L, Nikolic M, De La Monte S, Dikkes P, and Tsai LH. Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* 402: 615–622, 1999.

112. Perez RG, Soriano S, Hayes JD, Ostaszewski BL, Xia W. Selkoe DJ, Chen X, Stokin GB, and Koo EH. Mutagenesis identifies new signals for β-amyloid precursor protein endocytosis, turnover and the generation of secreted fragments, including A42. *J Biol Chem* 274: 18851–18856, 1999.

113. Perez RG, Zheng H, Van der Ploeg LH, and Koo EH. The beta-amyloid precursor protein of Alzheimer's disease enhances neuron viability and modulates neuronal polarity. *J Neurosci* 17: 9407–9414, 1997.

114. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, and Cotman CW. Neurodegeneration induced by β-amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci* 13: 1676–1687, 1993.

115. Podlisny MB, Citron M, Amarante P, Sherrington R, Xia W. Zhang J, Diehl T, Levesque G, Fraser P, Haass C, Koo EHM, Seubert P, St George-Hyslop P, Teplow DB, and Selkoe DJ. Presenilin proteins undergo heterogeneous endoproteolysis between Thr$_{291}$ and Ala$_{299}$ and occur as stable N- and C-terminal fragments in normal and Alzheimer brain tissue. *Neurobiol Dis* 3: 325–337, 1997.

116. Poduslo JF, Curran GL, Haggard JJ, Biere AL, and Selkoe DJ. Permeability and residual plasma volume of human, Dutch variant, and rat amyloid beta-protein 1–40 at the blood-brain barrier. *Neurobiol Dis* 4: 27–34, 1997.

117. Poduslo JF, Curran GL, Sanyal B, and Selkoe DJ. Receptor-mediated transport of human amyloid beta-protein 1–40 and 1–42 at the blood-brain barrier. *Neurobiol Dis* 6: 190–199, 1999.

118. Polvikoski T, Sulkava R, Haltia M, Kainulainen K, Vuorio A,

119. Verkkoniemi A, Niinisto L, Halonen P, and Kontula K. Apolipoprotein E, dementia, and cortical deposition of β-amyloid protein. *N Engl J Med* 333: 1242–1247, 1995.

119. Prasher VP, Farrer MJ, Kessling AM, Fisher EM, West RJ, Barber PC, and Butler AC. Molecular mapping of Alzheimer-type dementia in Down's syndrome. *Ann Neurol* 43: 380–383, 1998.

120. Probst A, Anderton BH, Brion JP, and Ulrich J. Senile plaque neurites fail to demonstrate anti-paired helical filament and anti-microtubule-associated protein-tau immunoreactive proteins in the absence of neurofibrillary tangles in the neocortex. *Acta Neuropathol* 77: 430–436, 1989.

121. Qian S, Jiang P, Guan XM, Singh G, Trumbauer ME, Yu H, Chen HY, Van de Ploeg LH, and Zheng H. Mutant human presenilin 1 protects presenilin 1 null mouse against embryonic lethality and elevates Aβ1–42/43 expression. *Neuron* 20: 611–617, 1998.

122. Qiu WQ, Ferreira A, Miller C, Koo EH, and Selkoe DJ. Cell-surface β-amyloid precursor protein stimulates neurite outgrowth of hippocampal neurons in an isoform-dependent manner. *J Neurosci* 15: 2157–2167, 1995.

123. Ratovitski T, Slunt HH, Thinakaran G, Price DL, Sisodia SS, and Borchelt DR. Endoproteolytic processing and stabilization of wild-type and mutant presenilin. *J Biol Chem* 272: 24536–24541, 1997.

124. Ray WJ, Yao M, Mumm J, Schroeter EH, Saftig P, Wolfe M, Selkoe DJ, Kopan R, and Goate AM. Cell surface presenilin-1 participates in the gamma-secretase-like proteolysis of notch. *J Biol Chem* 274: 36801–36807, 1999.

125. Ray WJ, Yao M, Nowotny P, Mumm J, Zhang W, Wu JY, Kopan R, and Goate AM. Evidence for a physical interaction between presenilin and Notch. *Proc Natl Acad Sci USA* 96: 3263–3268, 1999.

126. Rebeck GW, Reiter JS, Strickland DK, and Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* 11: 575–580, 1993.

127. Rogers J, Cooper NR, Webster S, Schultz J, McGeer PL, Styren SD, Civin WH, Brachova L, Bradt B, Ward P, and Lieberburg I. Complement activation by β-amyloid in Alzheimer disease. *Proc Natl Acad Sci USA* 89: 10016–10020, 1992.

128. Rogers J, Webster S, Lue L-F, Brachova L, Civin WH, Emmerling M, Shivers B, Walker D, and McGeer P. Inflammation and Alzheimer's disease pathogenesis. *Neurobiol Aging* 17: 681–686, 1996.

128a. Roher AE, Gowing E, Woods AS, Cotter RJ, Chaney M, Little SP, and Ball MJ. Chemical characterization of Aβ 17–42 peptide: a component of diffuse amyloid deposits of Alzheimer disease. *J Biol Chem* 269: 10987–10990, 1994.

129. Romas SN, Mayeux R, Rabinowitz D, Tang MX, Zadroga HR, Lantigua R, Medrano M, Tycko B, and Knowles JA. The deletion polymorphism and Val1000Ile in alpha-2-macroglobulin and Alzheimer disease in Caribbean Hispanics. *Neurosci Lett* 279: 133–136, 2000.

130. Rosen DR, Martin-Morris L, Luo L, and White K. A *Drosophila* gene encoding a protein resembling the human β-amyloid precursor protein. *Proc Natl Acad Sci USA* 86: 2478–2482, 1989.

131. Saftig P, Peters C, von Figura K, Craessaerts K, Van Leuven F, and De Strooper B. Amyloidogenic processing of human amyloid precursor protein in hippocampal neurons devoid of Cathepsin D. *J Biol Chem* 271: 27241–27244, 1996.

132. Saitoh T, Sundsmo M, Roch JM, Kimura N, Cole G, Schubert D, Oltersdorf T, and Schenk DB. Secreted form of amyloid β protein precursor is involved in the growth regulation of fibroblasts. *Cell* 58: 615–622, 1989.

133. Sambamurti K, Shioi J, Anderson JP, Pappolla MA, and Robakis NK. Evidence for intracellular cleavage of the Alzheimer's amyloid precursor in PC12 cells. *J Neurosci Res* 33: 319–329, 1992.

134. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-Machlachlan DR, Alberts MJ, Hulette C, Crain B, Goldgaber D, and Roses AD. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 43: 1467–1472, 1993.

135. Schellenberg GD, Bird TD, Wijsman EM, Orr HT, Anderson L, Nemens E, White JA, Bonnycastle L, Weber JL, Alonso ME, Potter H, Heston LH, and Martin GM. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* 258: 668–671, 1992.

136. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao

Z, LIEBERBURG I, MOTTER R, MUTTER L, SORIANO F, SHOPP G, VASQUEZ N, VENDEVERT C, WOGULIS SM, YEDNOCK T, GAMES D, AND SUEBERT P. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400: 173–177, 1999.

137. SCHEUNER D, ECKMAN C, JENSEN M, SONG X, CITRON M, SUZUKI N, BIRD TD, HARDY J, HUTTON M, KUKULL W, LARSON E, LEVY-LAHAD E, VIITANEN M, PESKIND E, POORKAJ P, SCHELLENBERG G, TANZI R, WASCO W, LANNFELT L, DELKOE DJ, AND YOUNKIN S. 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. *Nature Med* 2: 864–870, 1996.

138. SCHMECHEL DE, SAUNDERS AM, STRITTMATTER WJ, CRAIN BJ, HULETTE CM, JOO SH, PERICAK-VANCE M, GOLDGABER D, AND ROSES AD. Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci USA* 90: 9649–9653, 1993.

139. SCHROETER EH, KISSLINGER JA, AND KOPAN R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* 393: 382–386, 1998.

140. SCHUBERT D, JIN LW, SAITOH T, AND COLE G. The regulation of amyloid β protein precursor secretion and its modulatory role in cell adhesion. *Neuron* 3: 689–694, 1989.

141. SCHWARZMAN AL, SINGH N, TSIPER M, GREGORI L, DRANOVSKY A, VITEK MP, GLABE CG, ST. GEORGE-HYSLOP PH, AND GOLDGABER D. Endogenous presenilin 1 redistributes to the surface of lamellipodia upon adhesion of Jurkat cells to a collagen matrix. *Proc Natl Acad Sci USA* 96: 7932–7937, 1999.

142. SELKOE DJ. Alzheimer's disease: genotypes, phenotype, and treatments. *Science* 275: 630–631, 1997.

143. SELKOE DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* 399: A23–A31, 1999.

144. SELKOE DJ, ABRAHAM CR, PODLISNY MB, AND DUFFY LK. Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. *J Neurochem* 146: 1820–1834, 1986.

145. SELKOE DJ, IHARA Y, AND SALAZAR F. Alzheimer's disease: insolubility of partially purified helical filaments in sodium dodecyl sulfate and urea. *Science* 215: 1243–1245, 1982.

146. SELKOE DJ, PODLISNY MB, JOACHIM CL, VICKERS EA, LEE G, FRITZ LC, AND OLTERSDORF T. β-Amyloid precursor protein of Alzheimer disease occurs as 110–135 kilodalton membrane-associated proteins in neural and nonneural tissues. *Proc Natl Acad Sci USA* 85: 7341–7345, 1988.

147. SEUBERT P, OLTERSDORF T, LEE MG, BARBOUR R, BLOMQIST, DAVIS DL, BRYANT K, FRITZ LC, GALASKO D, THAI LJ, LIEBERBURG I, AND SCHENK DB. Secretion of β-amyloid precursor protein cleaved at the amino-terminus of the β-amyloid peptide. *Nature* 361: 260–263, 1993.

148. SEUBERT P, VIGO-PELFREY C, ESCH F, LEE M, DOVEY H, DAVIS D, SINHA S, SCHLOSSMACHER MG, WHALEY J, SWINDLEHURST C, MCCORMACK R, WOLFERT R, SELKOE DJ, LIEBERBURG I, AND SCHENK D. Isolation and quantitation of soluble Alzheimer's β-peptide from biological fluids. *Nature* 359: 325–327, 1992.

149. SHEN J, BRONSON RT, CHEN DF, XIA W, SELKOE DJ, AND TONEGAWA S. Skeletal and CNS defects in presenilin-1 deficient mice. *Cell* 89: 629–639, 1997.

150. SHERRINGTON R, ROGAEV EI, LIANG Y, ROGAEVA EA, LEVESQUE G, IKEDA M, CHI H, LIN C, LI G, HOLMAN K, TSUDA T, MAR L, FONCIN J-F, BRUNI AC, MONTESI MP, SORBI S, RAINERO I, PINESSI L, NEE L, CHUMAKOV I, POLLEN DA, ROSES AD, FRASER PE, ROMMENS JM, AND ST. GEORGE-HYSLOP PH. Cloning of a novel gene bearing missense mutations in early onset familial Alzheimer disease. *Nature* 375: 754–760, 1995.

151. SHOJI M, GOLDE TE, GHISO J, CHEUNG TT, ESTUS S, SHAFFER LM, CAI X, MCKAY DM, TINTNER R, FRANGIONE B, AND YOUNKIN SG. Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science* 258: 126–129, 1992.

152. SIMAN R, REAUME A, SAVAGE MJ, SCOTT RW, AND FLOOD DG. Presenilin 1 P264L knock-in mutation: effect on cortical neuronal vulnerability to degeneration. *J Neurosci Abstr* 25: 1046, 1999.

153. SINHA S, ANDERSON JP, BARBOUR R, BASI GS, CACCAVELLO R, DAVIS D, DOAN M, DOVEY HF, FRIGON N, HONG J, JACOBSON-CROAK K, JEWETT N, KEIM P, KNOPS J, LIEBERBURG I, POWER M, TAN H, TATSUNO G, TUNG J, SCHENK D, SEUBERT P, SUOMENSAARI SM, WANG S. WALKER D, AND JOHN V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 402: 537–540, 1999.

154. SINHA S, DOVEY HF, SEUBERT P, WARD PJ, BALCHER RW, BLABER M, BRADSHAW RA, ARICI M, MOBLEY WC, AND LIEBERBURG I. The protease inhibitory properties of the Alzheimer's β-amyloid precursor protein. *J Biol Chem* 265: 8983–8985, 1990.

155. SISODIA SS. β-Amyloid precursor protein cleavage by a membrane-bound protease. *Proc Natl Acad Sci USA* 89: 6075–6079, 1992.

156. SISODIA SS, KOO EH, BEYREUTHER K, UNTERBECK A, AND PRICE DL. Evidence that β-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* 248: 492–495, 1990.

157. SLUNT HH, THINAKARAN G, VON KOCH C, LO ACY, TANZI RE, AND SISODIA SS. Expression of a ubiquitous, cross-reactive homologue of the mouse β-amyloid precursor protein (APP). *J Biol Chem* 269: 2637–2644, 1994.

158. SMITH RP, HIGUCHI DA, AND BROZE GJ JR. Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. *Science* 248: 1126–1128, 1990.

159. SONG W, NADEAU P, YUAN M, YANG X, SHEN J, AND YANKNER BA. Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. *Proc Natl Acad Sci USA* 96: 6959–6963, 1999.

160. SPILLANTINI MG, MURRELL JR, GOEDERT M, FARLOW MR, KLUG A, AND GHETTI B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci USA* 95: 7737–7741, 1998.

161. STEINER H, CAPELL A, PESOLD B, CITRON M, KLOETZEK PM, SELKOE DJ, ROMIG H, MANDLA K, AND HAASS C. Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation. *J Biol Chem* 273: 32322–32331, 1998.

162. STEINER H, ROMIG H, PESOLD B, PHILIPP U, BAADER M, CITRON M, LOETSCHER H, JACOBSEN H, AND HAASS C. Amyloidogenic function of the Alzheimer's disease-associated presenilin 1 in the absence of endoproteolysis. *Biochemistry* 38: 14600–14605, 1999.

163. STRITTMATTER WJ, SAUNDERS AM, SCHMECHEL D, PERICAK-VANCE M, ENGHILD J, SALVESEN GS, AND ROSES AD. Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* 90: 1977–1981, 1993.

163a. STRUHL G AND ADACHI A. Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. *Mol Cell* 6: 625–636, 2000.

164. STRUHL G AND GREENWALD I. Presenilin is required for activity and nuclear access of Notch in *Drosophila*. *Nature* 398: 522–525, 1999.

165. SUZUKI N, IWATSUBO T, ODAKA A, ISHIBASHI Y, KITADA C, AND IHARA Y. High tissue content of soluble $\beta 1$–40 is linked to cerebral amyloid angiopathy. *Am J Pathol* 145: 452–460, 1994.

166. TAGLIAVINI F, GIACCONE G, FRANGIONE B, AND BUGIANI O. Preamyloid deposits in the cerebral cortex of patients with Alzheimer's disease and nondemented individuals. *Neurosci Lett* 93: 191–196, 1988.

167. TANZI RE, MCCLATCHEY AI, LAMPERTI ED, VILLA-KOMAROFF L, GUSELLA JF, AND NEVE RL. Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature* 331: 528–532, 1988.

168. TERRY RD, HANSEN LA, DETERESA R, DAVIES P, TOBIAS H, AND KATZMAN R. Senile dementia of the Alzheimer type without neocortical neurofibrillary tangles. *J Neuropath Exp Neurol* 46: 262–268, 1987.

169. THINAKARAN G, BORCHELT DR, LEE MK, SLUNT HH, SPITZER L, KIM G, RATOVITSKY T, DAVENPORT F, NORDSTEDT C, SEEGER M, HARDY J, LEVEY AI, GANDY SE, JENKINS NA, COPELAND NG, PRICE DL, AND SISODIA SS. Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* 17: 181–190, 1996.

170. THINAKARAN G, HARRIS CL, RATOVITSKI T, DAVENPORT F, SLUNT HH, PRICE DL, BORCHELT DR, AND SISODIA SS. Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *J Biol Chem* 272: 28415–28422, 1997.

171. THINAKARAN G, REGARD JB, BOUTON CML, HARRIS CL, PRICE DL, BORCHELT DR, AND SISODIA SS. Stable association of presenilin derivatives and absence of presenilin interactions with APP. *Neurobiol Dis* 4: 438–453, 1998.

172. THOMAS T, THOMAS G, MCLENDON C, SUTTON T, AND MULLAN M. β-Amyloid-mediated vasoactivity and vascular endothelial damage. *Nature* 380: 168–171, 1996.

173. TOKUDA T, FUKUSHIMA T, IKEDA S, SEKIJIMA Y, SHOJI S, YANAGISAWA N, AND TAMOAKA A. Plasma levels of amyloid beta proteins A$\beta 1$–40 and

Aβ1–42(43) are elevated in Down's syndrome. *Ann Neurol* 41: 271–273, 1997.

174. TOMITA T, MARUYAMA K, SAIDO TC, KUME H, SHINOZAKI K, TOKUHIRO S, CAPELL A. WALTER J, GRUNBERG J, HAASS C. IWATSUBO T, AND OBATA K. The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid β protein ending at the 42nd (or 43rd) residue. *Proc Natl Acad Sci USA* 94: 2025–2030, 1997.

175. TOMITA T, TOKUHIRO S, HASHIMOTO T, AIBA K, SAIDO TC, MARUYAMA K, AND IWATSUBO T. Molecular dissection of domains in mutant presenilin 2 that mediate overproduction of amyloidogenic forms of amyloid beta peptides. Inability of truncated forms of PS2 with familial Alzheimer's disease mutation to increase secretion of Abeta42. *J Biol Chem* 273: 21153–21160, 1998.

176. TROJANOWSKI JQ AND LEE VM. Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: focusing on phosphatases. *FASEB J* 9: 1570–1576, 1995.

177. VASSAR R, BENNETT BD, BABU-KHAN S, KHAN S, MENDIAZ EA, DENIS P, TEPLOW DB, ROSS S, AMARANTE P, LOELOFF R, LUO Y, FISHER S, FULLER J, EDENSON S, LILE J, JAROSINSKI MA, BIERE AL, CURRAN E, BURGESS T, LOUIS JC, COLLINS F, TREANOR J, ROGERS G, AND CITRON M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286: 735–741, 1999.

178. VERBEEK MM, VINTERS H, AND DE WAAL RM. *Cerebrovascular Amyloidosis in Alzheimer's Disease and Related Disorders*. Amsterdam: Kluwer, 2000. In press.

179. WALSH DM, LOMAKIN A, BENEDEK GB, MAGGIO JE, CONDRON MM, AND TEPLOW DB. Amyloid β-protein fibrillogenesis: detection of a protofibrillar intermediate. *J Biol Chem* 272: 22364–22374, 1997.

180. WALSH DM, TSENG BP, RYDEL RE, PODLISNY MB, AND SELKOE DJ. The oligomerization of amyloid β-protein begins intracellularly in cells derived from human brain. *Biochemistry* 39: 10831–10839, 2000.

181. WALTER J, CAPELL A, HUNG AY, LANGEN H, SCHNOLZER M, THINKARAN G, SISODIA SS, SELKOE DJ, AND HAASS C. Ectodomain phosphorylation of β-amyloid precursor protein at two distinct cellular locations. *J Biol Chem* 272: 1896–1903, 1997.

182. WASCO W, BUPP K, MAGENDANTZ M, GUSELLA J, TANZI RE, AND SOLOMON F. Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid β-protein precursor. *Proc Natl Acad Sci USA* 89: 10758–10762, 1992.

183. WEIDEMANN A, KONIG G, BUNKE D, FISCHER P, SALBAUM JM, MASTERS CL, AND BEYREUTHER K. Identification, biogenesis and localization of precursors of Alzheimer's disease A4 amyloid protein. *Cell* 57: 115–126, 1989.

184. WEIDEMANN A, PALIGA K, DURRWANG U, CZECH C, EVIN G, MASTERS CL, AND BEYREUTHER K. Formation of stable complexes between two Alzheimer's disease gene products: presenilin-2 and β-amyloid precursor protein. *Nature Med* 3: 328–332, 1997.

185. WISCHIK CM, NOVAK M, THOGERSEN HC, EDWARDS PC, RUNSWICK MJ, JAKES R, WALKER JE, MILSTEIN C, ROTHER M, AND KLUG A. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer's disease. *Proc Natl Acad Sci USA* 85: 4506–4510, 1988.

186. WOLFE MS, CITRON M, DIEHL TS, XIA W. DONKOR IO, AND SELKOE DJ. A substrate-based difluoro ketone selectively inhibits Alzheimer's gamma-secretase activity. *J Med Chem* 41: 6–9, 1998.

187. WOLFE MS, XIA W, MOORE CL, LEATHERWOOD DD, OSTASZEWSKI BL, RAHMATI T, DONKOR IO, AND SELKOE DJ. Peptidomimetic probes and molecular modeling suggest Alzheimer's γ-secretase is an intramembrane-cleaving aspartyl protease. *Biochemistry* 38: 4720–4727, 1999.

188. WOLFE MS, XIA W, OSTASZEWSKI BL, DIEHL TS, KIMBERLY WT, AND SELKOE DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* 398: 513–517, 1999.

189. WOLOZIN B, IWASAKI K, VITO P, GANJEI JK, LACANA E, SUNDERLAND T, ZHAO B, KUSIAK JW, WASCO W, AND D'ADAMIO L. Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. *Science* 274: 1710–1713, 1996.

190. WONG P, ZHEN H, CHEN H, BECHER MW, SIRINATHSIGHJI DJ, TRUMBAUER ME, PROCE DL, VAN DER PLOEG LHT, AND SISODIA SS. Presenilin 1 is required for Notch 1 and D111 expression in the paraxial mesoderm. *Nature* 397: 288, 1997.

191. WOOD JG, MIRRA SS, POLLOCK NL, BINDER LI. Neurofibrillary tangles of Alzheimer's disease share antigenic determinants with the axonal microtubule-associated protein tau. *Proc Natl Acad Sci USA* 83: 4040–4043, 1986.

192. WU G, HUBBARD EJ, KITAJEWSKI JK, GREENWALD I. Evidence for functional and physical association between *Caenorhabditis elegans* SEL-10, a Cdc4p-related protein, and SEL-12 presenilin. *Proc Natl Acad Sci USA* 95: 15787–15791, 1998.

193. XIA W, RAY WJ, OSTASZEWSKI BL, RAHMATI T, KIMBERLY WT, WOLFE MS, ZHANG J, GOATE AM, AND SELKOE DJ. Presenilin complexes with the C-terminal fragments of amyloid precursor protein at the sites of amyloid β-protein generation. *Proc Natl Acad Sci USA* 97: 9299–9304, 2000.

195. XIA W, ZHANG J, KHOLODENKO D, CITRON M, PODLISNY MB, TEPLOW DB, HAASS C, SEUBERT P, KOO EH, AND SELKOE DJ. Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J Biol Chem* 272: 7977–7982, 1997.

196. XIA W, ZHANG J, OSTASZEWSKI BL, KIMBERLY WT, SEUBERT P, KOO EH, SHEN J, AND SELKOE DJ. Presenilin 1 regulates the processing APP C-terminal fragments and the generation of amyloid β-protein in ER and Golgi. *Biochemistry* 37: 16465–16471, 1998.

197. XIA W, ZHANG J, PEREZ R, KOO EH, AND SELKOE DJ. Interaction between amyloid precursor protein and presenilins in mammalian cells: implications for the pathogenesis of Alzheimer's disease. *Proc Natl Acad Sci USA* 94: 8208–8213, 1997.

198. XU X, SHI Y, WU X, GAMBETTI P, SUI D, AND CUI MZ. Identification of a novel PSD-95/Dlg/ZO-1 (PDZ)-like protein interacting with the C terminus of presenilin-1. *J Biol Chem* 274: 32543–32546, 1999.

199. YAMAGUCHI H, HIRAI S, MORIMATSU M, SHOJI, AND HARIGAYA Y. Diffuse type of senile plaques in the brains of Alzheimer-type dementia. *Acta Neuropathol* 77: 113–119, 1988.

200. YAMAZAKI T, SELKOE DJ, AND KOO EH. Trafficking of cell surface β-amyloid precursor protein: retrograde and transcytotic transport in cultured neurons. *J Cell Biol* 129: 431–442, 1995.

201. YAN R, BIENKOWSKI MJ, SHUCK ME, MIAO H, TORY MC, PAULEY AM, BRASHIER JR, STRATMAN NC, MATHEWS WR, BUHL AE, CARTER DB, TOMASSELLI AG, PARODI LA, HEINRIKSON RL, AND GURNEY ME. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 402: 533–537, 1999.

202. YAN SD, CHEN X, FU J, CHEN M, ZHU H, ROHER A, SLATTERY T, ZHAO L, NAGASHIMA M, MORSER J, MIGHELI A, NAWROTH P, STERN D, AND SCHMIDT AM. RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. *Nature* 382: 685–691, 1996.

203. YE Y AND FORTINI ME. Characterization of *Drosophila* Presenilin and its colocalization with Notch during development. *Mech Dev* 79: 199–211, 1998.

204. YE Y, LUKINOVA N, AND FORTINI ME. Neurogenic phenotypes and altered Notch processing in *Drosophila* Presenilin mutants. *Nature* 398: 525–529, 1999.

205. YU G, CHEN F, LEVESQUE G, NISHIMURA M, ZHANG DM, LEVESQUE L, ROGAEVA E, XU D, LIANG Y, DUTHIE M, ST GEORGE-HYSLOP PH, AND FRASER PE. The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains beta-catenin. *J Biol Chem* 273: 16470–16475, 1998.

206. ZHANG J, KANG DE, XIA W, OKOCHI M, MORI H, SELKOE DJ, AND KOO EH. Subcellular distribution and turnover of presenilins in transfected cells. *J Biol Chem* 273: 12436–12442, 1998.

207. ZHENG H, JIANG M, TRUMBAUER ME, SIRINATHSINGHJI DJS, HOPKINS R, SMITH DW, HEAVESN RP, DAWSON GR, BOYCE S, CONNER MW, STEVENS KA, SLUNT HH, SISODIA SS, CHEN HY, AND VAN DER PLOEG LHT. β-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* 81: 525–531, 1995.

208. ZHOU J, LIYANAGE U, MEDINA M, HO C, SIMMONS AD, LOVETT M, AND KOSIK KS. Presenilin 1 interaction in the brain with a novel member of the Armadillo family. *Neuroreport* 8: 2085–2090, 1997.

209. ZLOKOVIC BV. Cerebrovascular transport of Alzheimer's amyloid beta and apolipoproteins J and E: possible anti-amyloidogenic role of the blood-brain barrier. *Life Sci* 59: 1483–1497, 1996.