

Alzheimer's Disease

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Over the last three decades, advances in biochemical pathology and human genetics have illuminated one of the most enigmatic subjects in biomedicine—neurodegeneration. Eponymic diseases of the nervous system such as Alzheimer's, Parkinson's, and Huntington's diseases that were long characterized by mechanistic ignorance have yielded striking progress in our understanding of their molecular underpinnings. A central theme in these and related disorders is the concept that certain normally soluble neuronal proteins can misfold and aggregate into oligomers and amyloid fibrils which can confer profound cytotoxicity. Perhaps the foremost example, both in terms of its societal impact and how far knowledge has moved toward the clinic, is that of Alzheimer's disease (AD). Here, we will review the classical protein lesions of the disorder that have provided a road map to etiology and pathogenesis. We will discuss how elucidating the genotype-to-phenotype relationships of familial forms of Alzheimer's disease has highlighted the importance of the misfolding and altered proteostasis of two otherwise soluble proteins, amyloid β -protein and tau, suggesting mechanism-based therapeutic targets that have led to clinical trials.

Among the human disorders marked by protein misfolding and aggregation, Alzheimer's disease looms large. This enormously common degeneration of limbic and association cortices and related subcortical nuclei slowly robs its victims of their most human qualities: memory, reasoning, abstraction, and language. The disease has no doubt existed for millennia but was often confused with other syndromes that also presented as "senile dementia," that is, progressive cognitive decline after middle age. The description of the clinico-pathological syndrome by the Bavarian psychiatrist, Alois Alzheimer, in 1906 established a neuropathological phenotype that has enabled considerable diagnostic specificity, although until recently

only at the end of the patient's life. The microscopic lesions that Alzheimer called attention to—senile (amyloid) plaques and neurofibrillary tangles—have also provided a crucial starting point for approaching molecular pathogenesis. Indeed, the principal reasons that substantial progress toward deciphering the disease has accrued are its high prevalence and the robustness of its histological signature.

Not surprisingly, the study of Alzheimer's disease has had its share of controversy. Given the cytological and biochemical complexity of the disorder, it has been difficult to come to agreement about the temporal sequence of events that leads to the dementia and which steps are most amenable to intervention.

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However, in recent years, a considerable consensus has developed that certain molecular events in the brain occur years or even decades prior to the first symptoms, and a rough outline of the pathogenic cascade has emerged. Although our understanding is certainly incomplete, advances in the field have led to the design of mechanism-based therapeutics that are now undergoing the painstaking process of clinical evaluation.

PROTEIN CHEMICAL NATURE OF THE DIAGNOSTIC BRAIN LESIONS

Progress in elucidating the biology of AD first arose from the compositional analyses of amyloid plaques and neurofibrillary tangles in the mid-1980s. Attempts to isolate the subunit proteins of these lesions were met with some skepticism, as it was argued that the plaques and tangles might be end-stage lesions that would provide little useful information about etiology and early pathogenesis. It has become increasingly apparent that this concern was ill-founded.

The amyloid deposits found in meningo-cerebral blood vessels and neuritic plaques in AD are composed of extracellular fibrils of the amyloid β -proteins ($A\beta$) (Glennner and Wong 1984a; Masters et al. 1985). Although these deposits contain skeins of insoluble amyloid fibrils (8–10 nm in diameter), these are intermixed with a poorly defined array of nonfibrillar (“amorphous”) forms of the peptide. Once it was established by protein sequencing of isolated amyloid deposits that $A\beta$ was the subunit protein of both vascular amyloid (Glennner and Wong 1984a) and plaque cores (Masters et al. 1985), immunohistochemistry with antibodies to $A\beta$ revealed innumerable plaque-like deposits in AD brain tissue that appeared to lack the surrounding dystrophic neurites and altered microglia and astrocytes which are features of the neuritic plaques. Such lesions, referred to as “diffuse” or “preamyloid” plaques, represent $A\beta$ deposits that are mostly in a nonfibrillar, apparently granular form in the neuropil (Tagliavini et al. 1988; Yamaguchi et al. 1988). Antibodies that selectively recognize the carboxyl termini

of various $A\beta$ peptides have shown that diffuse (nonneuritic) deposits are largely composed of the highly amyloidogenic 42-residue form ($A\beta_{42}$) (Iwatsubo et al. 1994), which has two extra hydrophobic amino acids (Ala and Ile) at its carboxyl terminus compared to the more abundantly generated $A\beta_{40}$ peptide. $A\beta$ deposits do not occur simply in these two extreme forms (diffuse and neuritic) but rather as a continuum in which complex mixtures of fibrillar, granular, and even soluble (nonparticulate) forms of the peptide are associated with highly varying degrees of surrounding glial and neuritic alteration. The extent of microvascular $A\beta$ deposition in AD brains usually correlates poorly with the amount of $A\beta$ plaques, and its importance in contributing to the dementia remains a subject of research (Verbeek et al. 2000).

In regions of the Alzheimer brain that are not strongly implicated in the clinical syndrome (e.g., cerebellum and thalamus), most $A\beta$ deposits are of the diffuse type and thus accompanied by relatively little peri-plaque gliosis and neuritic dystrophy. A frequently voiced concern of the “amyloid (or $A\beta$) hypothesis” of AD is that plaques can be found in the cortex of apparently healthy aged subjects (who were usually not tested for subtle cognitive dysfunction before death). However, these are primarily diffuse plaques that appear to be less bioactive (i.e., they lack significant surrounding neuritic and glial cytopathology). A rough analogy has been drawn to many cholesterol-rich fatty streaks in the coronary arteries of individuals who have not yet experienced clinically noticeable cardiovascular events.

Neurofibrillary tangles are generally intraneuronal cytoplasmic bundles of paired, helically wound ~ 10 -nm filaments (PHFs), often interspersed with straight ~ 10 -nm filaments (Geser et al. 2008). Neurofibrillary tangles usually occur in large numbers in AD brains, particularly in entorhinal cortex, hippocampal formation, amygdala, association cortices of the frontal, temporal, and parietal lobes, and certain subcortical nuclei that project to these regions. The subunit protein of the PHF is the microtubule-associated protein, tau. PHFs are not limited to the tangles found in the cell



bodies of neurons but also occur in many of the dystrophic neurites present within and outside of the amyloid plaques. The tau present in PHF comprises hyperphosphorylated, relatively insoluble forms of this normally highly soluble cytosolic protein (Grundke-Iqbal et al. 1986; Kosik et al. 1986; Nukina and Ihara 1986). The tau aggregates in the tangles are often complexed with ubiquitin, a feature they share with numerous other intraneuronal protein inclusions in etiologically diverse disorders such as Parkinson's disease and Lewy body dementia. If this ubiquitination represents an attempt to remove the tau filaments by way of degradation in the proteasome, it appears to be largely unsuccessful.

The two classical proteinaceous lesions of AD can occur independently in humans. Tangles composed of tau aggregates that are biochemically similar (though usually not identical) to those in AD have been described in a dozen or more neurodegenerative diseases in which one generally finds no A β deposits and neuritic plaques. Conversely, A β deposits (mostly of the diffuse type) can be seen in aged "normal" cortex in the virtual absence of tangles. There are some cases of AD itself that are "tangle-poor," that is, very few neurofibrillary tangles are found in the neocortex despite abundant A β plaques (Terry et al. 1987). In many such cases, one finds an alternate form of neuronal inclusion, the Lewy body, composed principally of fibrils of α -synuclein (Hansen et al. 1993). The fact that neurofibrillary tangles composed of altered, aggregated forms of tau protein occur in disorders (e.g., subacute sclerosing panencephalitis, Kuf's disease, and progressive supranuclear palsy) in the absence of A β deposition suggests that tangles can arise in the course of a variety of etiologically distinct neuronal insults.

A β IS GENERATED BY REGULATED PROTEOLYSIS OF A LARGE PRECURSOR PROTEIN

The purification and partial sequencing of the A β protein from meningovascular amyloid deposits in AD (Glenner and Wong 1984b)

and Down's syndrome (Glenner and Wong 1984b) enabled the subsequent cloning of the gene encoding the β -amyloid precursor protein (APP) (Kang et al. 1987). A β is derived from APP by sequential proteolytic cleavages by enzymes generally referred to as β -secretase and γ -secretase. APP comprises a group of ubiquitously expressed polypeptides whose heterogeneity arises from both alternative exon splicing and posttranslational modifications (e.g., N- and O-linked glycosylation, phosphorylation, and sulfation) (Weidemann et al. 1989). Deletion of the APP gene in mice results in neither early mortality nor appreciable early morbidity, although cerebral gliosis, changes in locomotor and cognitive behaviors and other deficits develop with age (Zheng et al. 1995; Ring et al. 2007), and knockdown of APP in rat embryos impairs radial glial-guided migration of immature neurons during cortical development (Young-Pearse et al. 2007). The lack of a lethal phenotype from APP deletion presumably results from mammals expressing two closely homologous Type 1 glycoproteins, the amyloid precursor-like proteins (APLPs) (Wasco et al. 1992; Slunt et al. 1994).

The recognition that the last 12–14 residues of A β derived from the transmembrane region of APP gave rise to a conundrum: How could A β be found as a free peptide in the extracellular amyloid deposits? It was generally assumed that neurons would need to undergo an initial injury to their membranes to allow the unknown protease that creates the carboxyl terminus of A β (γ -secretase) to access the intramembrane region of APP. In contrast, it was discovered in 1992 that A β is constitutively secreted by healthy cells throughout life and normally circulates in the cerebrospinal fluid (CSF) and plasma of humans and lower mammals (Haass et al. 1992; Seubert et al. 1992; Shoji et al. 1992). APP holoproteins undergo alternative processing events (Fig. 1).

The most common scission occurs between residues 16 and 17 of the A β region (12 residues amino-terminal to the transmembrane domain) and is affected by one of the α -secretases, which are members of the ADAM family of membrane-anchored metalloproteases (Sisodia

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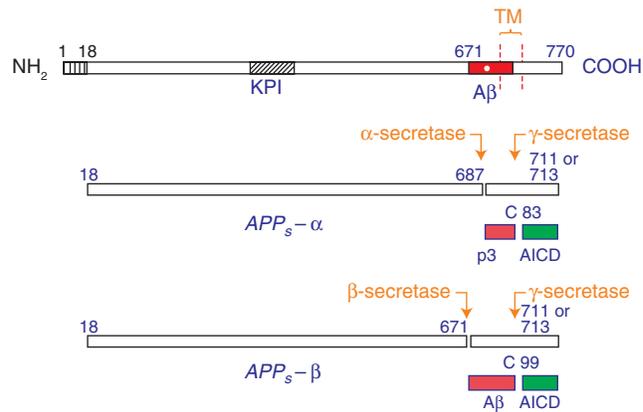


Figure 1. Schematic diagrams of the β -amyloid precursor protein (APP) and its principal metabolic derivatives. The *upper* diagram depicts the largest of the known APP alternate splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct relative positions. A 17-residue signal peptide occurs at the amino 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 β -protein ($A\beta$) 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 $A\beta$ 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 ($APP_s-\alpha$) into the medium and retention of the 83 residue carboxy-terminal fragment in the membrane. The C83 fragment can undergo cleavage by protease called γ -secretase at residue 711 or residue 713 to release the p3 peptides. The *lower* diagram depicts the alternative proteolytic cleavage after residue 671 by enzyme called β -secretase that results in the secretion of the slightly truncated $APP_s-\beta$ molecule and the retention of a 99-residue carboxy-terminal fragment. The C99 fragment can also undergo heterogenous cleavages by γ -secretase to release the $A\beta$ peptides.



1992; Buxbaum et al. 1998; Kuhn et al. 2010). The soluble ectodomain region ($APP_s-\alpha$) is released into vesicle lumens and from the cell surface, leaving behind a membrane-retained carboxy terminal fragment (CTF) of 83 amino acids (C83) (Fig. 1). Some APP holoproteins that do not undergo α -secretase cleavage may instead be cut by β -secretase, particularly in neurons, releasing a truncated form of APP_s ($APP_s-\beta$) from the cell (Seubert et al. 1993) and leaving a 99-residue CTF (C99) in the membrane (Fig. 1). C99 can subsequently be cleaved by the unusual proteolytic activity referred to as γ -secretase to release $A\beta$ into vesicle lumens and extracellular fluid. C83 can also undergo cleavage by γ -secretase to generate a peptide (p3) comprising the latter two-thirds of $A\beta$. In most cells, a much smaller portion of total cellular APP undergoes cleavage

by β - than by α -secretase. The β -secretase that generates the principal amino terminus of $A\beta$ (aspartate-1) is a single-transmembrane aspartyl protease (BACE) with its active site in its ectodomain (Sinha et al. 1999; Vassar et al. 1999; Yan et al. 1999).

Several functions have been attributed to the APP ectodomain, including the inhibition of certain serine proteases (in the case of those splice forms with a Kunitz protease inhibitor motif), enhancement of cell–cell and cell–substrate adhesion, neuritrophic and other growth-promoting effects, and neuroprotective properties (Anliker and Muller 2006; Zheng and Koo 2006). The hydrophilic APP intracellular domain (AICD) that is released into the cytoplasm by the intramembrane proteolysis of C83 or C99 (Fig. 1) could have a signaling function (analogous to another α - and γ -secretase

substrate, Notch), but putative activities of this type have not been widely confirmed (Hebert et al. 2006). No evidence has emerged that a fundamental function of APP is lost in AD patients. Instead, AD-causing APP mutations are all clustered near the secretase processing sites and seem to act by a gain-of-toxic-function mechanism, namely the increased production of the neurotoxic A β fragment.

THE GENETICS OF ALZHEIMER'S DISEASE

Estimates of the proportion of AD cases that are genetically based have varied widely from as low as 10% to as high as 40% or 50%, and some investigators believe that, in the fullness of time, virtually all cases will be shown to have some genetic determinants. The discovery that the ϵ 4 allele of apolipoprotein E is a normal polymorphism that greatly increases the risk of AD (Strittmatter et al. 1993) underscores that most genetic factors predisposing to AD do not occur in a simple medelian pattern and are thus difficult to identify in genetic epidemiological studies. There are four widely confirmed genes in which mutations or polymorphisms result in AD, and numerous additional candidate genes (e.g., clusterin [Apo J]; complement receptor 1) are in various stages of confirmation. Missense mutations in APP account for a tiny fraction (far less than 0.01%) of all Alzheimer's cases, but they appear to be informative about the pathogenic mechanisms of AD in general. Inheritance of one or two ϵ 4 alleles of ApoE is by far the most prevalent genetic factor predisposing to AD (Saunders et al. 1993; Strittmatter et al. 1993), accounting for upward of 30% of cases. Nevertheless, some humans homozygous for ApoE4 still show no AD symptoms in their ninth decade. The third and fourth genes implicated in familial forms of AD are presenilin 1 (PS1) and presenilin 2 (PS2); dominantly inherited missense mutations result in aggressive disease, with clinical onset between ages \sim 40 and \sim 65 (Levy-Lahad et al. 1995; Rogaeve et al. 1995; Sherrington et al. 1995).

Despite the prominence of tau accumulation in neurofibrillary tangles and dystrophic

neurites, the tau gene has not been found to be mutant in familial AD. Instead, mutations in tau cause a form of frontotemporal dementia (Hong et al. 1998; Hutton et al. 1998; Spillantini et al. 1998). The disorder, which generally occurs without amyloid deposits, is characterized by widespread tangle formation associated with specific biochemical alterations in the microtubule-binding properties of the mutant tau (Lee et al. 2001). The discovery of tau mutations in this distinct form of dementia has shown that severe neurofibrillary tangle formation does not lead to secondary accumulation of A β . Both APP and presenilin mutations in AD and tau mutations in frontotemporal dementia support the view that the profound alteration of wild-type tau in AD can follow A β accumulation, but not vice versa.

GENOTYPE-TO-PHENOTYPE RELATIONSHIPS IN FAMILIAL AD

Cultured cells and transgenic mice have been used to model the effects of each of the four genes that have been unequivocally implicated in familial AD (Table 1). In all cases, inherited alterations in the gene products have been linked to increases in the production and/or deposition of A β . This work provides the

Table 1. Genetic factors predisposing to AD: relationships to the β -amyloid phenotype

Chromosome	Gene defect	Phenotype
21	β APP mutations	Increased productions of all A β peptides or A β ₄₂ peptides
19	ApoE4 polymorphism	Increased density of A β ₄₀ plaques and vascular deposits
14	Presenilin 1 mutations	Increased production of A β ₄₂ peptides
1	Presenilin 2 mutations	Increased production of A β ₄₂ peptides

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strongest support for the hypothesis that cerebral A β accumulation can be causative of AD. The APP gene can cause AD in two distinct ways: via overexpression because of increased dosage of the wild-type gene (e.g., in trisomy 21 [Down's syndrome] and in patients with APP microduplications on chromosome 21q) or else via missense mutations that increase the amyloidogenic cleavages of APP by either β - or γ -secretase. In Down's syndrome, a life-long increase in APP expression and the resultant overproduction of both A β_{40} and A β_{42} peptides (Rumble et al. 1989; Tokuda et al. 1997) is responsible for the appearance of many diffuse plaques as early as age 12. Down's subjects usually develop diffuse plaques composed solely of A β_{42} in their teens and early 20s, with subsequent accrual of A β_{40} peptides onto these plaques and the gradual appearance of surrounding microgliosis, astrogliosis, neuritic dystrophy, and neurofibrillary tangles beginning in their 20s or 30s (Mann et al. 1995; Lemere et al. 1996a).

The more than 20 known APP missense mutations that cause AD are clustered at the β -secretase cleavage site, just after the γ -secretase site, or else in the middle of the A β region (Fig. 2). A double missense mutation immediately amino-terminal to A β allows

increased cleavage by β -secretase. Mutations occurring after the γ -secretase cleavage site enhance the relative production of the longer and more self-aggregating A β_{42} peptide. Mutations within A β (Levy et al. 1990; Hendriks et al. 1992) generally enhance the aggregation of the peptide. Importantly, no AD-causing mutations in the APP polypeptide away from the A β region have been documented.

The disease-promoting mechanism of inheriting one or two ApoE4 alleles is not settled. Considerable evidence suggests that this isoform leads to decreased clearance and/or enhanced aggregation of A β in the brain (Rebeck et al. 1993; Schmechel et al. 1993; Ma et al. 1994; Evans et al. 1995). A decrease in A β deposits occurs when APP transgenic mice are crossed with mice lacking ApoE (Bales et al. 1997). When these offspring are then crossed with mice expressing human ApoE3 or ApoE4, both resultant genotypes show more A β deposits than the mice lacking ApoE, but the ApoE4-expressing mice show significantly more than those with ApoE3 (Holtzman et al. 2000). Other possible mechanisms of the effect of ApoE4 in promoting the AD phenotype have been proposed (Mahley and Huang 2009). However, the observation in humans that not only parenchymal amyloid deposits

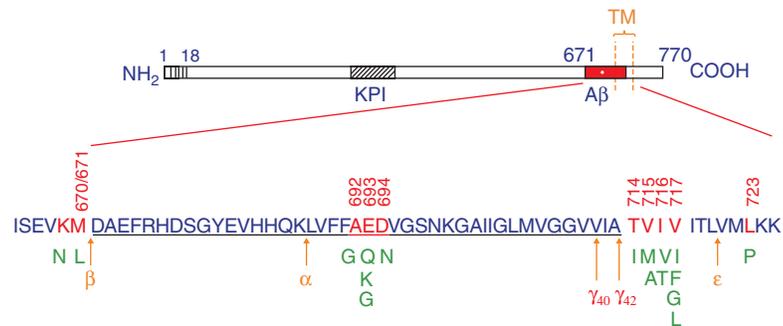


Figure 2. β APP mutations genetically linked to familial Alzheimer's disease or related disorders. The sequence within APP that contains the A β and transmembrane region is expanded and shown by the single-letter amino acid code. The underlined residues represent the A β 1-42 peptide. The vertical broken lines indicate the location of the transmembrane domain. The bold letters below the sequence indicate the currently known missense mutations identified in certain patients with familial Alzheimer's disease or hereditary cerebral hemorrhage with amyloidosis. The family with the AG92G mutation can experience cerebral hemorrhages from amyloid angiopathy and/or Alzheimer's disease as the phenotypes. Three-digit numbers refer to the residue number according to the β APP770 isoform.

but also those in meningeal vessels (and sometimes just the latter) accumulate as a function of rising ApoE4 gene dosage (Greenberg et al. 1995) strongly suggests that ApoE4 serves to increase tissue A β deposition.

When presenilin 1 and 2 were cloned in 1995, the mechanism by which missense mutations in these polytopic proteins produced AD was open and not necessarily expected to involve a change in A β economy. However, it soon became known that PS 1 or 2 missense mutations increase the ratio of A β_{42} to A β_{40} in the patients' plasma and cultured fibroblast media (Scheuner et al. 1996). Modeling of these mutations in cultured cells and mice confirmed this result (Borchelt et al. 1996; Citron et al. 1997). Crossing mice transgenic for mutant APP with mice expressing mutant PS1 leads to an accelerated AD-like phenotype, with A β_{42} plaques occurring as early as 3 mo of age (Holcomb et al. 1998). Moreover, the ability of PS mutations to selectively enhance A β_{42} deposition has been documented directly in patients' brains (Lemere et al. 1996b; Mann et al. 1996).

PS1 and PS2 each have nine transmembrane domains (TMD) and occur in ER, Golgi, and other vesicles in the secretory pathway, plasma membrane, and endosomes. Presenilin exists largely as a mature heterodimer of endoproteolytically generated fragments (NTF plus CTF) (Thinakaran et al. 1996). Studies in *Caenorhabditis elegans* led to the recognition that its presenilin (called SEL-12) is a facilitator of the Notch intercellular signaling pathway (Levitan et al. 1996). Accordingly, deleting the presenilin 1 gene in mouse causes profound Notch hypofunction (i.e., an embryonic lethal phenotype that includes severely disordered somitogenesis and axial skeletal development as well as abnormal neuronal differentiation in the forebrain [Shen et al. 1997; Wong et al. 1997]). In addition, the production of all A β peptides is markedly reduced in PS1 knockout embryos because of a loss of γ -secretase processing (De Strooper et al. 1998). APP was found by some investigators to interact with PS1 (Weidemann et al. 1997; Xia et al. 1997), suggesting that presenilin might either be a necessary cofactor of the γ -secretase reaction or might actually be

γ -secretase itself. The latter possibility was furthered by the finding that inhibiting γ -secretase activity in cells with APP peptidomimetic transition-state analogs suggested that γ -secretase was an aspartyl protease (Wolfe et al. 1999a). Close inspection of the presenilin sequence revealed two aspartates in adjacent TMDs of all presenilins down to *C. elegans*, and these flanked the cytoplasmic loop in which presenilin is normally cleaved into its heterodimer (Fig. 3). Mutation of either of the aspartates completely prevented both this endoproteolysis of presenilin and the γ -secretase processing of C99 to A β and C83 to p3 (Wolfe et al. 1999b). These results suggested that presenilin was a first-in-class intramembrane aspartyl protease that cleaves itself (an autoactivation step) and can then cleave intramembrane substrates such as C83 and C99 of APP. Separate work showed that presenilin is also required for the normal intramembrane cleavage of Notch following binding of its extracellular ligand (De Strooper et al. 1999). Consequently, mutating either one of the PS aspartates is lethal to *C. elegans* (Brockhaus et al. 1998) and all other animals. It was soon shown that γ -secretase inhibitors mimicking the transition state of a substrate with an aspartyl protease bound directly to presenilin (Esler et al. 2000; Li et al. 2000). These and many subsequent studies firmly establish presenilin as the catalytic site of γ -secretase. Thus, missense mutations in either the protease (presenilin) or the substrate (APP) of the γ -secretase reaction elevate the relative levels of A β_{42} and result in an aggressive AD phenotype. These findings are assumed to be relevant to conventional ("sporadic") forms of AD, which are usually indistinguishable neuropathologically from cases caused by PS mutations.

GENETICALLY ENGINEERED MOUSE MODELS OF AD

Given the many obstacles to studying disease progression dynamically in the human brain, significant effort has been expended to create mouse models that replicate aspects of AD pathogenesis. A sizable number of mouse lines

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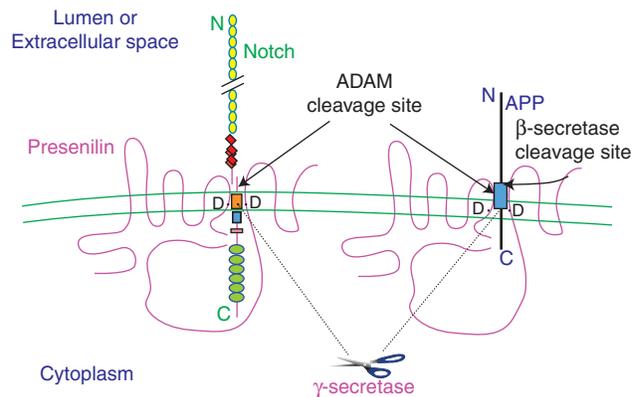


Figure 3. Model of the role of presenilin (PS) in Notch and APP processing based on current information. Polytopic PS protein, which occurs principally as a cleaved heterodimer. Some Notch and APP molecules form complexes with PS. Two aspartates (D) in TM6 and TM7 of PS are required for the cleavages of Notch and APP within their TM domains, and these align with the respective sites of cleavage in the two substrates. PS-mediated proteolysis of both Notch and APP is preceded by ectodomain shedding by an ADAM family protease (“ α -secretase”). Alternatively, APP can undergo ectodomain shedding by β -secretase. Several motifs are depicted in Notch: EGF-like repeats (yellow circles), LNG repeats (red diamonds), a single TM (orange box), the RAM23 domain (blue square), a nuclear localization sequence (pink rectangle), and six cdc10/ankyrin repeats (green ovals). Following 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 A β region (light blue box), which is released into the lumen after sequential cleavages of APP by β -secretase and then γ -secretase/PS. The APP intracellular domain is released into the cytoplasm.



transgenic for human APP (either without or with human PS1 coexpression) have been generated (Games et al. 1995; Hsiao et al. 1996; Holcomb et al. 1998; Mucke et al. 2000; Oddo et al. 2003). Most of these models recapitulate some but not all neuropathological features of the human disease. Typically, A $\beta_{42/40}$ peptide ratios are elevated and both diffuse and fibrillar (neuritic) plaques appear in an age-dependent fashion. No PHF in neurites or tangles are observed unless mutant human tau is also overexpressed. “Bigenic” mice expressing mutant human APP plus human tau bearing the P301L mutation that causes a form of frontotemporal dementia (Lewis et al. 2001) or “triple transgenic” mice expressing mutations in human APP, PS1, and tau (Oddo et al. 2003) develop NFTs reminiscent of those seen in AD and at a faster rate than mice expressing P301L tau alone.

In most APP transgenic models, significant neuronal loss is not observed (Irizarry et al. 1997). However, at least one mouse line (called

APP23) that expresses mutant human APP shows quantifiable neuronal loss in the CA1 region of hippocampus at age 14–18 mo (Calhoun et al. 1998). In the latter mice, A β is deposited almost exclusively in the form of Thioflavin-positive (amyloid fibril-rich) plaques (Sturchler-Pierrat et al. 1997), and the cell loss is observed primarily in the vicinity of such hippocampal deposits. In accord, another study reported a reduction in neuronal density around Thioflavin S-positive A β deposits in both AD brain and the brains of 12-mo-old mice coexpressing mutant human PS1 plus APP (Urbanc et al. 2002).

Although fibrillar A β in mature AD plaques may contribute to neurotoxicity, such plaques are dynamic structures and likely act as local reservoirs of small, diffusible oligomers (Koffie et al. 2009). Furthermore, a mouse line transgenic for mutant APP took \sim 12 mo to develop plaques, and yet the animals showed cognitive impairment and decreased hippocampal long-term potentiation (LTP) from 3 mo onward



(Moechars et al. 1999). Another APP mouse was found to have enhanced paired pulse facilitation, distorted responses to high frequency stimulation, and impaired LTP at just age 4–5 mo (Larson et al. 1999; Shinsky et al. 2002). Analyses of other mice have confirmed morphological, biochemical, and electrophysiological changes well in advance of amyloid plaque deposition (Mucke et al. 2000; Wu et al. 2004). In addition, studies in a *C. elegans* model also suggest that small oligomers of A β can confer cytotoxicity (Cohen et al. 2006).

PREFIBRILLAR FORMS OF A β PERTURB SYNAPTIC FORM AND FUNCTION

Early evidence from human studies that soluble, nonfibrillar assemblies of A β might play a principal role in cognitive impairment came from analyses that showed statistical correlations between cortical levels of soluble A β and the extent of synaptic loss and severity of cognitive symptoms (Lue et al. 1999; McLean et al. 1999; Wang et al. 1999). In such studies, the term “soluble A β ” is an operational definition, embracing all forms of A β that remain in aqueous solution following high-speed centrifugation of brain extracts (Kuo et al. 1996; Lue et al. 1999; McLean et al. 1999; Wang et al. 1999).

Experimental approaches to this concept in the investigator's laboratory have confirmed that abundant SDS-stable A β dimers (~8 kDa) and some SDS-stable trimers (~12 kDa) are detectable in buffer-soluble extracts of post-mortem AD cortex (Shankar et al. 2008). We subjected such extracts to size-exclusion chromatography, allowing the separation of monomers (~4 kDa) from dimers. Application of the respective SEC fractions to normal mouse hippocampal slices showed that the dimers potently inhibited LTP, an electrophysiological correlate of aspects of synaptic plasticity during learning and memory in rodents (Shankar et al. 2008). The dimers could also facilitate long-term synaptic depression (LTD) in the hippocampus (Li et al. 2009). Monomers isolated simultaneously from the same AD brains were without effect. The dimers also decreased dendritic spine density; this is relevant to

the strong correlations between AD neuropathology and synaptic loss in human brains. Monoclonal antibodies to A β (especially, to its free amino terminus) prevented these changes. Insoluble amyloid plaque cores isolated from the same AD brains did not impair LTP in hippocampal slices unless the cores were first solubilized in formic acid to release their constituent dimers (Shankar et al. 2008). Importantly, microinjection of the dimer-rich soluble AD extracts into the cerebral ventricles of healthy adult rats transiently impaired the memory of a learned behavior (Shankar et al. 2008). Further studies have recently shown that the isolated AD brain dimers potently induce hyperphosphorylation of endogenous tau in rat cortical neurons, followed by a progressive collapse of the neuritic cytoskeleton (M Jin and DJ Selkoe, unpublished data). Knocking down rat tau prevented the neuritic dystrophy, whereas expressing human tau accelerated it. Taken together, these findings support the hypothesis that small, soluble oligomers of human A β are sufficient to induce several features of the AD phenotype, including synaptic loss, tau hyperphosphorylation, neurofibrillary degeneration, and memory impairment, in the absence of amyloid plaques. Moreover, earlier *in vivo* studies that genetically deleted tau in APP transgenic mice strikingly protected these animals from A β -mediated behavioral deficits (Roberson et al. 2007).

Many studies employing supraphysiological concentrations of synthetic A β peptides in oligomeric form (generally larger than dimers/trimers) have also provided evidence of tau alteration (Zempel et al. 2010) and various neurotoxic effects. Because such synthetic intermediates can readily associate into higher order aggregates and fibrils can in turn dissociate, it is difficult to unambiguously ascribe cytopathological activity to a discrete species. Nonetheless, several groups have generated prefibrillar synthetic A β assemblies and probed their synaptotoxic activity. In 1998, Lambert and colleagues presented the first experimental evidence that certain soluble, nonfibrillar forms of synthetic A β (which they called A β -derived diffusible ligands, or ADDLs) could be

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neurotoxic (Lambert et al. 1998). ADDLs have been shown to cause neuronal death in culture, to block LTP (Lambert et al. 1998; Wang et al. 2004) and to inhibit reduction of MTT by neural cell lines (Lambert et al. 1998; Stine et al. 2003). Apparently distinct assembly intermediates of synthetic A β termed protofibrils (PFs) have been shown by other workers to alter neuronal function. Synthetic PFs range from spherical assemblies of \sim 5 nm diameter to short, flexible rods of up to 200 nm in length (Harper et al. 1997; Walsh et al. 1997). Synthetic PFs appear to behave as fibril intermediates in vitro in that they can both form fibrils and dissociate to lower molecular weight species (Harper et al. 1999; Walsh et al. 1999). Among other neurotoxic effects, PFs can induce an increase in excitatory postsynaptic currents (EPSCs) in rat cortical neurons (Hartley et al. 1999). An alternative to the use of synthetic peptides is to analyze the conditioned media of certain cultured cell lines that express mutant human APP and secrete low-nanomolar amounts of small, soluble oligomers. When such cell-derived oligomers were injected intraventricularly into healthy rats, they inhibited hippocampal LTP (Walsh et al. 2002) and also impaired the memory of a learned behavior (Cleary et al. 2005). Finally, yet another approach has been to identify—and then isolate—A β assembly intermediates (e.g., a metastable dcamer designated A β *56) from the brains of certain APP transgenic mice (Lesne et al. 2006). A β *56 has been shown to be associated with—and actually induce—transient learning deficits in water maze and other mouse behavioral assays (Lesne et al. 2006).

SEVERAL THERAPEUTIC OPPORTUNITIES ARE EMERGING FROM THE MECHANISTIC STUDY OF A β

Progress in deciphering the role of the presenilins in the proteolytic processing of APP, Notch and many other single-transmembrane proteins has provided a way of thinking about the question of how Alzheimer's disease arose in the human population. The presenilins, and specifically their two intramembrane

aspartates, were conserved during evolution because they confer a strong developmental advantage by mediating the Notch nuclear signaling pathway that is vital for cell fate decisions in all multicellular animals. But the similar processing of another, biologically less important substrate, APP, allows the lifelong production of A β , and this can lead in long-lived hosts to the accumulation of its most hydrophobic and oligomer-prone form (A β ₄₂). Because large numbers of humans now survive long past reproductive maturity, one sees a very substantial and rising prevalence of AD. A corollary of the latter concept is provided by the inheritance of missense mutations in either the substrate (APP) or the protease (presenilin) that biochemically accentuate—at least in relative terms—the cleavage of APP at the A β ₄₂₋₄₃ peptide bond, producing an early-onset form of AD.

Although many questions remain unanswered, sufficient progress in delineating the pathogenic cascade has been achieved to envision several discrete targets for treatment. Inhibitors of A β 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, namely in patients with minimal cognitive impairment or mild AD, and ultimately also in presymptomatic subjects with genetic predispositions. In the case of γ -secretase inhibitors, these could be designed to decrease APP cleavage by some 20% to 40%, but they must do so selectively, that is, without interfering in a quantitatively important way with Notch processing.

An alternate approach would be to use small molecules to bind A β monomers and prevent their assembly into potentially cytotoxic oligomers or else coat the small oligomers to mask their toxicity. However, if an antiaggregating 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 a properly designed antioligomer agent 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 certain aspects of the responses by microglia, astrocytes, or bone marrow-derived macrophages that occur in the AD brain. It would be necessary to design novel compounds that interfere with one or more specific steps in the $A\beta$ -induced inflammatory cascade of AD, rather than relying on conventional anti-inflammatory drugs that can have considerable toxicity when administered chronically to elderly humans.

One could also 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 $A\beta$ accumulation. The problem with this general approach may turn out to be that there are multiple ways in which neurons respond to $A\beta$ and the associated inflammatory process, and blocking one or two of these might not significantly decrease overall neuronal dysfunction and loss. An increasingly interesting possibility is to lower or neutralize the effects of tau, given the striking protective effects against the neurotoxicity of $A\beta$ that tau knockdown has shown in cell culture and mouse models (above). One can also consider the administration of neurorestorative factors, for example, neurotrophins and small compounds mimicking their actions that might rescue synapses and cell bodies undergoing active injury. However, this approach would need to operate successfully in the presence of ongoing new injury from the cytotoxic effects of $A\beta$.

Perhaps the clinically most advanced approach to lowering the levels of $A\beta$ monomers, oligomers, and amyloid deposits is active and passive immunotherapy. This idea arose from studies in APP transgenic mice immunized with synthetic human $A\beta$ peptide, leading to a humoral response and the movement of some of the antibodies across the blood brain barrier into the brain parenchyma, where they reduced both plaque burden and soluble $A\beta$ levels (Schenk et al. 1999). A subsequent study infusing monoclonal antibodies to $A\beta$ into

transgenic mice also led to $A\beta$ clearing (Bard et al. 2000). Many follow-up preclinical studies have robustly confirmed these effects and shown that abnormal behavior in APP mice can also be ameliorated. In one study, acute administration of a single injection of an antibody appeared to return certain cognitive deficits to baseline in older mice, obviously without reducing plaque burden (Dodart et al. 2002). The precise mechanisms of immunotherapy remain unclear but likely involve both the stimulation of plaque clearance by local microglia and/or exogenous macrophages (Bard et al. 2000) and the binding and neutralization of soluble forms of $A\beta$ in the brain (Shankar et al. Nat Med 2008) and perhaps in the systemic circulation (DeMattos et al. 2002). Success in APP transgenic mouse models led to initial human trials of active vaccination with a full-length $A\beta_{42}$ peptide (plus adjuvant), but in a phase 2 trial, 6% of the 300 immunized subjects developed a self-limited sterile meningo-encephalitis, apparently because of the entry of T-cells auto-reactive to $A\beta$ through the blood-brain barrier. Although this trial had to be halted, some subjects were followed and showed limited evidence of a biological effect, namely fewer declines than placebo-treated subjects on certain memory tests that correlated with their residual $A\beta$ antibody titers, plus a lowering of CSF tau levels in a small subset of recipients (Gilman et al. 2005). Given this mixed outcome, the next clinical approach was passive infusion of antibodies directed to the free $A\beta$ amino terminus, thus avoiding side effects of active vaccination. An 18-mo phase 2 trial again showed mixed results: (1) less declines in some cognitive measures in those who received all six antibody doses, again with lowering of mean CSF tau levels in the few subjects who had two lumbar punctures; and (2) the development in a small but significant percentage of a transient neuroradiological change called vasogenic edema, associated with transient worsening of the degree of dementia in a minority of these radiologically affected patients (Salloway et al. 2009). This amino-terminal antibody is now being tested in four large Phase 3 trials, and other passive and active immunotherapy trials are underway.

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In the future, it is probable 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, identification of specific predisposing genetic factors, structural and functional brain imaging (including amyloid PET scans) to detect evidence of presymptomatic lesions, and measurement of A β ₄₂, tau, and other markers of the neuropathology in CSF and perhaps (in the case of A β) even in blood. Based on further epidemiologic experience with such assessment measures in large populations of healthy elderly, MCI and 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 appreciable risk could be offered preventative treatments, assuming one or more of the agents contemplated in the previous paragraphs prove efficacious and safe. 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 many additional clinical trials suggest that some level of practical success may come sooner than one might think.

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