Translating cell biology into therapeutic advances in Alzheimer's disease

Dennis J. Selkoe

Studies of the molecular basis of Alzheimer's disease exemplify the increasingly blurred distinction between basic and applied biomedical research. The four genes so far implicated in familial Alzheimer's disease have each been shown to elevate brain levels of the self-aggregating amyloid-β protein, leading gradually to profound neuronal and glial alteration, synaptic loss and dementia. Progress in understanding this cascade has helped to identify specific therapeutic targets and provides a model for elucidating other neurodegenerative disorders.

Until the last decade, degenerative diseases of the brain were considered to be among the most obscure and intractable disorders in medicine. Alzheimer's disease (AD) epitomized the mechanistic ignorance and therapeutic nihilism that pervaded the study of neurodegeneration in humans. But research advances in two broad areas—biochemical pathology and molecular genetics—have combined to offer new hope and to stimulate research. Determining the composition of the classical brain lesions and identifying at least four genes that predispose individuals to the disorder have increased our understanding of the genotype-to-phenotype relationships that underlie inherited forms of AD. It is therefore timely to review and attempt to integrate the disparate elements of the disease into a coherent whole, perhaps helping to focus future investigative efforts on developing rational treatments.

Three central questions about Alzheimer's syndrome

It is now clear that AD is, in reality, a multifactorial syndrome, rather than a single disease. Given the complex array of factors that may initiate or propagate the syndrome, it sometimes seems that researchers are pursuing many ostensibly unrelated clues to its pathogenesis. Yet almost all investigations of AD during the past two decades have sought to provide information about one or more of three interrelated questions. First, what are the causes of the disorder? Second, regardless of cause, is there a common cell biological and biochemical mechanism that leads to the dementia in essentially all cases? And third, what is the phenotype of the degenerating neurons affected by this mechanism: where are they located, what are their neural connections and neurotransmitter specificities, and what behavioural symptoms do they mediate? When thought of in this way, the profusion of initially distinct observations about the syndrome can be evaluated with respect to the particular step in the disease cascade that each study addresses. Here I review the progress made in attempting to synthesize the answers to these questions into a mechanistic pathway. In doing so, I hope to show that the elucidation of AD represents an emerging triumph of reductionist biology applied to a chronic disorder of the most complex of biological systems, the human cerebral cortex.

Biochemistry of the classical brain lesions

Senile (neuritic) plaques and neurofibrillary tangles, observed in Alzheimer's original patient of 1906, comprise the major neuropathological lesions that, when present in sufficient numbers in limbic and association cortices, allow a definitive diagnosis of AD after the patient's death (Fig. 1). Although there are other distinct pathological changes that often appear to be physically separate from the plaques and tangles (for example, microvascular amyloidosis and dystrophic cortical neurites), evidence indicates that even these are related to the pathogenesis of one or both of the classical lesions. Early attempts to purify the tangle and plaque proteins and determine their respective compositions were met with considerable scepticism as it was argued that, because the plaques and tangles were end-stage lesions that apparently represented the tombstones of the pathogenic process, such knowledge would provide little useful information about aetiology and early pathogenesis. It has become apparent in recent years that this concern was ill-founded.

Neuritic plaques are spherical, multicellular lesions that are usually found in moderate or large numbers in limbic structures and association neocortex (reviewed in ref. 1). They contain extracellular deposits of amyloid-β protein (Aβ) that include abundant amyloid fibrils (7–10 nm) intermixed with non-fibrillar forms of this peptide. Considered to be mature lesions that are generally associated with full-blown clinical disease, neuritic plaques have degenerating axons and dendrites (neurites) within and intimately surrounding the amyloid deposit (Fig. 1). Such plaques also contain variable numbers of activated microglia that are often situated within and near the fibrillar amyloid core, as well as reactive

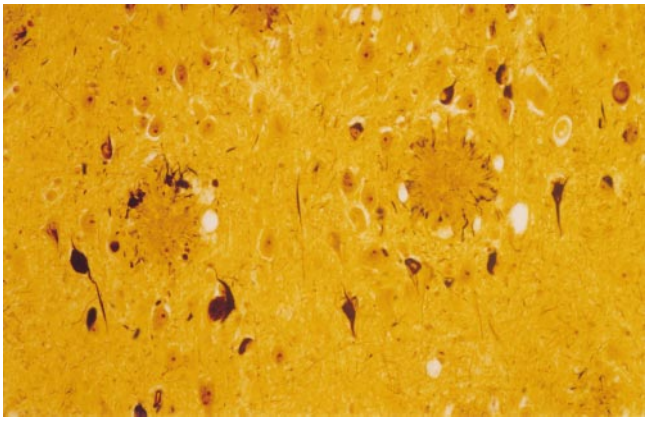

Figure 1 High-power photomicrograph of a section of the amygdala from an Alzheimer's patient showing the classical neuropathological lesions of the disorder. The modified Bielschowsky silver stain demonstrates two senile (neuritic) plaques consisting of compacted, spherical deposits of extracellular amyloid immediately surrounded by a halo of silver-positive dystrophic neurites, which can include both axonal terminals and dendrites. Some of the pyramidal neurons in this field contain neurofibrillary tangles, which are darkly staining masses of abnormal filaments occupying much of the perinuclear cytoplasm. Electron microscopy of such neurons generally reveals large, non-membrane-bound bundles of paired helical filaments.

astrocytes surrounding the core. Immunohistochemistry using antibodies against Aβ reveals an even larger number of deposits in the Alzheimer's brain that seem to lack altered microglia and astrocytes and surrounding dystrophic neurites. These lesions are referred to as diffuse plaques, and within these the Aβ occurs in a predominantly non-fibrillar, amorphous form in the neuropil[2]. Diffuse deposits are almost exclusively composed of the highly amyloidogenic 42-amino-acid-residue form of the peptide ($A\beta_{42}$). This form is normally produced by cells in much lower quantities than the 40-residue form ($A\beta_{40}$), which represents roughly 90% of total secreted Aβ. Aβ deposits do not occur simply in these two extreme forms (diffuse and neuritic), but rather as a continuum in which mixtures of fibrillar and non-filamentous forms of the peptide can be associated with varying degrees of local glial and neuritic alteration.

In regions of the Alzheimer brain that are generally not implicated in the clinical syndrome, for example the cerebellum and thalamus, almost all Aβ deposits seem to be diffuse, with little evidence of local glial and neuritic reaction. Likewise, the brains of aged, cognitively normal humans often contain Aβ deposits, but these are primarily of the diffuse type, with few neuritic plaques and neurofibrillary tangles present in limbic and association cortices. Aβ also accumulates in the basement membranes of some cerebral capillaries, arterioles and venules and some meningeal arterioles. The extent of this microvascular β-amyloidosis usually does not correlate closely with the number of Aβ plaques in a brain, and its importance in contributing to the dementia remains a subject of active research.

Neurofibrillary tangles are intraneuronal cytoplasmic lesions consisting of non-membrane-bound bundles of paired, helically wound ~10-nm filaments (PHF), sometimes interspersed with straight filaments[3]. Neurofibrillary tangles generally occur in large numbers in the Alzheimer brain, particularly in entorhinal cortex, hippocampus, 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. Biochemical studies have shown that the tau found in the tangles and also in many of the dystrophic neurites within and outside the plaques comprises hyperphosphorylated, insoluble forms of this normally highly soluble cytosolic protein. The insoluble tau aggregates in the tangles are often conjugated with ubiquitin, a feature they share with other intraneuronal proteinaceous inclusions in aetiologically diverse disorders such as Parkinson's disease and diffuse Lewy-body disease. If this ubiquitination represents an attempt to remove the tau filaments by way of the proteasome, it seems to be largely unsuccessful. Tangles also occur in more than a dozen relatively uncommon neurodegenerative diseases in which one usually finds no Aβ deposits and neuritic plaques.

Therefore, the two classical lesions of AD can occur independently of each other. As I shall discuss, there is growing evidence that the formation of tangles represents one of several cytological responses by cells to the gradual accumulation of Aβ and Aβ-associated proteins.

The genetics of Alzheimer's disease

It has been known for several decades that AD can occur in a familial form that transmits as an autosomal dominant trait. Estimates of the proportion of Alzheimer's 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 almost all cases will be shown eventually to have genetic determinants. It is difficult to resolve this question in a late-onset disorder that, up until the past two decades, was often not explicitly diagnosed. Moreover, the discovery that the ε4 allele of apolipoprotein E (ApoE) is a normal polymorphism that confers increased risk for developing AD[4] indicates that genetic factors predisposing individuals to AD need not occur in a simple, autosomal dominant pattern. Such factors can therefore be difficult to recognize in epidemiological studies.

At present, there are four well confirmed genes in which mutations or polymorphisms can result in AD, and several other candidates are in various stages of confirmation. The first AD-causing gene to be identified was that encoding the precursor of Aβ, the β-amyloid precursor protein (APP). Missense mutations in APP account for a tiny fraction (less than 0.1%) of all Alzheimer's cases, but they have proved to be highly informative as regards the pathogenic mechanisms of AD in general. For example, expression of mutant APP transgenically in mice provided the first reproducible and robust animal models of the disease. Inheritance of one or two ε4 alleles of ApoE is a far more prevalent genetic basis for AD. ApoE4 helps precipitate the disorder primarily in subjects in their sixties and seventies, thus lowering the typical age of late-onset AD[5]. There is also evidence that an alternative ApoE allele, ε2, confers some protection from the development of AD. It should be emphasized that ApoE4 is a risk factor for, not an invariant cause of, AD. Some humans who are homozygous for this isoform continue to show no Alzheimer symptoms in their nineties. The third and fourth genes implicated in familial forms of AD are designated presenilin-1 (PS1) and presenilin-2 (PS2), because missense mutations result in an aggressive, early-onset form of the disorder, usually beginning between the age of 40 and 60 years[6–8]. PS1 and PS2 are homologous polytopic proteins that are believed to span certain membranes of cells eight times (see below). More than 50 missense mutations have been identified in PS1 and 2–3 in PS2; these are widely scattered in the molecule but tend to cluster within and adjacent to the transmembrane domains.

The inheritance of a polymorphism in the gene encoding α2-macroglobulin, a large multifunctional protein that can act as a special kind of protease inhibitor, has been associated with increased risk of late-onset AD[9], and genetic epidemiological studies are underway to confirm the occurrence and frequency of this polymorphism in AD. Families with multiple AD members that show no linkage to any of the above five genes are also under study in an attempt to identify or confirm additional genetic risk factors or autosomal dominant mutations. Within a decade or two, a sizeable number of additional genes will be implicated, most of them probably acting as polymorphic risk factors in some populations.

Despite the prominence of tau accumulation in the neurofibrillary tangles and dystrophic neurites of a high percentage of AD cases, the tau gene has so far not been found to be the site of mutations in familial AD. Instead, mutations in tau have been discovered in families with a less common dementia: frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)[10–12]. This disorder is characterized by widespread neurofibrillary tangle formation associated with specific biochemical alterations in the microtubule-binding properties of tau[13] in the absence of amyloid deposits. The discovery of tau mutations in this distinct form of dementia proves that a primary alteration of tau structure and function can lead to progressive, severe neuronal degeneration and, ultimately, to the death of the patient. This finding also shows that even severe neurofibrillary tangle formation does not lead to secondary accumulation of Aβ as diffuse and neuritic plaques. The latter point addresses a recurring controversy in the study of AD, that is, whether plaques or tangles have temporal precedence in the pathogenesis of the disorder. Both the APP and presenilin mutations in AD and the tau mutations in FTDP-17 support the conclusion that the tau alteration in AD follows Aβ accumulation rather than vice versa.

Cell biology of APP

During the past few years, progress in understanding the transport and unusual proteolytic processing of APP, together with the importance of the presenilins in these pathways, has provided insights into the molecular basis of familial AD. The results of

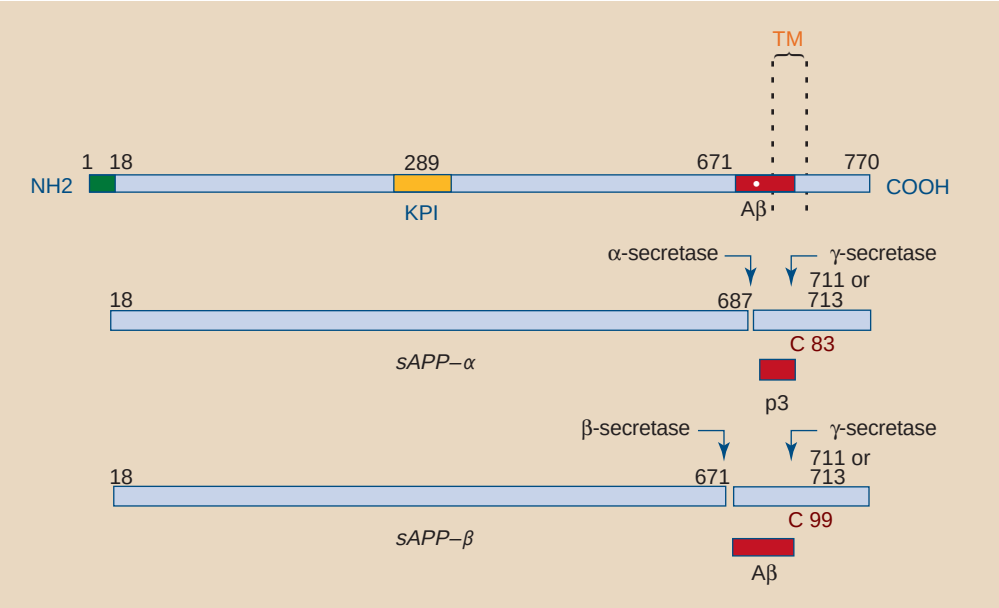

Figure 2 Diagrams of APP and its principal metabolic derivatives. The upper diagram depicts the largest of the known APP alternate splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct relative positions in the linear sequence. A 17-residue signal peptide occurs at the N terminus. An alternatively spliced exon of 56 amino acids is inserted at residue 289; it 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 dashed lines. The Aβ region is indicated in red. In the middle diagram, the left arrow indicates the site (after residue 687; this is the same site as that indicated by the white dot in the Aβ region of the upper diagram) of a constitutive proteolytic cleavage made by a protease(s) designated α-secretase, which enables secretion of the large, soluble ectodomain (sAPP-α) into the medium and retention of the 83-residue C-terminal fragment (C83) in the membrane. The latter can undergo cleavage by the activity termed γ-secretase at residues 711 or 713 to release the $p3_{40}$ and $p3_{42}$ peptides. The lower diagram depicts the alternative proteolytic cleavage after residue 671 by the activity termed β-secretase. This results in the secretion of the slightly truncated sAPP-β molecule and the retention of C99. The latter can also undergo cleavage by γ-secretase at 711 or 713 to release the $Aβ_{40}$ and $Aβ_{42}$ peptides.

these studies have implications also for the mechanism of the phenotypically similar 'sporadic' form of the disease and have provided new information about fundamental features of protein structure and function.

The cloning of the gene on chromosome 21 that encodes APP[14] was made possible by the purification and sequencing of its Aβ fragment from the microvascular amyloid deposits of AD and Down's syndrome patients[15]. APP comprises a group of ubiquitously expressed polypeptides whose heterogeneity arises from both alternative splicing and post-translational processing (reviewed in ref. 16). In addition to the 751- and 770-residue splice forms expressed in non-neuronal cells throughout the body, neurons express a more abundant 695-residue isoform. The difference between the 751/770- and the 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 inhibitors, indicating one potential function of these longer APP isoforms. Indeed, the 751/770 forms of APP present in human platelets serve as inhibitors of Factor XIa (a serine protease) in the coagulation cascade. Nevertheless, deletion of the gene in mice results in neither early mortality nor appreciable morbidity; cerebral gliosis and changes in locomotor behaviour occur later in adult life[17], and neurons cultured at birth may have diminished viability and retarded neurite outgrowth[18].

The lack of a vital consequence of APP deletion *in vivo* may result from mammals expressing proteins that are closely homologous to APP—the amyloid precursor-like proteins (APLPs)[19,20]—but which do not contain the Aβ sequence. Although some activities of holoAPP or its major secreted derivative, sAPP-α, have been inferred using cell-culture studies, the principal function(s) of the molecule *in vivo* remain unclear. Functions that have been described *in vitro* include inhibition of certain serine proteases (for the $APP_{751/770}$ isoforms), enhancement of cell-substrate adhesion, neuritotrophic and other growth-promoting effects, and neuroprotective properties[16]. No evidence has emerged that a fundamental cellular function of APP is lost in AD patients; instead, APP mutations seem to act by a gain-of-function mechanism, namely the increased production of the potentially cytotoxic Aβ fragment (see below).

APP has one ~23-residue hydrophobic stretch near its carboxy-terminal region (Fig. 2) that anchors it in internal membranes (for example, endoplasmic reticulum (ER), Golgi, *trans*-Golgi network and endosome) and in the plasmalemma. Both during and after its transport through the secretory pathway to the cell surface, a subset of APP molecules undergoes specific endoproteolytic cleavages, most frequently by a scission between amino acids 16 and 17 of the Aβ region, that is, 12 residues amino terminal to the transmembrane sequence (Fig. 2). This principal secretory cleavage is effected by a protease(s) designated α-secretase(s). The cut creates a large, soluble ectodomain fragment (sAPP-α) that is released into vesicle lumens and from the cell surface, and a membrane-retained C-terminal fragment (CTF) of 83 amino acids (C83) (Fig. 2). α-Secretase(s) are probably membrane-anchored proteases capable of cleaving diverse single transmembrane proteins, and they seem to cleave APP at a specific distance from the outer membrane surface while showing little sequence specificity[21]. Although the constitutive α-secretases are not yet clearly defined, the regulated cleavage of APP (for example, as enhanced by phorbol esters) may be carried out by certain metalloprotease disintegrins that are capable of shedding the ectodomains of proteins such as tumour-necrosis factor-α (TNF-α)[22,23]. In most cell types, a minority of all APP molecules undergoes α-secretory cleavage, so that any increase in this scission would still leave many APP polypeptides that could be subjected to the alternative cleavages (made by the β- and γ-secretases) that lead to Aβ formation.

The generation of amyloid-β protein

Aβ is secreted constitutively by normal cells in culture and detected as a circulating peptide in the plasma and cerebrospinal fluid (CSF) of healthy humans and other mammals[24–27]. When this unexpected observation was made in 1992, it was also recognized that a smaller fragment, which had a relative molecular mass of 3,000 (M_r 3K) and which comprised the latter two-thirds of Aβ (designated p3), was released constitutively by APP-expressing cells during normal metabolism[24]. These and other studies have shown that the N terminus of p3 is generated when α-secretase cleaves APP, and its C terminus is generated when the resultant C83 CTF is cleaved by the unusual activity referred to as γ-secretase(s) (Fig. 2). In an analogous fashion, other APP holoproteins are instead cleaved by β-secretase just before the Aβ region to create its N terminus, followed by cleavage of the resultant 99-residue CTF (C99) by γ-secretase(s) to create Aβ (Fig. 2). The scission by β-secretase releases a truncated form of sAPP (sAPP-β) from the cell[28]. Although precise quantification is not available, it seems that a substantially smaller portion of total cellular APP undergoes cleavage by β- than by α-secretase. Moreover, not all of the resultant C99 and C83 fragments are processed by γ-secretase to Aβ and p3, respectively; alternative proteolytic pathways can fully degrade these CTFs, probably in late endosomes and lysosomes (Box 1).

Box 1 Complexity of the amyloidogenic processing of APP

Aβ peptides present in culture medium, human CSF and the brain amyloid deposits of AD subjects show heterogeneity in both their amino- and carboxy-terminal regions[24,26,88–90]. The C-terminal heterogeneity of Aβ has special importance for its aggregation. Immunohistochemistry with antibodies that selectively recognize either the Val 40 or the Ala 42 C terminus have revealed that the first Aβ form deposited as diffuse plaques in AD and Down's syndrome brains ends at residue 42 (refs 47, 90). In studies of the temporal progression of plaque formation in the brains of Down's syndrome patients of increasing age, $Aβ_{42}$ peptides can form numerous diffuse plaques as early as age 12 years, whereas $Aβ_{40}$ is first detected in the plaques almost 20 years later[47]. This evidence of initial $Aβ_{42}$ deposition in AD and Down's syndrome brains fits well with biochemical studies[91] showing that the $Aβ_{42}$ peptide, with its two additional hydrophobic residues, aggregates far more rapidly into amyloid fibrils (as well as into intermediate assemblies called protofibrils[84,85]) than does the $Aβ_{40}$ peptide.

Because the β- and γ-secretases have yet to be identified definitively, it is difficult to determine their precise location in the cell. A portion of Aβ peptides appears to be generated in recycling endosomes after internalization of APP molecules from the cell surface. That surface APP can indeed undergo clathrin-mediated endocytosis and then recycle rapidly to the surface has been established in both non-neural[92] and neuronal[93] cells. The clearest evidence that Aβ can be generated from reinternalized APP molecules has come from experiments in which APP on the plasma membrane of intact cells was radioiodinated and allowed to internalize at 37 °C; this led to the release within 15–30 minutes of radioiodinated Aβ that could have arisen only from the surface-labelled molecules[94]. As regards the loci for $Aβ_{42}$ formation, the chemical retardation of APP transport through the secretory pathway and the use of sensitive Aβ enzyme-linked immunosorbent assays (ELISAs) on isolated vesicle fractions have suggested that $Aβ_{42}$ can be generated early during secretory processing (for example, in the ER and Golgi)[62,95,96]. Direct quantification of both Aβ peptides in subcellular fractions indicates that $Aβ_{42}$ is the most abundant species in ER-rich fractions, whereas more $Aβ_{40}$ than $Aβ_{42}$ is detectable in Golgi-rich fractions[62]. That Aβ can be generated and/or accumulate at various points during the secretory processing of APP is further supported by biochemical and immunocytochemical experiments detecting sAPP-β in ER[97] and post-Golgi secretory vesicles[98], Aβ42 in ER[99] and Aβ peptides in detergent-insoluble glycolipid (DIG) membranes[100].

Cell biology of the presenilins

PS1 and PS2 are homologous, polytopic membrane proteins that have been localized so far to ER and Golgi in mammals. A member of the *Caenorhabditis elegans* presenilin family, *sel-12*, is a facilitator of *lin-12*/Notch signalling during the determination of cell fate in development[29]. Wild-type human PS1 can rescue the lethal phenotype caused by *sel-12* mutations in *C. elegans*, whereas most AD-linked mutant PS1 molecules examined in this bioassay confer only partial functional recovery[30,31]. Despite their structural and functional homologies, the precise cellular activities of the presenilin/*sel-12* proteins are not yet known (but see later). PS1 can interact with a novel neuron-specific member of the Armadillo family, δ-catenin, in the yeast two-hybrid system, and both δ- and β-catenins co-immunoprecipitate with PS1 (refs 32, 33). These results indicate that, in addition to facilitating Notch activity, PS1 may interact with members of the Armadillo family that are known to serve as intracellular components of cell–cell adhesion complexes. Deletion of the PS1 gene in mice produces an embryonic-lethal phenotype characterized by severely disordered somitogenesis and axial skeletal development[34,35] as well as by neurodevelopmental changes in the forebrain[35]. Both wild-type and AD-linked mutant PS1 can rescue this knockout phenotype in mice[36]. Thus, the AD-linked presenilin mutations do not confer loss of function in mammals, but instead result in a dominantly transmitted gain of function.

Presenilins are expressed at low abundance in most cell types, including neurons. Steady-state levels of the presenilin holoproteins are low because the precursor undergoes endoproteolysis to generate stable N- and C-terminal fragments[37]. The constitutive proteolytic cleavage site[38] occurs in a hydrophobic portion of the cytoplasmic loop between the sixth and seventh of the eight[39] transmembrane (TM) domains believed to exist in PS1. The steady-state levels of the presenilin N-terminal fragments (NTFs) and CTFs seem to be tightly regulated, as overexpression of PS1 in transfected cells or transgenic mice generally does not increase the levels of the fragments[40]; the excess holoproteins are rapidly degraded, mainly by the proteasome[41]. Once formed, the PS1 fragments associate into higher molecular mass (~150K) complexes that may represent the principal form in which presenilin functions in cells[33,42]. Subcellular fractionation indicates that the PS1 holoprotein is found principally in ER vesicles, where the constitutive endoproteolysis can first be detected; the fragments accumulate subsequently in Golgi-type vesicles, where they are highly stable[43].

By using the yeast two-hybrid system and/or co-immunoprecipitation, several known or newly identified proteins have been shown to interact *in vitro* with PS1 or PS2, but their importance for the normal and pathogenic functions of the presenilins is unclear. In particular, those proteins that interact with either PS1 or PS2 alone seem unlikely to be crucial in the pathogenic mechanism of the presenilins in AD, because mutations of conserved residues in both proteins produce elevation of Aβ42 production[44] and lead to a similar clinicopathological phenotype. Sequences that diverge between PS1 and PS2 (such as the distal TM6 → TM7 'loop' domain of PS1, 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 the C terminus[45]).

Genotype-to-phenotype relationships

Cultured cells and transgenic mice have been used to model the biochemical and neuropathological effects of each of the four genes implicated so far in familial AD. The results have been compared to the actual phenotypes observed in the brains of patients with the respective gene defects. In all four cases, inherited alterations in the gene products have been linked to increases in the cerebral production and/or deposition of the Aβ peptides (reviewed in ref. 46). This work has provided strong support for the importance of cerebral Aβ

accumulation as an early, invariant and necessary event in the genesis of familial AD.

Alterations in the APP gene can lead to the AD syndrome in at least two ways: either by overexpression (owing to a gene dosage effect in trisomy 21 (Down's syndrome)) or by missense mutations that increase the amyloidogenic cleavages of APP at either the β-secretase site (resulting in excessive production of both $A\beta_{40}$ and $A\beta_{42}$) or the γ-secretase site (resulting in selectively increased production of $A\beta_{42}$). In trisomy 21, a lifelong increase in APP expression and the resultant overproduction of both $A\beta_{40}$ and $A\beta_{42}$ peptides is assumed to be responsible for the early appearance of some or many $A\beta_{42}$ diffuse plaques, which occur as early as age 12 years and accumulate with time. Because Down's patients invariably develop the full-blown neuropathology of AD by their forties or fifties, the temporal progression of AD-type lesions between the early teens and the forties has been considered to represent the sequence of pathogenesis in conventional AD. Down's subjects often display diffuse plaques composed solely of $A\beta_{42}$ in their teens and twenties, with accrual of $A\beta_{40}$ peptides onto these plaques and the appearance of associated microgliosis, astrocytosis and surrounding neuritic dystrophy beginning in their late twenties or thirties[47,48]. This observation exemplifies the importance of $A\beta_{42}$ deposition as a potentially seminal event in the development of AD pathology. The appearance of neurofibrillary tangles is also delayed until the late twenties or thirties 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 further loss of cognitive and behavioural functions after the age of 35 or so.

All eight reported APP missense mutations linked to AD are clustered at the β-secretase cleavage site, just after the α-secretase site or just after the γ-secretase site (Fig. 3). Studies in cell cultures and transgenic mice have shown that these mutations enhance either the β-secretase or the γ-secretase cleavage of APP, resulting in chronically elevated levels of $A\beta_{42}$. For the two missense mutations located internally in Aβ (Fig. 3), one produces particularly severe microvascular β-amyloidosis with relatively minor parenchymal deposition (the E22Q mutation, in which glutamine is substituted for glutamic acid at position 22 and which causes hereditary cerebral haemorrhage with amyloidosis of the Dutch type)[49]. The other mutation (glycine substituted for alanine at position 21 (A21G)) leads to a mixed phenotype of AD-type plaque and tangle formation associated with progressive dementia and severe microvascular β-amyloidosis with occasional cerebral haemorrhages[50]. These adjacent mutations within the Aβ sequence may alter α-secretase processing to favour β-secretase cleavage of APP, but they are also likely to increase the propensity of the mutant peptides to aggregate into amyloid fibrils. In short, the APP mutations result in increased production and deposition of Aβ in the brain and its microvasculature. No other APP mutations away from the sites of the secretase cleavages have been discovered in AD families. If APP mutations caused familial AD by perturbing the normal function of the precursor (as has sometimes been hypothesized), then one would expect AD-linked mutations to be more widely distributed in the molecule, not exclusively clustered at the secretase-processing sites.

In the case of the *ApoE4* polymorphism, co-expression of each of the three human *ApoE* alleles with APP in cultured cells shows no differential change in the proteolytic processing of APP to Aβ (ref. 51). Instead, the disease-promoting effect of inheriting one or two *ApoE4* alleles seems to involve enhanced aggregation and/or decreased clearance of Aβ (refs 52–55). The resultant increase in steady-state levels of cerebral Aβ has been demonstrated by crossing APP transgenic mice with knockout mice that lack ApoE: far less Aβ plaque formation is observed in these offspring than when ApoE is present in the brain[56].

Perhaps the most interesting genotype-to-phenotype relationship in AD involves the presenilin mutations. Even before PS1 and PS2 mutations were expressed in cultured cells and transgenic mice, assays of $A\beta_{40}$ and $A\beta_{42}$ in the plasma and skin fibroblast media of humans bearing these mutations revealed a selective 1.5–3-fold elevation in $A\beta_{42}$ (ref. 44). Modelling these mutations *in vitro* and *in vivo* confirmed this result (reviewed in ref. 57). Indeed, crossing mice transgenic for mutant APP with mice expressing a PS1 mutation results in a substantially accelerated AD-like phenotype, with $A\beta_{42}$ plaques (both diffuse and mature) occurring as early as 3–4 months of age[58]. Moreover, the ability of presenilin mutations selectively to enhance $A\beta_{42}$ deposition in the brain has been demonstrated directly in patients carrying these mutations[59,60].

How do mutations in the eight-transmembrane (TM) presenilin proteins cause selective $A\beta_{42}$ hypersecretion? Two broad hypotheses about the mechanism have emerged. One suggests that the presenilin molecule regulates the transport of γ-secretase or APP to each other without any physical interaction with APP. This mechanism has been

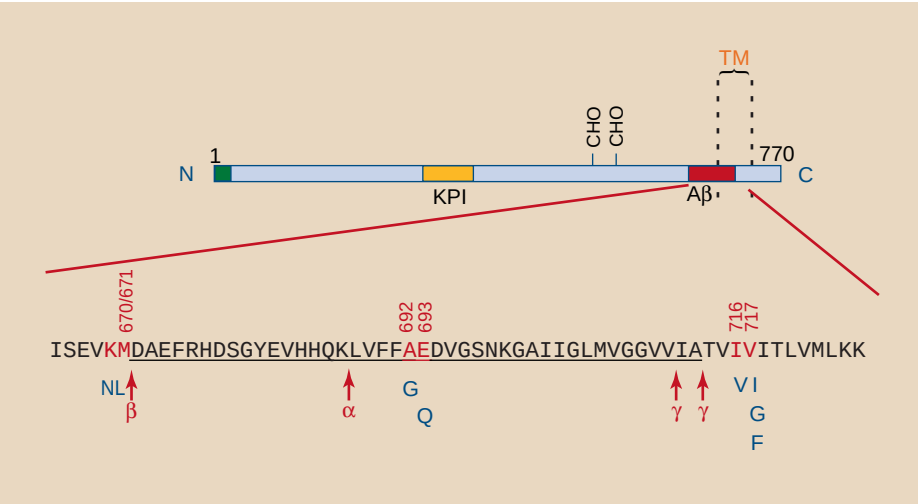

Figure 3 APP mutations causing AD or hereditary cerebral haemorrhage. The wild-type sequence of Aβ and regions that immediately flank it in human APP is shown by the single-letter code. The underlining indicates the $A\beta_{1-42}$ peptide. The bold residues below the wild-type sequence indicate the reported missense mutations linked to familial AD, and their respective codon numbers are given above the sequence. The major sites of the β-, α- and γ-secretase cleavages are indicated.

suggested by investigators who have failed to co-immunoprecipitate APP with PS1 (ref. 61). The alternate hypothesis, physical involvement of the presenilins in the γ-secretase cleavage of APP, has been proposed by those who have detected small amounts of APP co-precipitating with presenilin in whole-cell lysates and in isolated ER and Golgi vesicles[62–64]. If presenilins were required to bring APP and γ-secretase together without actually contacting APP, this transport role must involve membranous microdomains within a subclass of vesicles, as it has already been shown that APP, PS1 and the γ-secretase-mediated products $A\beta_{42}$ and $A\beta_{40}$ can all be recovered together within purified, calnexin-rich ER vesicles[43,62].

Evidence has emerged recently that supports a direct involvement of presenilins in the γ-secretase cleavage of APP[65]. Because mice deficient in PS1 show markedly decreased γ-secretase processing of C99 to Aβ (ref. 66), it seemed possible that PS1 and PS2 could themselves be γ-secretases. However, the multi-transmembrane structure of the presenilins made them unlikely candidates for proteases. Nevertheless, the recognition that all members of the presenilin gene family have two aspartic acid residues in TM6 and TM7, respectively, which flank the constitutive presenilin cleavage site in the proximal part of the TM6 → TM7 cytoplasmic loop, led to the hypothesis that these residues might represent the active site of an unprecedented intramembranous aspartyl protease[65]. Mutation of either transmembrane aspartate to alanine resulted in both the abolition of presenilin endoproteolysis and the marked inhibition of γ-secretase processing of C99 to Aβ (and C83 processing to p3)[65]. Apparently, the Asp → Ala mutant isoforms, when transfected into several cell types, act as dominant negatives to suppress endogenous PS1 and obviate both PS endoproteolysis and γ-secretase cleavage of C99 and C83. These unexpected results indicate that the two transmembrane aspartates either allow presenilins to serve as essential diaspartyl co-factors for both the 'presenilinase' and γ-secretase cleavages, or function as the active site of an intramembranous aspartyl protease by cleaving the $A\beta_{40-41}$ and $A\beta_{42-43}$ peptide bonds within C99 and C83 to generate $A\beta_{40}$ and $A\beta_{42}$ and $p3_{40}$ and $p3_{42}$. Two additional findings are consistent with the second mechanism. First, mutating either of the aspartates to glutamate again disrupts the presenilinase and γ-secretase cleavages, indicating that the conserved, charged glutamate residue cannot substitute for the aspartate[65]. Second, Aβ can be generated from recombinantly expressed C99 *in vitro* using microsomes containing wild-type but not Asp-mutant PS1 and at mildly acidic but not neutral pH[65].

Although these various findings suggest that the presenilins represent the long-sought γ-secretases, definitive proof will require reconstitution of Aβ generation from pure C99 and pure wild-type (but not Asp-mutant) PS1 in phospholipid vesicles, something that may be difficult to accomplish until other protein factors that are believed to regulate presenilin fragments are discovered[40]. If presenilins are, indeed, γ-secretases, they could effect the intramembranous cleavage of other substrates, in particular the Notch proteins, which apparently undergo intramembranous proteolysis[67] and which require the presenilins for their signalling function[29,34]. In this regard, mutation of just one of the PS1 transmembrane aspartates to asparagine has been found to destroy the ability of human PS1 to rescue the lethal phenotype of the *sel-12* mutation in *C. elegans*[68].

Confirmation that presenilins are γ-secretases would provide further support for the amyloid cascade hypothesis of AD. The most common mutations causing familial (autosomal dominant) AD would therefore occur in the very protease that generates Aβ, and mutations in either the substrate or the protease would result in a markedly accelerated and severe AD phenotype.

It is important to emphasize that each new gene implicated in familial forms of AD will need to be similarly analysed to establish its specific effect on the production, deposition or clearance of Aβ, in order to determine whether all genetic forms of the disorder involve an elevation of steady-state levels of Aβ.

The inflammatory and neurotoxic cascade

I have focused on some of the ways in which genetic alterations can lead to chronically elevated concentrations of Aβ in the brain, but the putative downstream effects of this accumulation remain the subjects of intensive study (Fig. 4). Chronic elevation of $A\beta_{42}$ in brain interstitial fluid (and perhaps also inside neurons[69]), caused by defects in the genes encoding APP and the presenilins, is assumed to lead gradually to oligomerization and, eventually, fibrillization of the peptide and its deposition as diffuse and, later, mature plaques. Based on studies of Down's syndrome patients and transgenic mice that express mutant APP and/or PS1, it is hypothesized that $A\beta_{42}$ accumulation and diffuse plaque formation is associated with local microglial activation, cytokine release, reactive astrocytosis and a multi-protein inflammatory response[70–72], including the binding of the C1q component of the classical complement cascade by Aβ (ref. 73) and the triggering of this cascade[74]. It has been proposed that such a glial inflammatory process and/or any direct neurotoxic effects of oligomeric and fibrillar Aβ could produce the multifaceted biochemical and structural changes in surrounding axons, dendrites and neuronal cell bodies that characterize the limbic and association cortices in AD. There is considerable evidence that the effects of an Aβ-initiated inflammatory and neurotoxic process include excessive generation of free radicals and peroxidative injury to proteins and other macromolecules in neurons[75,76]. In this regard, a therapeutic trial of the antioxidant, vitamin E, seemed to result in slower clinical progression of the disease, although actual amnestic symptoms were not noticeably improved[77]. Among the many possible metabolic consequences of Aβ accumulation and aggregation, altered ionic homeostasis, particularly excessive calcium entry into neurons, could well contribute to selective neuronal dysfunction and cell death, based on studies of the *in vitro* effects of aggregated Aβ (refs 78–80). Establishing definitively that Aβ accumulation triggers the hyperphosphorylation of tau, which precedes the assembly of these molecules into PHF[13], must await the production of neurofibrillary tangles in transgenic mice that overexpress both mutant APP and human tau. Although tangles have not been described in existing APP mouse models[81–83], crossing APP/presenilin doubly transgenic mice with mice that overexpress human tau bearing one of the tangle-promoting mutations might be expected to lead to a 'plaque-plus-tangle' phenotype that closely resembles the AD state.

Although substantial evidence supports the Aβ-mediated cytopathological cascade summarized briefly in the preceding paragraph, many questions remain unanswered. First, what are the relative contributions of extracellular compared with intraneuronal Aβ accumulation in initiating the neurotoxic response? Whereas immunohistochemistry shows abundant extracellular Aβ in AD brains, the same antibodies seem not to detect any intracellular accumulation. Nevertheless, small amounts of Aβ dimers or higher oligomers could well be accumulating intracellularly[69], and their role in neuronal dysfunction will need to be established. Second, are Aβ fibrils the principal toxic moiety in the disease, or do they instead represent a relatively inert aggregate, so that smaller assembly forms such as protofibrils[84,85] or even diffusible dimers or other oligomers[86] actually serve as the microglia-activating and neuron-injuring species? In this context, are highly fibrillar 'mature' plaques simply a consequence of the local accumulation of Aβ to high levels, allowing smaller, diffusable species of the peptide in equilibrium with the fibrils to serve as the toxic moieties?

Another pressing question is whether apoptosis of neurons is important for producing AD brain dysfunction. Although presenilins (particularly mutant PS2) have been associated with enhanced apoptosis in cell-culture studies[87], whether and how presenilins mediate apoptosis *in vivo* will need to be considered in light of the possibility that presenilin is an aspartyl protease that processes APP and probably other intramembranous substrates such as Notch[65]. Moreover, the ultrastructural appearance of the

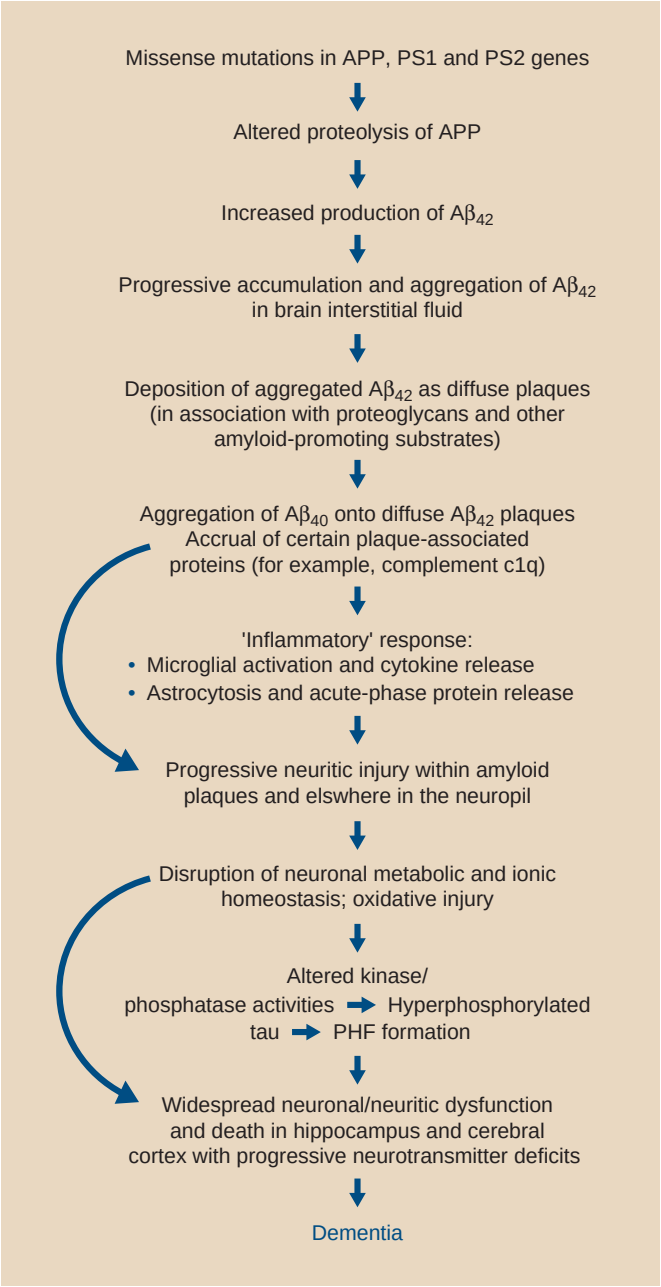

Figure 4 A hypothetical sequence of the pathogenetic steps of familial forms of AD.

many tangle-bearing neurons present in the AD brain suggests a gradual dysfunction and slow death of the affected neurons rather than a sudden, apoptotic loss. Tangle-bearing neurons often show relatively well preserved organelle structure by electron microscopy, with intact ER, nuclear membranes and mitochondria, which suggests that the cells are chronically dysfunctional but not near apoptosis. Yet another unresolved question concerns the selective vulnerability of neuronal populations in AD. Local and regional differences in the pathogenic process arise on at least two broad levels. First, $A\beta_{42}$ can accumulate chronically in some brain regions (for example, cerebellum, striatum and thalamus) with little or no evolution to amyloid fibrils and their associated neuritic and glial cytopathology. At this level, regionally specific factors (for example, pro- or anti-aggregating proteins) may exist that enable Aβ to proceed into aggregated forms or prevent it from doing so. Anti-aggregating factors that are present in regions spared from mature plaque formation could be of potential therapeutic interest. The second level at which selective vulnerability may act relates to the observation that not all neurons and neurites in the vicinity of mature, fibril-rich plaques undergo neurotoxic changes. The complex issue of what allows some neurons to succumb and others to survive may be among the most difficult to resolve, as it has been in many other selective neurodegenerative disorders of varying aetiology.

Notwithstanding the many unanswered questions about the inflammatory and neurotoxic cascade of AD, studies of disease progression in Down's syndrome and transgenic mouse models continue to support Aβ accumulation as an early event in most or all forms of the syndrome. Interference with early steps in the process, such as Aβ production or Aβ assembly, may therefore prove a more attractive therapeutic strategy than attempting to block the multiple downstream effects of the peptide and its many associated proteins.

Predictions for pharmacological intervention

Despite our incomplete understanding of AD, sufficient progress in delineating the disease cascade has been achieved to suggest several discrete targets for treatment. These include: (1) inbititors of Aβ production (that is, small compounds that decrease but do not eliminate β- or γ-secretase activity); (2) inhibitors of Aβ oligomerization or fibrillization; (3) anti-inflammatory drugs that could interfere with aspects of the microglial and astrocytic responses in the brain; (4) antioxidants, free-radical scavengers, calcium-channel blockers and modulators of signal transduction that could protect neurons from the downstream effects of the accumulation of Aβ and its associated proteins; and (5) neurorestorative factors (for example, neurotrophins and small compounds mimicking their action; oestrogens) that could conceivably rescue synapses and cell bodies undergoing active injury. All of these approaches should be pursued, because success of one particular strategy cannot be predicted and two or more approaches might ultimately be combined to treat a patient, depending on the temporal stage in the disorder. Current, largely symptomatic treatments aimed at enhancing the levels of depleted neurotransmitters such as acetylcholine may still be used, even if more specific treatments aimed at early steps in the disease are forthcoming. Based on the emerging importance of Aβ, γ-secretase inhibitors or other Aβ-lowering compounds may be the first agents to reach clinical trials, although it is possible that partial inhibition of this highly unusual protease may have serious side effects. Although downregulating Notch and APP processing could be considered as dangerous consequences of a γ-secretase inhibitor, the therapeutic goal is to induce partial (perhaps 30–40%) inhibition of the protease, just as has been safely accomplished for another vital enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, in chronically lowering cholesterol.

In the future, it is likely that individuals reaching their fifties or beyond will be offered a specific risk-assessment profile to determine their likelihood of developing AD. Such an assessment, perhaps modelled on that now widely used to judge the risk of serious atherosclerotic disease, would include inquiry about a positive family history, identification of specific predisposing genetic factors, structural and functional brain imaging to detect evidence of presymptomatic lesions, and measurement of $A\beta_{42}$, tau and other markers of the neuropathology in the spinal fluid and perhaps (in the case of Aβ) even in the blood. Based on further epidemiological experience with such assessment measures in large populations of elderly 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 particularly high risk could be offered preventative treatment with one or more of the agents contemplated in the previous paragraph. Although the achievement of an integrated diagnostic

and therapeutic approach to this complex and tragic disorder may still seem remote, the current rate of scientific progress indicates that some level of practical success may come sooner than one might think. If so, the knowledge gained from mechanistic and therapeutic research on AD should greatly facilitate efforts to understand and treat other brain disorders, for example, Parkinson's and Huntington's diseases, that may also involve abnormal protein aggregation. □

Dennis J. Selkoe is at the Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA.

1. Dickson, D. W. The pathogenesis of senile plaques. *J. Neuropathol. Exp. Neurol.* **56,** 321–339 (1997).
2. Yamaguchi, H., Nakazato, Y., Hirai, S., Shoji, M. & Harigaya, Y. Electron micrograph of diffuse plaques: initial stage of senile plaque formation in the Alzheimer brain. *Am. J. Pathol.* **135,** 593–597 (1989).
3. Goedert, M., Trojanowski, J. Q. & Lee, V. M.-Y. in *The Molecular and Genetic Basis of Neurological Disease*, 2nd edn (eds Rosenberg, R. N., Prusiner, S. B., DiMauro, S. & Barchi, R. L.) 613–627 (Butterworth-Heinemann, Boston, 1996).
4. Strittmatter, W. J. *et al.* 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).
5. Saunders, A. M. *et al.* Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43,** 1467–1472 (1993).
6. Sherrington, R. *et al.* Cloning of a novel gene bearing missense mutations in early onset familial Alzheimer disease. *Nature* **375,** 754–760 (1995).
7. Levy-Lahad, E. *et al.* Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269,** 973–977 (1995).
8. Rogaev, E. I. *et al.* Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376,** 775–778 (1995).
9. Blacker, D. *et al.* Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nature Genet.* **19,** 357–360 (1998).
10. Spillantini, M. G. *et al.* Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl Acad. Sci. USA* **95,** 7737–7741 (1998).
11. Hutton, M. *et al.* Association of missense and 5′-splice-site mutations in tau with the inherited FTDP-17. *Nature* **393,** 702–705 (1998).
12. Poorkaj, P. *et al.* Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol.* **43,** 815–825 (1998).
13. Goedert, M. Tau mutations cause frontotemporal dementias. *Neuron* **21,** 955–958 (1998).
14. Kang, J. *et al.* The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325,** 733–736 (1987).
15. Glenner, G. G. & Wong, C. W. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun.* **122,** 1131–1135 (1984).
16. Selkoe, D. J. Cell biology of the amyloid β-protein precursor and the mechanism of Alzheimer's disease. *Annu. Rev. Cell Biol.* **10,** 373–403 (1994).
17. Zheng, H. *et al.* β-amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* **81,** 525–531 (1995).
18. Perez, R. G., Zheng, H., Van der Ploeg, L. H. & Koo, E. H. The beta-amyloid precursor protein of Alzheimer's disease enhances neuron viability and modulates neuronal polarity. *J. Neurosci.* **17,** 9407–9414 (1997).
19. Wasco, W. *et al.* 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).
20. Slunt, H. H. *et al.* Expression of a ubiquitous, cross-reactive homologue of the mouse β-amyloid precursor protein (APP). *J. Biol. Chem.* **269,** 2637–2644 (1994).
21. Sisodia, S. S. β-amyloid precursor protein cleavage by a membrane-bound protease. *Proc. Natl Acad. Sci. USA* **89,** 6075–6079 (1992).
22. Black, R. A. *et al.* A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. *Nature* **385,** 729–733 (1997).
23. Buxbaum, J. D. *et al.* Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem.* **273,** 27765–27767 (1998).
24. Haass, C. *et al.* Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature* **359,** 322–325 (1992).
25. Shoji, M. *et al.* Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science* **258,** 126–129 (1992).
26. Seubert, P. *et al.* Isolation and quantitation of soluble Alzheimer's β-peptide from biological fluids. *Nature* **359,** 325–327 (1992).
27. Busciglio, J., Gabuzda, D. H., Matsudaira, P. & Yankner, B. A. Generation of β-amyloid in the secretory pathway in neuronal and nonneuronal cells. *Proc. Natl Acad. Sci. USA* **90,** 2092–2096 (1993).
28. Seubert, P. *et al.* Secretion of β-amyloid precursor protein cleaved at the amino-terminus of the β-amyloid peptide. *Nature* **361,** 260–263 (1993).
29. Levitan, D. & Greenwald, I. Facilitation of *lin-12*-mediated signalling by *sel-12*, a *Caenorhabditis elegans S182* Alzheimer's disease gene. *Nature* **377,** 351–354 (1995).
30. Levitan, D. *et al.* Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **93,** 14940–14944 (1996).
31. Baumeister, R. *et al.* 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).
32. Zhou, J. *et al.* Presenilin 1 interaction in the brain with a novel member of the Armadillo family. *NeuroReport* **8,** 2085–2090 (1997).
33. Yu, G. *et al.* 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).
34. Wong, P. *et al.* Presenilin 1 is required for Notch 1 and D111 expression in the paraxial mesoderm. *Nature* **397,** 288 (1997).
35. Shen, J. *et al.* Skeletal and CNS defects in presenilin-1 deficient mice. *Cell* **89,** 629–639 (1997).
36. Qian, S. *et al.* Mutant human presenilin 1 protects presenilin 1 null mouse against embryonic lethality and elevates Abeta1-42/43 expression. *Neuron* **20,** 611–617 (1998).
37. Thinakaran, G. *et al.* Endoproteolysis of presenilin 1 and accumulation of processed derivatives *in vivo*. *Neuron* **17,** 181–190 (1996).
38. Podlisny, M. B. *et al.* 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).
39. Li, X. & 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).
40. Thinakaran, G. *et al.* Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *J. Biol. Chem.* **272,** 28415–28422 (1997).
41. Steiner, H. *et al.* Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation. *J. Biol. Chem.* **273,** 32322–32331 (1998).
42. Capell, A. *et al.* The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100-150-kDa molecular mass complex. *J. Biol. Chem.* **273,** 3205–3211 (1998).
43. Zhang, J. *et al.* Subcellular distribution and turnover of presenilins in transfected cells. *J. Biol. Chem.* **273,** 12436–12442 (1998).
44. Scheuner, D. *et al.* 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).
45. Tomita, T. *et al.* 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).
46. Selkoe, D. J. Alzheimer's disease: genotypes, phenotype, and treatments. *Science* **275,** 630–631 (1997).
47. Lemere, C. A. *et al.* 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).
48. Mann, D. M. *et al.* Microglial cells and amyloid beta protein (A beta) deposition; association with A beta 40-containing plaques. *Acta Neuropathol. (Berl.)* **90,** 472–477 (1995).
49. Levy, E. *et al.* Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. *Science* **248,** 1124–1126 (1990).
50. Hendriks, L. *et al.* 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).
51. Biere, A. L. *et al.* Co-expression of β-amyloid precursor protein (βAPP) and apolipoprotein E in cell culture: analysis of βAPP processing. *Neurobiol. Dis.* **2,** 177–187 (1995).
52. Schmechel, D. E. *et al.* 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).
53. Rebeck, G. W., Reiter, J. S., Strickland, D. K. & Hyman, B. T. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* **11,** 575–580 (1993).
54. Ma, J., Yee, A., Brewer, H. B. Jr, Das, S. & Potter, H. The amyloid-associated proteins α1-antichymotrypsin and apolipoprotein E promote the assembly of the Alzheimer β-protein into filaments. *Nature* **372,** 92–94 (1994).
55. Evans, K. C., Berger, E. P., Cho, C.-G., Weisgraber, K. H. & Lansbury, P. T. 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).
56. Bales, K. R. *et al.* Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. *Nature Genet.* **17,** 263–264 (1997).
57. Hardy, J. The Alzheimer family of diseases: many etiologies, one pathogenesis? *Proc. Natl Acad. Sci. USA* **94,** 2095–2097 (1997).
58. Holcomb, L. *et al.* Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Med.* **4,** 97–100 (1998).
59. Lemere, C. A. *et al.* The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. *Nature Med.* **2,** 1146–1148 (1996).
60. Mann, D. M. A. *et al.* 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).
61. Thinakaran, G. *et al.* Stable association of presenilin derivatives and absence of presenilin interactions with APP. *Neurobiol. Dis.* **4,** 438–453 (1998).
62. Xia, W. *et al.* 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).
63. Weidemann, A. *et al.* Formation of stable complexes between two Alzheimer's disease gene products: presenilin-2 and β-amyloid precursor protein. *Nature Med.* **3,** 328–332 (1997).
64. Xia, W., Zhang, J., Perez, R., Koo, E. H. & Selkoe, D. J. 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).
65. Wolfe, M. S. *et al.* Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* **398,** 513–517 (1999).
66. De Strooper, B. *et al.* Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391,** 387–390 (1998).
67. Schroeter, E. H., Kisslinger, J. A. & Kopan, R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* **393,** 382–386 (1998).
68. Brockhaus, M. *et al.* Caspase-mediated cleavage is not required for the activity of presenilins in amyloidogenesis and NOTCH signaling. *NeuroReport* **9,** 1481–1486 (1998).
69. Skovronsky, D. M., Doms, R. W. & Lee, V. M.-Y. Detection of a novel intraneuronal pool of insoluble amyloid β protein that accumulates with time in culture. *J. Cell Biol.* **141,** 1031–1039 (1998).
70. Eikelenboom, P., Zhan, S. S., van Gool, W. A. & Allsop, D. Inflammatory mechanisms in Alzheimer's disease. *Trends Pharmacol. Sci.* **15,** 447–450 (1994).
71. McGeer, P. L. & McGeer, E. G. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res. Rev.* **21,** 195–218 (1995).
72. Rogers, J. *et al.* Inflammation and Alzheimer's disease pathogenesis. *Neurobiol. Aging* **17,** 681–686 (1996).
73. Rogers, J. *et al.* Complement activation by β-amyloid in Alzheimer disease. *Proc. Natl Acad. Sci. USA* **89,** 10016–10020 (1992).
74. Itagaki, S., Akiyama, H., Saito, H. & McGeer, P. L. Ultrastructural localization of complement membrane attack complex (MAC)-like immunoreactivity in brains of patients with Alzheimer's disease. *Brain Res.* **645,** 78–84 (1994).
75. Behl, C., Davis, J. B., Lesley, R. & Schubert, D. Hydrogen peroxide mediates amyloid β protein toxicity. *Cell* **77,** 817–827 (1994).
76. Harris, M. E., Hensley, K., Butterfield, D. A., Leedle, R. A. & Carney, J. M. 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).
77. Sano, M. *et al.* A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N. Engl. J. Med.* **336,** 1216–1222 (1997).
78. Mattson, M. P. *et al.* β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci.* **12,** 379–389 (1992).

79. Pike, C. J., Burdick, D., Walencewicz, A. J., Glabe, C. G. & Cotman, C. W. Neurodegeneration induced by β-amyloid peptides in vitro: the role of peptide assembly state. *J. Neurosci.* **13,** 1676–1687 (1993).
80. Lorenzo, A. & Yankner, B. β-amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. *Proc. Natl Acad. Sci. USA* **91,** 12243–12247 (1994).
81. Games, D. *et al.* Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* **373,** 523–527 (1995).
82. Hsiao, K. *et al.* Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* **274,** 99–102 (1996).
83. Sturchler-Pierrat, C. *et al.* Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc. Natl Acad. Sci. USA* **94,** 13287–13292 (1997).
84. Harper, J. D., Wong, S. S., Lieber, C. M. & Lansbury, P. T. Jr Observation of metastable Aβ amyloid protofibrils by atomic force microscopy. *Chem. Biol.* **4,** 119–125 (1997).
85. Walsh, D. M. *et al.* Amyloid β-protein fibrillogenesis: detection of a protofibrillar intermediate. *J. Biol. Chem.* **272,** 22364–22374 (1997).
86. Lambert, M. P. *et al.* Diffusible, nonfribrillar ligands derived from $A\beta_{1-42}$ are potent central nervous system neurotoxins. *Proc. Natl Acad. Sci. USA* **95,** 6448–6453 (1998).
87. Wolozin, B. *et al.* Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. *Science* **274,** 1710–1713 (1996).
88. Miller, D. L., Papayannopoulos, I. A., Styles, J. & Bobin, S. A. Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. *Arch. Biochem. Biophys.* **301,** 41–52 (1993).
89. Roher, A. E. *et al.* β-amyloid-(1–42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **90,** 10836–10840 (1993).
90. Iwatsubo, T. *et al.* Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). *Neuron* **13,** 45–53 (1995).
91. Jarrett, J. T., Berger, E. P. & Lansbury, P. T. 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).
92. Yamazaki, T., Koo, E. H. & Selkoe, D. J. Trafficking of cell-surface amyloid β-protein precursor. II. Endocytosis, recycling and lysosomal targeting detected by immunolocalization. *J. Cell Sci.* **109,** 999–1008 (1996).
93. Yamazaki, T., Selkoe, D. J. & Koo, E. H. Trafficking of cell surface β-amyloid precursor protein: retrograde and transcytotic transport in cultured neurons. *J. Cell Biol.* **129,** 431–442 (1995).
94. Koo, E. H. & Squazzo, S. Evidence that production and release of amyloid β-protein involves the endocytic pathway. *J. Biol. Chem.* **269,** 17386–17389 (1994).
95. Wilde-Bode, C. *et al.* Intracellular generation and accumulation of amyloid β-peptide terminating at amino acid 42. *J. Biol. Chem.* **272,** 16085–16088 (1997).
96. Cook, D. G. *et al.* Alzheimer's Aβ (1–42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nature Med.* **3,** 1021–1023 (1997).
97. Chyung, A. S. C., Greenberg, B. D., Cook, D. G., Doms, R. W. & Lee, V. M.-Y. Novel β-secretase cleavage of β-amyloid precursor protein in the endoplasmic reticulum/intermediate compartment of NT2N cells. *J. Cell Biol.* **138,** 671–680 (1997).
98. Haass, C. *et al.* The Swedish mutation causes early-onset Alzheimer's disease by β-secretase cleavage within the secretory pathway. *Nature Med.* **1,** 1291–1296 (1995).
99. Hartmann, T. *et al.* Distinct sites of intracellular production for Alzheimer's disease Aβ-40/42 amyloid peptides. *Nature Med.* **3,** 1016–1020 (1997).
100. Lee, S. J. *et al.* A detergent-insoluble membrane compartment contains Aβ *in vivo*. *Nature Med.* **4,** 730–734 (1998).