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Neurobiology of Alzheimer's Disease

Philip C. Wong, Alena Savonenko, Tong Li, Donald L. Price

OUTLINE

ALZHEIMER'S DISEASE IS THE MOST PREVALENT NEURODEGENERATIVE DISEASE OF THE ELDERLY

Alzheimer's disease (AD) manifests as progressive memory loss and cognitive impairments (Perrin et al., 2009; Price et al., 1998; Price & Sisodia, 1998; Citron, 2010; Buckner et al., 2008; Querfurth et al., 2010; McKhann et al., 1984; Golde et al., 2011; Ashe et al., 2010; Bishop et al., 2010). AD affects more than 4 million elderly individuals in the United States (Brookmeyer et al., 1998; Ashe et al., 2010). The syndrome results from dysfunction and death of specific populations of neurons, particularly those in neural systems involved in memory, cognition and self-reflection (McKhann et al., 1984; Buckner et al., 2008; Perrin et al., 2009). In AD, CNS abnormalities are demonstrable by laboratory assessments of levels of Aβ (beta amyloid; decreased) and of tau (increased) in CSF (Perrin et al., 2009) and by imaging studies (Perrin et al., 2009; Buckner et al., 2008; Klunk et al., 2004). The neuropathology of AD is characterized by intracellular and extracellular protein aggregates (tau- and Aβ-related abnormalities) in neurofibrillary tangles (NFT) and neuritic amyloid plaques, respectively (Morris & Price, 2001; Braak et al., 1991; Goedert et al., 2006; Ballatore et al., 2007; Price et al., 1998; Price & Sisodia, 1998; Perrin et al., 2009; Brookmeyer et al., 1998; Ashe et al., 2010j; Querfurth et al., 2010; Golde et al., 2011).

Genetic evidence indicates that the inheritance of mutations in several genes. Those for amyloid precursor protein (*APP*), presenilins 1 and 2 (*PS1* and *PS2*), cause autosomal dominant familial AD (fAD) (Bertram et al., 2008). In contrast,

the presence of certain alleles of the apolipoprotein E (ApoE) are significant dose-dependent risk factors for putative sporadic disease *(ApoE4)*, while the presence of others *(ApoE2)* shows lower risk for AD (Bertram et al., 2008; Bu, 2009; Kim et al., 2009a). Other genes may represent additional risk factors for AD, but some 95% of cases are classified as sporadic AD and many details of pathogenesis remain poorly understood.

Due to increased life expectancy and the postwar baby boom, the elderly are the most rapidly growing segment of our society and over the next several decades, the number of persons with AD in the United States will triple (Brookmeyer et al., 1998). Because of prevalence, the paucity of mechanism-based treatments, the expense of care and the significant impacts on individuals, caregivers, and society-at-large, AD is one of the most challenging diseases in medicine (Bertram et al., 2008; Frisardi et al., 2010; Perrin et al., 2009).

The clinical syndrome, ranging from mild cognitive impairments to severe dementia, reflects biochemical and cellular abnormalities in specific regions and circuits in the brain

The disease often initially manifests as a syndrome termed mild cognitive impairment (MCI), which is usually characterized by memory complaints and mild impairments of cognition on formal testing, with intact general cognition, preserved daily activities and absence of overt dementia (Petersen et al., 2001; Perrin et al., 2009; Morris et al., 2001; Petersen, 2003; McKhann et al., 1984). MCI is regarded as a transitional stage between normal aging and early AD. The clinical manifestations of symptomatic AD include increasing difficulties with memory and other cognitive functions (executive skills, language, attention, judgment, sense of self, orientation, fund of general information, etc.) (Perrin et al., 2009h; Petersen et al., 2001; Petersen, 2003; Morris et al., 2001; Buckner et al., 2008). Some patients develop psychotic symptoms. As the disease evolves, a variety of mental functions and activities of daily living become increasingly impaired (Perrin et al., 2009; Buckner et al., 2008; Golde et al., 2011; McKhann et al., 1984). In the late stages, affected individuals become profoundly demented and usually die of intercurrent illnesses. For a diagnosis of AD, clinicians rely on histories; physical, neurological and psychiatric examinations; neuropsychological tests; and a variety of laboratory studies (Perrin et al., 2009; Buckner et al., 2008; Sunderland et al., 2003; Klunk et al., 2004; McKhann et al., 1984; Morris & Price, 2001; Golde et al., 2011; Nestor et al., 2004).

Advances in laboratory measurements and imaging are of value in establishing the diagnosis of AD

In cases of AD, the levels of Aβ peptides in CSF are often low, and levels of tau may be higher than in controls (Sunderland et al., 2003; Perrin et al., 2009). Values vary between individuals and serial measures may be of great diagnostic value. Over time, the clinical profile, in concert with laboratory assessments and results from imaging studies (described below), allow the clinicians to make a diagnosis of possible or probable AD (McKhann et al., 1984; Perrin et al., 2009).

In cases of AD, functional magnetic resonance imaging (fMRI) often discloses progressive evidence of brain atrophy, particularly involving hippocampus and entorhinal cortex (Nestor et al., 2004). Rates of atrophy may correlate with changes in clinical status and may have predictive value for diagnosis. Positron emission tomography (PET) with ^{18}F deoxyglucose or single photon emission computerized tomography (SPECT) can be utilized to demonstrate decreased glucose utilization and reductions in regional blood flow in the parietal and temporal lobes of patients with AD.

Recent advances in imaging have influenced our thinking about AD. First, PET imaging, using radiolabeled Pittsburgh Compound B (PIB), which is a brain penetrant ^{11}C-labeled uncharged thioflavin derivative that binds to Aβ with high affinity, was used to visualize Aβ amyloid burden *in vivo* (Klunk et al., 2004). The patterns of PIB labeling are interpreted to reflect the amounts and distributions of Aβ in the brain (Klunk et al., 2004; Buckner et al., 2008; Perrin et al., 2009). In comparison with controls, subjects with AD show retention of label in areas of brain found to accumulate amyloid in postmortem AD brain. This approach should prove useful for enhancing accuracy of diagnosis, particularly in early stages of the disease, and, potentially, allow assessments of the efficacies of new anti-amyloid therapeutics (Perrin et al., 2009; Bateman et al., 2006; Bateman et al., 2009; Citron, 2010; Frisardi et al., 2010; Golde et al., 2011).

Second, in 2001, Raichle and colleagues, using fMRI approaches (Zhang & Raichle, 2010), delineated an integrated system of circuits and hubs, termed the "default mode network" (DMN), which exhibits high levels of synchronous neuronal activity occurring while individuals are engaged in internally focused tasks, including recall of past events; envisioning the future; and considering the perspectives of other persons (Buckner et al., 2008; Perrin et al., 2009; Sperling et al., 2009). Subsequently, correlations were shown to exist between the neuroanatomical distributions of the default network and the localization of amyloid in the brain (Buckner et al., 2008). Because the formation of Aβ appears to be linked to synaptic activity (i.e., in simple terms, activity increases amyloid) (Buckner et al., 2008; Perrin et al., 2009; Zhang & Raichle, 2010), it has been hypothesized that the intrinsic high levels of synaptic activity and the increased metabolism of the DMN might play a critical role in the formation and release of Aβ at synaptic terminals in the circuits of this system. Buckner and colleagues have shown that Aβ amyloid accumulates in brain regions of this network (Buckner et al., 2008). Elevated levels of amyloid have been documented to occur in the default network of asymptomatic and minimally impaired older individuals (Sperling et al., 2009). Increasingly, results from these several imaging approaches will be combined with information from analyses of biomarkers in CSF (Bateman et al., 2006; Bateman et al., 2009; Golde et al., 2011; Perrin et al., 2009; Sunderland et al., 2003) and, possibly, of new markers in plasma (Reddy et al., 2011) to help establish the diagnoses of early AD and to assess outcomes of therapy (Bateman et al., 2006; Bateman et al., 2009; Perrin et al., 2009).

Familial forms of AD are associated with mutations in select genes inherited as autosomal dominants, while variants in other genes can lead to increased risk of sporadic AD

Genetic risk factors for AD include mutations in APP (chromosome 21); mutations in presenilin 1 (PS1) (chromosome 14) and PS2 (chromosome 1); and different susceptibility alleles of ApoE (chromosome 19) (Bu, 2009; Bertram et al., 2008; Kim et al., 2009a). Other influences include variant haplotypes of sortilin-related receptor 1 (SORLI); and variants of clusterin (CLU) and phosphatidylinositol-binding clathrin assembly protein (PICALM) (Harold et al., 2009; Rogaeva et al., 2007).

Autosomal dominant mutations in APP, PS1 and PS2 usually cause disease in younger people than those who acquire the sporadic disease. The majority of mutations in APP, PS1 and PS2 influence BACE1 (β-site APP cleaving enzyme 1) and γ-secretase cleavages to increase the levels of all Aβ species or the relative amounts of toxic Aβ42 (Citron, 2010; Ashe et al. 2010; Bertram et al., 2008; Golde et al., 2011; Price et al., 1998). Individuals with trisomy 21 or Down syndrome (DS) have an extra copy of APP (and other genes) in the putative obligate DS region; these individuals develop AD pathology relatively early in life. Some families have duplications of APP (Bertram et al., 2008; Price et al., 1998). The presence of Apo E4 predisposes to later onset sporadic AD and in some cases to late-onset fAD (Kim et al., 2009a; Bertram et al., 2008; Bu, 2009). Recent research has identified other loci that confer risk (Bertram et al., 2008; Harold et al., 2009; Rogaeva et al., 2007).

APP Mutations are Linked to fAD

Encoded by a gene on chromosome 21, APP—a type I transmembrane protein existing as several isoforms—is abundant in the nervous system, enriched in neurons, and transported anterograde in axons to nerve terminals (Buxbaum et al., 1998; Koo et al., 1990; Sisodia et al., 1993). The specific functions of APP remain to be defined (Cao et al., 2001; Kamenetz et al., 2003). As described below, APP is cleaved by the enzymatic activities of BACE1 (β-site APP-cleaving enzyme 1) and the γ-secretase complex, which generate the N- and C- termini of Aβ peptides, respectively (Cai et al., 2001; De Strooper, 2003; Haass, 2004; Iwatsubo, 2004; Marjaux et al., 2004; Selkoe & Kopan, 2003; Takasugi et al., 2003; Vassar et al., 1999). The APPswe mutation, a double mutation involving codons 670 and 671, enhances many-fold the BACE1 cleavage at the N-terminus of Aβ; the result is substantial elevation in levels of all Aβ peptides. With APP717 mutations, γ-secretase cleavages are altered, leading to increased secretion of Aβ42, which is a neurotoxic peptide. Thus, some of the APP mutations linked to fAD can change the processing of APP and influence the biology of Aβ by altering the production of Aβ peptides or the amounts of the more toxic Aβ42; other APP mutations may promote local fibril formation.

Mutations in PS1 and PS2 are Linked to fAD

Encoded by two genes, PS1 and PS2, two highly homologous and conserved 43- to 50-kDa multi-pass transmembrane proteins (Doan et al., 1996; Price et al., 1998; Sherrington et al., 1995; De Strooper, 2003; Haass, 2004; Iwatsubo, 2004; Marjaux et al., 2004; Selkoe & Kopan, 2003), are involved in the Notch 1 signaling pathways critical for many developmental functions, including cell fate decisions (Selkoe & Kopan, 2003; Shen et al., 1997; Sisodia et al., 2002; Wong et al., 1997). These proteins are endoproteolytically cleaved to form an N-terminal ~28-kDa fragment and a C-terminal ~18-kDa fragment (Doan et al., 1996; Thinakaran et al., 1997). PS, along with several other proteins described below, are critical components of the γ-secretase complex (De Strooper, 2003; Haass, 2004; Iwatsubo, 2004; Marjaux et al., 2004; Selkoe & Kopan, 2003; Takasugi et al., 2003; Wolfe, 2002).

Nearly 50% of cases of early-onset fAD are linked to one of more than 90 different mutations in PS1 (Bertram et al., 2008; Price et al., 1998; Sherrington et al., 1995; Sisodia et al., 2002). A small number of *PS2* mutations cause autosomal dominant fAD (Bertram et al., 2008; Price et al., 1998; Sherrington et al., 1995; Sisodia et al., 2002). The majority of abnormalities in *PS* genes are single amino acid missense mutations that alter γ-secretase activities and increase the ratio of the Aβ42/Aβ40 peptides.

ApoE carries cholesterol and other lipids in the blood. In humans, three alleles exist: *ApoE2*, A*poE3* and A*poE4* (Bertram et al., 2008; Bu, 2009; Kim et al., 2009a). The *ApoE3* allele is most common in the general population (frequency of 0.78), whereas the allelic frequency of *ApoE4* is 0.14. However, in clinic-based studies, the *ApoE4* allelic frequency in patients with AD (onset after 65 years of age) is 0.50 (Bertram et al., 2008; Bu, 2009; Kim et al., 2009a). Thus, the presence of *apoE4* increases the risk for AD. Significant differences exist in the abilities of ApoE isoforms to bind Aβ and these features are hypothesized to differentially influence aggregation, deposition and/or clearance of Aβ by the different ApoE isoforms (Bertram et al., 2008; Bu, 2009; Kim et al., 2009a).

Multiple neurotransmitter circuits and brain networks are damaged in AD

The clinical signs of AD reflect the distributions of abnormalities among different populations of neurons in brain regions and systems critical for memory, learning and cognitive performance. Circuits damaged by the disease include basal forebrain cholinergic system, monoamine neurons in the brain stem, hippocampus, entorhinal cortex, limbic cortex and neocortex. In these regions and circuits, neurodegeneration is reflected by the abundance of NFT and neuritic plaques involving these systems. These lesions are the results of cytoskeletal abnormalities, particularly involving conformational alterations in tau, hyperphosphorylated tau or fragments of tau, leading to formation of PHF in neurons (Braak et al., 1991; Ballatore et al., 2007; Goedert et al., 2006) and the presence of neurites around Aβ plaques (sites of synaptic disconnection) in brain regions receiving inputs from these neurons. Both generic and transmitter-specific synaptic markers are reduced in the target fields of these cells (Price & Sisodia, 1998) and there is evidence of death of neurons in these regions (Ballatore et al., 2007; Braak et al., 1991; Goedert et al., 2006; Whitehouse et al.,1981). Local glial and inflammatory responses are associated with the plaques. Disruption of synaptic communications between cells and circuits—associated with degeneration of axons and, eventually,

cell bodies of neurons—has profound clinical consequences. Abnormalities that damage the circuits involving the entorhinal cortex, medial temporal cortex and hippocampus are thought to contribute significantly to memory impairments. Pathology in the neocortex is reflected by deficits in higher cognitive functions, such as disturbances in language, calculation, problem solving and judgment. The cellular pathology involving these regions is linked to the loss of functions performed by these circuits. Alterations in the basal forebrain cholinergic system may contribute to difficulties in memory, arousal and attention (Whitehouse et al., 1981), while involvement of the DMN, limbic cortex, amygdala, thalamus and monoaminergic systems results in a variety of disturbances in emotion, thought (Buckner et al., 2008; Perrin et al., 2009; Sperling et al., 2009; Zhang & Raichle, 2010) and sense of self.

Neuritic plaques, one of the pathological hallmarks of AD, are composed of swollen neurites, extracellular deposits of Aβ 40-42 peptides derived from β- and γ-secretase cleavages of APP

Neuritic Aβ plaques are characterized by deposits of amyloid, extracellular β-pleated sheet fibrillar Aβ peptides, surrounded by swollen neurites (nerve terminals) and reactive glial cells. Aβ amyloid peptides, derived by β- and γ-secretase cleavages of APP to generate Aβ1-40, 42 and 11-40, 42 peptides, accumulate in the extracellular space of the neuropil of the neocortex and hippocampus. In neurons, APP is carried by fast anterograde axonal transport to nerve terminals (Buxbaum et al., 1998; Koo et al., 1990; Sisodia et al., 1993). At these sites, APP in endocytic compartments is cleaved by β and γ-secretases that liberate extracellular monomeric Aβ peptides into the extracellular space (Buxbaum et al., 1998). Moreover, some Aβ may be derived from postsynaptic processes. Aβ multimers assemble into β sheets, into protofilaments and into amyloid fibrils (Caughey et al., 2003; Ashe et al., 2010; Golde et al., 2011; Gong et al., 2003; Iwatsubo et al., 1994; Lue et al., 1999; Selkoe, 2002); these fibrillar aggregates are birefringent when stained with Congo Red or thioflavin dyes and viewed in polarized light or fluorescence illumination, respectively. Considerable debate exists concerning the Aβ species and conformational state exhibiting the greatest toxicity. Previously, plaques, fibrils and protofibrils were proposed as principal offenders (Caughey et al., 2003; Gong et al., 2003; Ashe et al., 2010; Iwatsubo et al., 1994; Lue et al., 1999; Selkoe, 2002) but it is now thought that multimers, sometimes termed Aβ-derived diffusible ligands (ADDLs), are the principal toxic entities (Caughey et al., 2003; Gong et al., 2003; Iwatsubo et al., 1994; Lue et al., 1999; Selkoe, 2002).

Because APP and pro-amyloidogenic secretases are present in neurons and transported to synapses, it is thought that neuronal APP is a major source of APP that gives rise to Aβ occurring in proximity to terminals (Wong et al., 2001; Buxbaum et al., 1998; Koo et al., 1990; Sisodia et al., 1993). At these sites, BACE1 cleaves APP in endocytic compartments to form amyloidogenic C-terminal derivatives, which are then cleaved by γ-secretase to generate secreted Aβ 40, 42, 43 peptides. Released normally at terminals, Aβ may influence synaptic functions (Kamenetz et al., 2003), perhaps behaving as a modulator depressing activity at excitatory, glutaminergic synapses via influences on the glutamate receptors (Kamenetz et al., 2003). With increasing accumulations of Aβ42 multimers at terminals, synaptic functions, including long-term potentiation (LTP), are disrupted. In this scenario, neuritic amyloid plaques are complex structures, representing sites of Aβ-mediated damage to synapses associated with disconnection of terminals from their targets and degeneration of neurites. Plaques are surrounded by astrocytes and microglia, which produce cytokines, chemokines and other factors (including complement components) involved in inflammatory processes. The degree to which these cells and their products are beneficial or damaging is the subject of ongoing investigations.

Neurofibrillary tangles (NFT), another characteristic feature of AD, are composed of intracellular bundles of paired helical filaments (PHF), which represent aggregated β-pleated sheet assemblies of tau peptides

While mounting evidence indicates that accumulation of Aβ amyloid is the major initiator of disease in the pathogenesis of AD, available evidence suggests that the presence of neurofibrillary pathology appears to correlate more closely with cognitive deficits in AD than does the amount of amyloid. NFT are fibrillar intracytoplasmic inclusions in cell bodies/proximal dendrites of affected neurons, while neuropil threads and neurites are predominantly swollen, filament-containing dendrites and distal axons/terminals, respectively (Ballatore et al., 2007; Braak et al., 1991; Goedert et al., 2006). These intracellular lesions are rich in PHF, which consists of poorly soluble tau, a low-molecular-weight microtubule-associated protein (Ballatore et al., 2007; Goedert et al., 2006). In human brain, alternative splicing from a single gene leads to formation of six tau isoforms, consisting of three isoforms of three-repeat tau (3-R) and three isoforms of four-repeat (4-R) tau, the latter derived by inclusion of exon 10 in the transcript (Ballatore et al., 2007; Goedert et al., 2006). Normally tau, synthesized in neuronal cell bodies, is transported anterograde in axons, where it interacts via repeat regions with tubulin to stabilize tubulin polymers critical for microtubule assembly and stability (Edbauer et al., 2002). Containing one more microtubule-binding domain, 4R tau shows a higher affinity for microtubules than 3R tau (Ballatore et al., 2007; Goedert et al., 2006). Binding of tau to microtubules is also regulated by post-translational modifications such as phosphorylation, glycosylation, glycation, ubiquitylation, sumoylation, nitration and proteolysis (Ballatore et al., 2007) (see Chs 1, 7, 8).

Aggregation of tau peptide fragments and full-length tau occurs not only in AD but also in a number of other neurodegenerative diseases, including frontotemporal dementia (FTD), Pick disease, corticobasal degeneration, progressive supranuclear palsy and dementia pugilistica (Ballatore et al., 2007; Goedert et al., 2006) (see also Chs. 41, 47). In these various disorders, collectively termed tauopathies, aberrations of tau are the principal pathological features. Post-translationally

modified tau in NFT, which exhibits abnormal conformations, differs somewhat in the different tauopathies: in cases of AD, the PHF are composed of six isoforms of tau; in contrast, the fibrillar inclusions occurring in cases of progressive supranuclear palsy (PSP) and cortical basal degeneration (CBD) are characterized by 4-R tau, while the inclusions seen in individuals with Pick disease (PD) are enriched in 3-R tau (Ballatore et al., 2007; Goedert et al., 2006). The understanding of perturbations in the biology of tau in neurodegenerative diseases was dramatically advanced by the discovery of mutations in the tau gene in FTD patients (Ballatore et al., 2007; Goedert et al., 2006; Gotz et al., 2008). Among these mutations, more than half involve changes in the pre-mRNA splicing of exon 10 alone, which appear to be associated with changes of R4/R3 ratio of tau isoforms (Ballatore et al., 2007; Goedert et al., 2006). Other mutations can increase the degree of phosphorylation, alter the ability of tau to bind to microtubules, change the stability of microtubules, or increase the formation of filamentous tau (Goedert et al., 2006; Ballatore et al., 2007). While neurofibrillary pathology appears to correlate with cognitive decline more significantly than does the Aβ amyloid pathology, the preponderance of evidence suggests that Aβ amyloidosis is an early event in the disease process of AD.

Studies of cell cultures and transgenic mouse models indicate that Aβ accumulation can initiate or accelerate tau pathology. In one hypothetical model linking Aβ and phosphorylated tau, Aβ42 damage to terminals leads to synaptic disconnection, which in turn leads to retrograde signaling, which ultimately triggers the activation of kinases (or the suppression of phosphatases) whose activities lead to hyperphosphorylation of tau at certain residues. Subsequently, conformational changes in the tau protein or peptide fragments (see below) are associated with the formation of PHF. Since the cytoskeleton is essential for maintaining cell geometry and for the intracellular trafficking and transport of proteins and organelles, disturbances of the cytoskeleton can lead to alterations in axonal transport which, in turn, can compromise the functions and viability of neurons. Eventually, affected nerve cells die (possibly by apoptosis) (Braak et al., 1991; Goedert et al., 2006; Whitehouse et al., 1981): extracellular tangles remain as "tombstones" of the nerve cells destroyed by disease. See also discussion of tau in Ch. 7, 8 and 47.

While hyperphosphorylation is a notable modification of tau in cases of AD, recent studies suggest that proteolysis of tau may initiate formation of PHF and play an important role at an early stage of neuronal degeneration. Since tau is a natively unfolded protein, it is susceptible to protease digestion (Wang et al., 2010). The truncation of tau at Asp421 by caspase 3 has been validated both *in vitro* and *in vivo;* cleavage of tau by caspase could initiate tangle formation, with tau peptide fragments recruiting normal tau to misfold and to form PHF-NFT (de Calignon et al., 2010; Golde et al., 2011). Interestingly, treatment of cultured neurons with fibrillar Aβ appears to induce cleavage of tau by caspases, which makes fibrilogenic tau (Gamblin et al., 2003), indicating a potential connection between pathologies of Aβ and tau.

In addition to caspases and calpain, other not-yet-defined proteases may also be involved in cleavages of tau. For example, mutant tau harboring deletion of Lys280 (tauRDΔK) can be sequentially cleaved around the repeat domain to generate small amyloidogenic tau fragments, which in turn can induce the aggregation of tauRDΔK or full-length tau, leading to cytotoxicity (Khlistunova et al., 2006; Wang et al., 2007). Blocking the generation of these amyloidogenic tau fragments by mutating the residues at the cleavage site disrupts the aggregation of tauRDΔK, indicating that the limited proteolysis is necessary for tau aggregation in this cell model (Wang et al., 2007).

In one hypothetical model linking Aβ and phosphorylated tau, elevated concentrations of Aβ42 damage terminals, resulting in synaptic disconnections that lead to a retrograde signal that ultimately triggers the activation of kinases (or the suppression of phosphatases) whose activities ultimately produce excessive phosphorylation of tau at certain residues. Subsequently, conformational changes in the protein cause the formation of PHF, in this view. Since the cytoskeleton is essential for maintaining cell geometry and for the intracellular trafficking and transport of proteins and organelles, disturbances of the cytoskeleton can lead to alterations in axonal transport which, in turn, can compromise the functions and viability of neurons. Eventually, affected nerve cells die (possibly by apoptosis) (Koo et al., 2004; Whitehouse et al., 1981) and extracellular tangles remain as "tombstones" of the nerve cells destroyed by disease (see Chs 7, 8).

Aspartyl proteases carry out the β- and γ-secretase cleavages of APP to generate Aβ peptides

APP is processed by β- and γ-secretase enzymes, resulting in release of the soluble ectodomain of APP (APPs), the production of a cytosolic fragment termed APP intracellular domain (AICD), and the generation of several Aβ peptides. In the CNS, Aβ peptides are generated by sequential endoproteolytic cleavages of neuronal APP by two membrane-bound enzymes: as described below, BACE1 cleaves APP at the Aβ +1 and +11 sites to generate APP-β carboxyl terminal fragments (APP-βCTFs) (Cai et al., 2001; Citron, 2010; Luo et al., 2001; Vassar et al., 1999; Yan et al., 1999), while γ-secretase complex cleaves, via regulated intramembranous proteolysis, APP-βCTFs at several sites, including Aβ 40, 42, and 43, to form these peptides (Citron, 2010; De Strooper, 2003; Haass, 2004; Iwatsubo, 2004; Yan et al., 1999; Ma et al., 2005; Marjaux et al., 2004; Takasugi et al., 2003). The γ-secretase cleavages of APP-βCTF or αCTF release AICD, which forms a multimeric complex with Fe65, a nuclear adaptor protein (Cao et al., 2001). It has been suggested that the complex of Fe65 and AICD or Fe65 alone (in a new conformation), enters the nucleus and binds to histone acetyltransferase Tip60 to influence gene transcription (Cao et al., 2001) (see histone acetylation in Ch.27). A signaling mechanism analogous to that occurring in the Notch1 pathway follows the S3 cleavage of NEXT to produce NICD (Iwatsubo, 2004; Selkoe & Kopan, 2003). In other cells or other organs, APP can also be cleaved endoproteolytically within the Aβ sequence through alternative, non-amyloidogenic pathways involving α-secretase (TNF-alpha converting enzyme or TACE) or BACE2 (Farzan et al., 2000; Haass, 2004). The α-secretase and BACE2 cleavages, which occur in non-neural tissues, preclude the formation of

Aβ peptides and thus are thought to protect these organs from Aβ amyloidosis (Wong et al., 2001).

BACE1 and BACE2, encoded by genes on chromosomes 11 and 21, respectively, are transmembrane aspartyl proteases that are directly involved in the cleavages of APP (Citron, 2010; Cai et al., 2001; Farzan et al., 2000; Haass, 2004; Luo et al., 2001; Vassar et al., 1999; Yan et al., 1999). Analyses of cells and brains from BACE1-/- mice (Cai et al., 2001; Luo et al., 2001) disclose that Aβ1-40/42 and Aβ11- 40/42 are not secreted in these samples (Cai et al., 2001; Luo et al., 2001). BACE1 is expressed in the CNS; immunoreactivity is visualized in some synaptic regions. BACE1 preferentially cleaves APP at the +11 > +1 sites of Aβ in APP (Cai et al., 2001) and this enzyme is essential for the generation of Aβ (Cai et al., 2001; Luo et al., 2001). Significantly, APPswe is cleaved perhaps 100-fold more efficiently at the +1 site than is wild-type APP. Thus, the presence of this mutation greatly increases BACE1 cleavage and accounts for the elevation of Aβ species in the presence of this mutation. It has been reported that the expression of BACE1 is increased in certain regions of brain from some cases of sporadic AD (Li et al., 2004; Yang et al., 2003). All available evidence indicates that BACE1 is the principal neuronal β-secretase and is responsible for the critical penultimate pro-amyloidogenic cleavage of APP. Although BACE1 mRNA is present in a variety of tissues (particularly the pancreas), levels of this protein are low in most non-neural tissues. Interestingly, in the pancreas, BACE1 mRNA is high, but the transcript is alternatively spliced to produce a smaller protein incapable of cleaving APP.

BACE2 mRNA, present in a variety of organs, is expressed at very low levels in neural tissues, except for scattered nuclei in the hypothalamus and brainstem. BACE2 activity appears to be virtually undetectable in brain regions involved in AD. BACE2 is responsible for generation of anti-amyloidogenic cleavages at +19/+20 of Aβ (Farzan et al., 2000). Thus, BACE2 is an anti-amyloidogenic enzyme, acting like α-secretase (ADAM10 or TACE) that cleaves between residues 16 and 17 of the Aβ peptide (Sisodia et al., 1990; Sisodia et al., 2002) and reducing production of amyloidogenic forms of Aβ.

γ-Secretase, essential for the regulated intramembranous proteolysis of a variety of transmembrane proteins, is a multiprotein catalytic complex that includes PS; Nicastrin (Nct), a type I transmembrane glycoprotein (De Strooper, 2003; Edbauer et al., 2002; Li et al., 2003; Sisodia et al., 2002); and Aph-1 and Pen-2, which are two multipass transmembrane proteins (De Strooper, 2003; Edbauer et al., 2002; Li et al., 2003; Sisodia et al., 2002; Goutte et al., 2002; Haass, 2004; Iwatsubo, 2004; Ma et al., 2005; Marjaux et al., 2004; Selkoe & Kopan, 2003; Steiner et al., 2002; Takasugi et al., 2003). PS1 is isolated with γ-secretase under specific detergent soluble conditions; it is selectively cross-linked or photoaffinity-labeled by transition state inhibitors (Esler et al., 2000; Li et al., 2000). Substitutions of aspartate residues at D257 in TM 6 and at D385 in TM 7 reduce the secretion of Aβ and the cleavage of Notch1 *in vitro*; PS1−/− cells show decreased levels of secretion of Aβ (De Strooper, 2003; Li et al., 2003; Naruse et al., 1998; Takasugi et al., 2003). Aph-1 and Pen-2 (De Strooper, 2003; Goutte et al., 2002; Takasugi et al., 2003) are novel transmembrane proteins: Aph-1 has seven predicted transmembrane domains, and Pen-2 has two predicted transmembrane regions (De Strooper, 2003; Takasugi et al., 2003).

The functions of these proteins and their interactions with each other in the complex and in γ-secretase activity are not yet fully defined. PS1 initially was proposed to act as an aspartyl protease itself, to function as a co-factor critical for the activity of γ-secretase or to play a role in trafficking of APP or proteins critical for enzyme activity to the proper compartment for γ-secretase cleavage (Esler et al., 2000; Naruse et al., 1998; Wolfe, 2002). Recent reconstitution studies of recombinant PS1 and PEN2 or PS1DE9 in liposomes have demonstrated that PS1 is the γ-secretase and that PEN2, which converts the PS1 zymogen, is necessary and sufficient to activate the endoproteolysis of PS1 to generate the active enzyme (Ahn et al., 2010).

Significantly, γ-secretase cleaves both Notch-1 and APP generating intracellular peptides termed NICD and AICD. (Cao et al., 2001; Selkoe & Kopan, 2003) As described above, the AICD is believed to interact with FE65, a cytosolic adapter. This interaction leads to a signal that influences transcription (Cao et al., 2001; Selkoe & Kopan, 2003). Results of targeting of PS1, Nct and Aph-1 in mice (Cao et al., 2001; Li et al., 2003; Ma et al., 2005; Selkoe & Kopan, 2003; Shen et al., 1997; Wong et al., 1997), which are described below, are consistent with the concept that PS1, Nct and Aph-1 (Capell et al., 2000; De Strooper, 2003; Haass, 2004; Takasugi et al., 2003), along with PEN2, are critical components of the γ-secretase complex. The phenotypes of targeted PS1, Nct and APH-1 mice are interpreted to be the result of impaired Notch1 signaling (Li et al., 2003; Ma et al., 2005; Shen et al., 1997; Wong et al., 1997).

Transgenic strategies have been used to create models of Aβ amyloidosis and tauopathies

In mice, expression of APPswe or APP717 minigenes (Chui et al., 1999) leads to an Aβ amyloidosis in the CNS (Sturchler-Pierrat et al., 1997), with the severity of pathology influenced by the nature and levels of the expressed transgene and the specific mutations. Mice expressing both mutant APP and PS1 develop accelerated disease. In these animals, levels of Aβ (particularly Aβ42) in the brain are elevated, and diffuse Aβ deposits and neuritic plaques appear in the hippocampus and cortex. In lines of transgenic mice generated by Borchelt and associates (Borchelt et al., 1996; Borchelt et al., 1997; Jankowsky et al., 2004), the pathology evolves in stages. Levels of Aβ peptides are elevated in the brain and increase with age; over time, Aβ deposits become increasingly abundant; swollen neurites appear in proximity to these deposits; and, as neuritic plaques evolve, they are associated with glial responses. In these mice, aggregated tau and tangles are not detectable. The density of synaptic terminals is reduced and several neurotransmitter markers are decreased; in some lines of mice, these abnormalities appear linked to deficiencies in synaptic transmission (Hsia et al., 1999). Moreover, some lines of mice show degeneration of subsets of neurons. (Liu et al., 2008) Amyloid has been routinely detected *in vivo* by invasive techniques (Bacskai et al., 2003), but advances in MRI and PET imaging have made it possible to detect Aβ in the animals *in vivo* (Maeda et al., 2007; Wengenack et al., 2011).

Recently developed transgenic models of Aβ amyloidosis employ inducible expression of a transgene based on

tetracycline-regulated promoters. In several of these lines memory formation is influenced by regulated expression of a CaMKII-driver transgene. These conditional transgenic mouse models exhibit high levels of transgene expression; the levels can be reduced by several orders of magnitude by treatment with doxycycline (Kistner et al., 1996). A number of transgenic lines expressing different levels of mutated APP have been developed by Borchelt and Jankowsky (Jankowsky et al., 2005). Swedish and Indiana APP mutations have been incorporated into the *APP* transgene to alter processing of the mutated protein by β- and γ-secretases, respectively. The suppression of *APP* expression and Aβ production is achieved in these models by feeding APP *swe/ind* transgenic mouse diets containing doxycycline. These models allow for testing of the potential for highly toxic effects of inhibiting production of Aβ and the influence of these manifestations on the evolution of amyloid pathology and cognitive deficits. It also allows investigators to ask whether amyloid pathology can be reversed following such treatment. In these conditional models, long-term (3–6 months) suppression of Aβ production discloses that many amyloid deposits are stable and persist for months after expression is inhibited (Jankowsky et al., 2005).

Despite successes in modeling amyloidosis, tau-related pathology was not observed in these amyloidogenic models. The paucity of tau abnormalities in the various lines of mutant APP and PS1 mice may be related to differences in tau isoforms expressed in mice versus those in primates. Early efforts to express mutant tau transgenes in mice did not lead to striking clinical phenotypes or pathology. Some lines of mice overexpressing tau show clinical signs, attributed to degeneration of motor axons (Lee et al., 2001) (see Ch. 47). For example, when the prion or Thy1 promoters are used to drive tauP301L (a mutation linked to autosomal dominant frontotemporal dementia with parkinsonism), tangles develop in neurons of the brain and spinal cord (Gotz et al., 2001; Gotz et al., 2008). Mice expressing APPswe/tauP301L exhibit enhanced tangle-like pathology in limbic system and olfactory cortex. (Lewis et al., 2001) Moreover, when Aβ42 fibrils are injected into specific brain regions of tauP301L mice, the number of tangles is increased in those neurons projecting to sites of injection of Aβ. A triple transgenic mouse (3xTg-AD), created by microinjecting APPswe and tauP301L into single cells derived from monozygous PS1M146V knock-in mice (Oddo et al., 2003) develop age-related plaques and tangles as well as deficits in long-term potentiation (LTP), which appear to antedate overt pathology. (Oddo et al., 2003) However, mice bearing both mutant tau and APP (or APP/PS1) or mutant tau mice injected with Aβ are not fully faithful models of fAD because the presence of the tau mutation alone is associated with the development of tangles.

Learning deficits, problems in object recognition and fear memory, and difficulties in performing tasks assessing spatial reference and working memory have been identified in some of the lines of mutant mice with high levels of expression of mutant transgenes (Oddo et al., 2003; Chapman et al., 1999; Chen et al., 2000). Some of the behavioral abnormalities are hypothesized to be associated with disconnection of synaptic terminals from their targets. Although these mice do not fully recapitulate the complete phenotype of AD, they are useful subjects for research designed to examine disease mechanisms and to test novel therapies.

Gene targeting approaches have identified and validated targets for therapy

To begin to understand the functions of some proteins thought to play roles in the biology of APP and in Aβ amyloidogenesis, investigators have targeted a variety of genes encoding APP and members of the APP family of amyloid precursor–like proteins (APLPs) (Heber et al., 2000; Walsh & Selkoe, 2004) and by knocking out genes encoding the secretase proteins (BACE1, PS,; Nct; and Aph-1) (Heber et al., 2000; Walsh & Selkoe, 2004). Homozygous APP−/− mice are viable and fertile, but appear to have subtle decreases in locomotor activity and forelimb grip strength (Heber et al., 2000). The absence of substantial phenotypes in APP−/− mice appears to be related, in part, to functional redundancy of two amyloid precursor-like proteins (APLP1 and APLP2) (Walsh & Selkoe, 2004) that are homologous to APP. APLP2−/− mice appear relatively normal, while APLP1−/− mice exhibit a postnatal growth deficit (Heber et al., 2000). APLP1−/− mice are viable, but APP/APLP2−/− mice and APLP1/APLP2−/− mice do not survive the perinatal period (Heber et al., 2000). These observations support the concept that some redundancy exists between members of this interesting family of proteins (Heber et al., 2000).

BACE1−/− mice are viable, have no obvious phenotype or pathology and can mate successfully (Cai et al., 2001; Luo et al., 2001). Importantly, cortical neurons cultured from BACE1−/− embryos do not show cleavages at the +1 and +11 sites of Aβ, and the secretion of Aβ peptides is abolished even in the presence of elevated levels of exogenous wild-type (wt) or mutant APP (Cai et al., 2001). The brains of BACE1−/− mice appear morphologically normal and Aβ peptides are not produced (Cai et al., 2001; Luo et al., 2001). These results establish that BACE1 is the neuronal β-secretase required to generate the N-termini of Aβ (Xia et al., 2010). Significantly, behavioral studies of the BACE1−/− mice indicate that these animals show altered performance on some tests of cognition and emotion (Savonenko et al., 2008). Moreover, BACE1-null mice exhibit hypomyelination, attributed to the developmental influence of neuregulin (NRG), which requires BACE1 for normal processing. Nevertheless, BACE1 appears to be an outstanding target for development of an anti-amyloidogenic therapy (Citron, 2010; Vassar et al., 1999; Vassar et al., 2009; Wong et al., 2002).

In contrast to BACE1−/− mice, PS1−/− mice do not survive beyond the early postnatal period and show severe developmental abnormalities of the axial skeleton, ribs and spinal ganglia; this outcome resembles a partial Notch 1−/− phenotype (Shen et al., 1997; Wong et al., 1997) because PS1, along with Nct, Aph-1 and Pen-2, are components of the γ-secretase complex that carries out the S3 intramembranous cleavage of Notch1 (De Strooper et al., 1999; De Strooper, 2003; Edbauer et al., 2002; Esler et al., 2002; Li et al., 2003; Selkoe & Kopan, 2003; Takasugi et al., 2003) Without this cleavage, the NICD is not released from the plasma membrane and the appropriate signal does not reach the nucleus to initiate transcriptional processes essential for cell fate decisions. PS2−/− mice are viable and fertile, though they develop age-associated mild pulmonary fibrosis and hemorrhages. Mice lacking PS1 and PS2 die midway through gestation, showing a Notch1−/− phenotype.

Nct−/− embryos die by embryonic day 10.5 and exhibit several developmental patterning defects, including abnormal segmentation of somites; the phenotype closely resembles that seen in embryos lacking Notch1 or PS (Li et al., 2003). Importantly, secretion of Aβ peptides is abolished in Nct−/− fibroblasts, whereas it is reduced by ~50% in Nct+/− cells (Li et al., 2003). The failure to generate Aβ peptides in Nct−/− cells is accompanied by apparent destabilization of the γ-secretase complex and by accumulation of APP C-terminal fragments. Moreover, analysis of APP trafficking in Nct−/− fibroblasts discloses a significant delay in the rate of APP re-internalization compared with that of control cells.

Three murine Aph-1 alleles—termed Aph-1a, Aph-1b and Aph-1c—encode four distinct Aph-1 isoforms: Aph-1aL and Aph-1aS, derived from differential splicing of Aph-1a; Aph-1b; and Aph-1c (Ma et al., 2005). To determine the contributions of various mammalian Aph-1 homologues in formation of functional γ-secretase complexes, our laboratory generated Aph-1a−/− mice (Ma et al., 2005). As compared to littermate controls, the development of Aph-1a/− embryos was dramatically retarded by embryonic day 9.5 and these animals exhibited patterning defects that resemble, but are not identical to those of Notch1, Nct or PS−/− embryos. Moreover, in immortalized Aph-1a−/− fibroblasts, the levels of Nct, PS fragments and Pen-2 are dramatically decreased. Consequently, deletion of Aph-1a results in significant reductions in levels of the high-molecular-weight γ-secretase complex and in the secretion of Aβ. Importantly, complementation analysis reveals that all mammalian Aph-1 isoforms are capable of restoring the levels of Nct, PS and Pen-2 in Aph-1a−/− cells. Taken together, the findings establish that Aph-1a is the major mammalian Aph-1 homologue present in PS-dependent γ-secretase complexes during embryogenesis; these data support the view that mammalian Aph-1 isoforms define a set of functional γ-secretase complexes. Significantly, as mentioned earlier, *in vitro* reconstitution experiments indicate that PS and PEN2 are critical for γ-secretase activity (Ahn et al., 2010). (see Box)

Transgenic mouse models are being used to test a variety of novel therapies

Many experimental therapeutic efforts have focused on influencing Aβ production (by inhibiting or modulating secretase activities), blocking the aggregation of or enhancing the clearance of Aβ and targeting the neurotoxicity of Aβ42 (Citron, 2010; Monsonego et al., 2003; Ashe et al., 2010a; Bard et al., 2000; Brody et al., 2008; Chow et al., 2010a; Chow et al., 2010b; Dodart et al., 2002; Golde et al., 2011; Kim et al., 2009b; Kotilinek et al., 2002; Kounnas et al., 2010c; Morgan et al., 2000; Perrin et al., 2009v; Sagare et al., 2011; Schenk et al., 1999; Wong et al., 2002; Walsh & Selkoe, 2004). Although mutant transgenic mice do not recapitulate the full phenotype of AD, they represent excellent models of Aβ amyloidosis and are highly suitable for identification of therapeutic targets and for testing new treatments *in vivo*. Because it is not possible to discuss all treatment efforts in transgenic mice, we focus on a few selected studies to illustrate experimental therapeutic strategies, including γ-secretase inhibitors, BACE1 inhibitors, immunotherapy and other agents influencing clearance.

BACE1 is the principal β-secretase in neurons *in vitro*, and BACE1-deficient neurons fail to secrete Aβ even when co-expressing the APPswe and mutant PS1 genes (Luo et al., 2001). Significantly, BACE1−/−; APPswe; PS1 E9 mice do not develop the Aβ deposits and age-associated abnormalities in working memory that occur in the APPswe; PS1ΔE9 model of Aβ amyloidosis (Borchelt et al., 1996). Similarly, BACE1−/− TG2576 mice appear to be rescued from age-dependent memory deficits and physiological abnormalities (Ohno et al., 2004). These data indicate that BACE1 is a very attractive therapeutic target. However, BACE1−/− mice develop abnormalities in performance of tasks assessing cognition and emotion, which can be rescued if APP is overexpressed in brain (Savonenko et al., 2008). In trials, it will be important to critically examine the influences of BACE1 inhibitors on memory, cognition and emotion.

The γ-secretase complex catalyzes the final cleavage of APP, which liberates the Aβ peptide into the extracellular space (amyloid deposits). As demonstrated by the gene targeting strategies described above, this complex is critically dependent upon the presence of PS and Pen-2 (as well as Nct, Aph-1a). Because reductions in levels of these γ-secretase components decrease levels of Aβ, the enzyme is a significant target for therapy. In cell-free and cell-based systems and in mutant mice with Aβ amyloidosis, inhibition of γ-secretase activity decreases production of Aβ. However γ-secretase activity is also essential for Notch processing critical for lineage specification and cell growth during embryonic development. Therefore, γ-secretase inhibitors can also influence these latter activities. For example, LY – 411, 575 not only reduces production of Aβ, but it also has effects on T and B cell development and on the appearance of intestinal mucosa (proliferation of goblet cells, increased mucin in gut lumen and crypt necrosis). Moreover, Nct+/− mice develop skin cancers with age (related to the importance of notch signaling as tumor suppression in the skin). Clinicians will have to be alert to the problem. It is hoped that γ-secretase modulator will circumvent some of the problems (Citron, 2010; Golde et al., 2011). Thus, several adverse affects could occur following inhibition of these secretase enzymes, and it will be important for investigators to be alert for these effects.

It is likely that AD will require combinatorial therapy initiated in the earliest stages of disease (Ashe et al. 2010; Brody et al., 2008; Chow et al., 2010; Citron, 2010; Golde et al., 2011; Perrin et al., 2009). For example, a combination genetic approach to reduce β- and γ-secretase activities demonstrated that moderate reductions of either the γ-secretase or BACE1 activities provided modest benefits and were not associated with major mechanism-based toxicities (Chow et al., 2010). Decreasing the levels of both enzymes had significant beneficial effects on amyloid burden and on cognitive performance with no associated evidence of adverse influences. On the basis of these experiments, we suggest that lowering levels of both γ-secretase and BACE1 activities may provide an effective and safe treatment of the human disease. Moreover, lowering of enzyme activities can be complemented by strategies designed to promote clearance (see below) (Brody et al., 2008; Bertram et al., 2008). A variety of companies have attempted to identify and develop potent and selective γ-secretase inhibitors that lower the formation of Aβ in the brain. In addition to focusing attention on

brain penetration and efficacy of these agents, investigators are concerned about the potential adverse impacts of lowering γ-secretase cleavages involved the processing of Notch and other important signaling proteins that are cleaved by this enzyme, but some progress has been made in identifying selective inhibitor that preserve Notch cleavage while reducing APP cleavage.

Many pharmaceutical and biotechnology companies and some academic laboratories are using high-throughput screening and molecular modeling strategies to discover compounds that inhibit (or modulate) these enzyme activities (Citron, 2010; Marjaux et al., 2004; Walsh & Selkoe, 2004; Wolfe, 2002). Once lead compounds are identified, medicinal chemists have modified these compounds to enhance efficacy, to allow entry through the blood–brain barrier and to reduce any potential toxicities.

In a recent study (Kounnas et al., 2010), the authors describe the screening (in cell-based assays) for modulators of γ-secretase activity (GSMs). They identified small molecules that were able to preferentially decrease the levels of Aβ42 without impacting on the ϵ-cleavage sites. A library (10,000) of diverse composition was screened for compounds that decreased Aβ42, did not alter levels of Aβ40, and increased levels of Aβ37 and 38 without impacting on ϵ-cleavage. Selected aminothiazole agents, which were bioavailable following oral administration, were tested for efficacy in TG 2576 mice; levels of Aβ42 were decreased without inhibiting ϵ-site cleavage or altering the generation of APP and Notch intracellular domains. Oral administration of one of these potent GSM to TG 2576 mice showed a dose response lowering of plasma membrane Aβ 42. Moreover, chronic daily administration of this compound was associated with significant decrements in both diffuse and neuritic plaques. There was no evidence of Notch-related abnormalities that often confound use of γ-secretase inhibitors. These aminothiazole γ-secretase modulators are termed AGSM's (Kounnas et al., 2010) are intriguingly, similar to other compounds of a similar class being developed for the treatment of abnormalities of the prion protein (PrP), i.e. the generation of PrPsc associated with prion diseases (Ghaemmaghami et al., 2010).

Active and passive Aβ immunotherapy and other approaches have been used in both prevention and treatment trials in mutant mice (Perrin et al., 2009; Brody et al., 2008). Both Aβ immunization and passive transfer of anti-Aβ antibodies reduce levels of Aβ and plaque burden in mutant APP and APP/PS1 transgenic mice (Jankowsky et al., 2005; Ashe et al., 2010c; Golde et al., 2011; Perrin et al., 2009; Bard et al., 2000; DeMattos et al., 2002; Dodart et al., 2002; Kotilinek et al., 2002; Monsonego et al., 2003; Morgan et al., 2000; Schenk et al., 1999). Efficacy seems to be related to antibody titer. The mechanisms of enhanced clearance are not certain, but at least two not mutually exclusive hypotheses have been suggested: (1) A small amount of anti-Aβ antibody reaches the brain, binds to Aβ peptides, promotes disassembly of fibrils, and, via the Fc antibody domain, encourages activated microglia to enter the affected region and remove Aβ (Schenk et al., 1999); and/or (2) serum antibodies serve as a sink to bind the amyloid peptides exiting from brain into the circulation, thus changing the equilibrium of Aβ in different compartments and promoting removal of Aβ from the brain (Cirrito et al., 2003; DeMattos et al., 2002; Dodart et al., 2002; Morgan et al., 2000). Significantly, immunotherapy in transgenic mice is successful in partially clearing Aβ, and attenuating learning and behavioral deficits in at least a number of mutant APP mice (Jankowsky et al., 2005; Ashe et al. 2010i; Golde et al., 2011; Perrin et al., 2009; Bard et al., 2000; DeMattos et al., 2002; Dodart et al., 2002; Kotilinek et al., 2002; Monsonego et al., 2003; Morgan et al., 2000; Schenk et al., 1999).

Another approach to the clearance of Aβ from the brain has utilized delivery of low-density lipoprotein receptor–related protein 1 (LRP) into the circulation (Sagare A et al., 2011). This strategy promotes clearance of Aβ from the brain by soluble circulating LRP acting as sink for Aβ in the plasma. Another approach takes advantage of the observations that apolipoprotein E (APOE) is a genetic risk factor for AD (Bu, 2009; Kim et al., 2009a) and that this protein plays a role in the pathogenesis of AD, possibly by influencing aggregation and/or clearance of Aβ. Thus, experimental manipulations of APOE metabolism may allow insights into potential therapeutic targets for regulating levels of Aβ (Bu, 2009; Kim et al., 2009b; Kim et al., 2009a). Because LDLR, a member of the L D L receptor family, binds to APOE, it has been hypothesized that overexpression of LDLR might decrease levels of APOE and enhance the clearance of Aβ and decrease levels of amyloid (Bu, 2009; Kim et al., 2009b; Kim et al., 2009a). To test this hypothesis, mice were generated to overexpress LDLR in the brain; the levels of APOE were decreased in these mice (Dodart et al., 2002). Additional experiments showed that mice overexpressing LDLR showed significant reductions in aggregation of Aβ and enhancement of Aβ clearance from the brain (Kim et al., 2009b). Moreover, inflammatory responses associated with plaques were reduced in these mice. The authors suggest that approaches to increase the levels of LDLR may have value in developing new treatments for the disease (Kim et al., 2009b).

Presently available therapies for patients with AD include cholinesterase inhibitors, agents that influence glutamate neurotransmission, neuroprotective approaches and pharmacological agents, which are useful for behavioral disturbances. However, these approaches do not directly influence the disease process, and new disease-modifying treatments are the major unmet need in AD. In addition to the selected studies described briefly above, a variety of other treatments are being tested in model systems.

CONCLUSIONS

The lines of research described above have greatly enhanced our understanding of AD. We are now on the threshold of implementation of novel treatments based on an understanding of the pathogenesis of amyloidogenic mechanisms leading to the illness. Progress is being made in understanding the tauopathies. We anticipate that future discoveries will lead to the design of new mechanism-based therapies that can be tested in models of AD and that eventually these approaches, if effective and safe, can be introduced into clinical settings for the benefit of patients with this devastating disease. We are confident that parallel strategies will provide similar benefits for the treatment of other neurodegenerative diseases of the nervous system. A major problem in designing therapy or

γ-SECRETASE AND PRESENILINS: NOT JUST FOR Aβ AND ALZHEIMER'S ANYMORE

Scott T. Brady

For most people, the presenilins are familiar from their roles in Alzheimer's disease (AD) pathogenesis. Not only are Presenilin 1 and 2 (PSEN1 and PSEN2) often mutated in patients with familial AD, but the role of presenilins as components of the γ-secretase proteolytic complex also makes them essential for the production of Aβ peptides from APP. However, the biological roles of the presenilins extend well beyond APP processing. Evidence for a more extensive list of functions is seen in the fact that the PSEN1 knockout is embryonic lethal (Wong et al., 1997), while the phenotype of the APP null mouse is mild (Zheng et al., 1995).

As noted in the text, γ-secretase is a complex of four polypeptides with an aspartyl protease activity (Small, et al., 2010). The γ-secretase complex has the unusual property of working on membrane proteins and producing intramembranous cleavages in a select set of proteins (Hass, et al., 2009). There may be more than 90 type I integral membrane protein substrates of γ-secretase (Lleo et al., 2011). There is no clear consensus sequence for cleavage and the example of APP indicates that γ-secretase cleavages can be imprecise, yielding multiple distinct fragments. There is nonetheless selectivity, which may be based on conformation of the transmembrane domain. The functional significance of γ-secretase cleavage is uncertain for many of these substrates, but known functions include signaling and regulation of protein functions. Several of these substrates are of particular interest.

The best-known γ-secretase substrates after APP are notch and its ligands, delta and jagged (Woo, et al., 2009). Notch is a four member gene family in humans that plays an important role in neurogenesis, neurite growth, and differentiation. Notch is also implicated in synaptic plasticity as well as learning and memory. Notch is subject to multiple regulatory proteolytic events. The critical one is γ-secretase cleavage following ligand binding to release the notch intercellular cytoplasmic domain (NICD), which translocates to the nucleus and binds DNA to regulate transcription. The notch ligands Delta and Jagged are also substrates for γ-secretase (Lleo et al., 2011). The loss of notch signaling is considered to be a major barrier to the use of γ-secretase inhibitors for treatment of Alzheimer's. However, other substrates that may also present complications.

There are other receptors important for differentiation of cells in the nervous system that are processed by γ-secretase. Two receptors of particular interest are ErbB4 and some members of the Ephrin receptor family (Lleo et al., 2011). Both are receptor tyrosine kinases (see chapter 26) that play key roles in the nervous system. ErbB4 and its ligands, the neuregulins, are important for control of glial development and myelination (Newbern et al., 2010). The Eph/Ephrin pathway plays important roles in synaptic development and plasticity (Lai et al., 2009). Several Eph receptors (A4, B2 and B4) as well as some of their Ephrin ligands (B1, B2) can be processed by γ-secretase. As with APP and Notch, the action of γ-secretase on these receptors is to generate an intracellular cytoplasmic domain involved in signaling either to modulate transcription or through Rac1.

LDL receptor related proteins are particularly interesting substrates given the identification of ApoE4 as a major risk factor for late onset Alzheimer's disease (Bu, 2009). Several of the family members (LRP1, ApoER2, VLDL and megalin can be processed by γ-secretase (Lleo et al., 2011). Much like APP and Notch, cleavage of LRP1 gives rise to an intracellular cytoplasmic domain that can be translocated to the nucleus and affect transcription (Bu, 2009). LRP1 and ApoER2 also interact with APP, while ligand ApoE can bind Aβ. The extent to which LDL receptor processing by γ-secretase contributes to AD pathology is a matter of speculation at present.

Although these examples touch on only a fraction of the substrates cleaved by the presenilins and γ-secretase, they illustrate the complexities of presenilin biology in the brain. For example, some familial AD mutations of PSEN1 can differentially affect processing of some of substrates, but not others. Consistent with this, a recent report identified a γ-secretase activating protein (GSAP) that selectively enhances APP processing and Aβ production without altering Notch processing (He et al., 2010). Before γ-secretase inhibitors can be used to treat AD, we need to understand the biology of these proteases. Such information may provide new strategies as well as imposing constraint on therapeutics.

References

Bu, G. (2009). Apolipoprotein E and its receptors in Alzheimer's disease: Pathways, pathogenesis and therapy. (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review). *Nature Reviews. Neuroscience*, *10*(5), 333–344. 10.1038/nrn2620.

Hass, M. R., Sato, C., Kopan, R., & Zhao, G. (2009). Presenilin: RIP and beyond. (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review). *Seminars in Cell & Developmental Biology*, *20*(2), 201–210. 10.1016/j.semcdb.2008.11.014.

He, G., Luo, W., Li, P., Remmers, C., Netzer, W. J., Hendrick, J., et al. (2010). Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease. *Nature*, *467*(7311), 95–98. (doi: nature09325; pii: 10.1038/nature09325).

Lai, K. O., & Ip, N. Y. (2009). Synapse development and plasticity: Roles of ephrin/Eph receptor signaling. (Research Support, Non-U.S. Gov't Review). *Current Opinion in Neurobiology*, *19*(3), 275–283. 10.1016/j.conb.2009.04.009.

Lleo, A., & Saura, C. (2011). γ-secretase substrates and their implication for drug development in Alzheimer's disease. *Current Topics in Medicinal Chemistry* (11) (in press).

Newbern, J., & Birchmeier, C. (2010). Nrg1/ErbB signaling networks in Schwann cell development and myelination. (Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review). *Seminars in Cell & Developmental Biology*, *21*(9), 922–928. 10.1016/j.semcdb.2010.08.008.

Small, D. H., Klaver, D. W., & Foa, L. (2010). Presenilins and the gamma-secretase: Still a complex problem. (Research

γ-SECRETASE AND PRESENILINS: NOT JUST FOR Aβ AND ALZHEIMER'S ANYMORE (*cont'd*)

Support, Non-U.S. Gov't Review). *Molecular Brain*, *3*, 7. 10.1186/1756-6606-3-7.

Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E., et al. (1997). Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature*, *387*(6630), 288–292. 10.1038/387288a0.

Woo, H. N., Park, J. S., Gwon, A. R., Arumugam, T. V., & Jo, D. G. (2009). Alzheimer's disease and Notch signaling Research Support, Non-U.S. Gov't Review). *Biochemical and Biophysical Research Communications*, *390*(4), 1093–1097. 10.1016/j.bbrc.2009.10.093.

Zheng, H., Jiang, M., Trumbauer, M. E., Sirinathsinghji, D. J., Hopkins, R., Smith, D. W., et al. (1995). beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell*, *81*(4), 525–531. (doi: 0092-8674(95)90073-X; pii).

prevention is presented by the growing realization that the earliest neurocellular damage begins possibly decades before any clinical symptoms appear. Thus, research into clinical biomarkers that may identify the earliest stage in the pathogenesis of Alzheimer's disease is crucial.

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References

Ahn, K., Shelton, C. C., Tian, Y., Zhang, X., Gilchrist, M. L., Sisodia, S. S., et al. (2010). Activation and intrinsic {gamma}-secretase activity of presenilin 1. *Proceedings of the National Academy of Sciences of the United States of America*

Ashe, K. H., et al. (2010). Probing the biology of Alzheimer's disease in mice. *Neuron*, *66*, 631–645.

Bacskai, B. J., Hickey, G. A., Skoch, J., Kajdasz, S. T., Wang, Y., Huang, G. F., et al. (2003). Four-dimensional multiphoton imaging of brain entry, amyloid binding, and clearance of an amyloid-beta ligand in transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 12462–12467.

Ballatore, C., Lee, V. M. Y., & Trojanowski, J. Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nature Reviews Neuroscience*, *8*, 663–672.

Bard, F., Cannon, C., Barbour, R., Burke, R. L., Games, D., Grajeda, H., et al. (2000). Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nature Medicine*, *6*, 916–919.

Bateman, R. J., Munsell, L. Y., Morris, J. C., Swarm, R., Yarasheski, K. E., & Holtzman, D. M. (2006). Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid *in vivo*. *Nature Medicine*, *12*, 856–861.

Bateman, R. J., Siemers, E. R., Mawuenyega, K. G., Wen, G., Browning, K. R., Sigurdson, W. C., et al. (2009). A gamma-secretase inhibitor decreases amyloid-beta production in the central nervous system. *Annals of Neurology*, *66*, 48–54.

Bertram, L., & Tanzi, R. E. (2008). Thirty years of Alzheimer's disease genetics: The implications of systematic meta-analyses. *Nature Reviews. Neuroscience*, *9*, 768–778.

Bishop, N. A., Lu, T., & Yankner, B. A. (2010). Neural mechanisms of ageing and cognitive decline. *Nature*, *464*, 529–535.

Borchelt, D. R., Ratovitski, T., Van Lare, J., Lee, M. K., Gonzales, V., Jenkins, N. A., et al. (1997). Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron*, *19*, 939–945.

Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitski, T., et al. (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron*, *17*, 1005–1013.

Braak, H., & Braak, E. (1991). Neuropathological staging of Alzheimer-related changes. *Acta Neuropathologica*, *82*, 239–259.

Brody, D. L., & Holtzman, D. M. (2008). Active and passive immunotherapy for neurodegenerative disorders. *Annual Review of Neuroscience*, *31*, 175–193.

Brookmeyer, R., Gray, S., & Kawas, C. (1998). Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *American Journal of Public Health*, *88*, 1337–1342.

Bu, G. (2009). Apolipoprotein E and its receptors in Alzheimer's disease: Pathways, pathogenesis and therapy 1. *Nature Reviews. Neuroscience*, *10*, 333–344.

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The brain's default network: Anatomy, function, and relevance to disease 1. *Annals of the New York Academy of Sciences*, *1124*, 1–38.

Buxbaum, J. D., Thinakaran, G., Koliatsos, V., O'Callahan, J., Slunt, H. H., Price, D. L., et al. (1998). Alzheimer amyloid protein precursor in the rat hippocampus: Transport and processing through the perforant path. *The Journal of Neuroscience*, *18*, 9629–9637.

Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D. R., Price, D. L., et al. (2001). BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nature Neuroscience*, *4*, 233–234.

Cao, X., & Sudhof, T. C. (2001). A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science*, *293*, 115–120.

Capell, A., Steiner, H., Romig, H., Keck, S., Baader, M., Grim, M. G., et al. (2000). Presenilin-1 differentially facilitates endoproteolysis of the beta-amyloid precursor protein and Notch. *Nature Cell Biology*, *2*, 205–211.

Caughey, B., & Lansbury, P. T. (2003). Protofibrils, pores, fibrils, and neurodegeneration: Separating the responsible protein aggregates from the innocent bystanders. *Annual Review of Neuroscience*, *26*, 267–298.

Chapman, P. F., White, G. L., Jones, M. W., Cooper-Blacketer, D., Marshall, V. J., Irizarry, M., et al. (1999). Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nature Neuroscience*, *2*, 271–276.

Chen, G., Chen, K. S., Knox, J., Inglis, J., Bernard, A., Martin, S. J., et al. (2000). A learning deficit related to age and b-amyloid plaques in a mouse model of Alzheimer's disease. *Nature*, *408*, 975–979.

Chow, V., Savonenko, A., Melnikova, T., Kim, H., Price, D., Li, T., et al. (2010a). Modeling an anti-amyloid combination therapy for Alzheimer's disease. Ref Type: Serial (Book, Monograph).

Chow, V. W., Savonenko, A. V., Melnikova, T., Kim, H., Price, D. L., Li, T., et al. (2010). Modeling an anti-amyloid combination therapy for Alzheimer's disease. *Science Translational Medicine*, *2*, 13ra1.

Cirrito, J. R., May, P. C., O'Dell, M. A., Taylor, J. W., Parsadanian, M., Cramer, J. W., et al. (2003). *In vivo* assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. *The Journal of Neuroscience*, *23*, 8844–8853.

Citron, M. (2010). Alzheimer's disease: Strategies for disease modification. *Nature Reviews. Drug Discovery*, *9*, 387–398.

de Calignon, A., Fox, L. M., Pitstick, R., Carlson, G. A., Bacskai, B. J., Spires-Jones, T. L., et al. (2010). Caspase activation precedes and leads to tangles. *Nature*, *464*, 1201–1204.

De Strooper, B. (2003). Aph-1, Pen-2, and Nicastrin with presenilin generate an active gamma-secretase complex. *Neuron*, *38*, 9–12.

De Strooper, B., Annaert, W. G., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., et al. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature*, *398*, 518–522.

DeMattos, R. B., Bales, K. R., Cummins, D. J., Paul, S. M., & Holtzman, D. M. (2002). Brain to plasma amyloid-beta efflux: A measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science*, *295*, 2264–2267.

Doan, A., Thinakaran, G., Borchelt, D. R., Slunt, H. H., Ratovitski, T., Podlisny, M., et al. (1996). Protein topology of presenilin 1. *Neuron*, *17*, 1023–1030.

Dodart, J. C., Bales, K. R., Gannon, K. S., Greene, S. J., DeMattos, R. B., Mathis, C., et al. (2002). Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. *Nature Neuroscience*

Edbauer, D., Winkler, E., Haass, C., & Steiner, H. (2002). Presenilin and nicastrin regulate each other and determine amyloid beta- peptide production via complex formation. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 8666–8671.

Esler, W. P., Kimberly, W. T., Ostaszewski, B. L., Diehl, T. S., Moore, C. L., Tsai, J. Y., et al. (2000). Transition-state analogue inhibitors of gamma-secretase bind directly to presenilin-1. *Nature Cell Biology*, *2*, 428–434.

Esler, W. P., Kimberly, W. T., Ostaszewski, B. L., Ye, W., Diehl, T. S., Selkoe, D. J., et al. (2002). Activity-dependent isolation of the presenilin-y-secretase complex reveals nicastrin and a y substrate. *Proceedings of the National Academy of Sciences*, *14*, 1–6.

Farzan, M., Schnitzler, C. E., Vasilieva, N., Leung, D., & Choe, H. (2000). BACE2, a b-secretase homolog, cleaves at the b site and within the amyloid-b region of the amyloid-b precursor protein. *Proceedings of the National Academy of Sciences*, *97*, 9712–9717.

Frisardi, V., Solfrizzi, V., Imbimbo, P. B., Capurso, C., D'Introno, A., Colacicco, A. M., et al. (2010). Towards disease-modifying treatment of Alzheimer's disease: Drugs targeting beta-amyloid. *Current Alzheimer Research*, *7*, 40–55.

Gamblin, T. C., Chen, F., Zambrano, A., Abraha, A., Lagalwar, S., Guillozet, A. L., et al. (2003). Caspase cleavage of tau: Linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 10032–10037.

Ghaemmaghami, S., Ullman, J., Ahn, M., St, Martin, S., & Prusiner, S. B. (2010). Chemical induction of misfolded prion protein conformers in cell culture. *The Journal of Biological Chemistry*, *285*, 10415–10423.

Goedert, M., & Spillantini, M. -G. (2006). Neurodegenerative alpha-Synucleinopathies and tauopathies Siegel Albers Brady Price (Eds.), *Basic neurochemistry: Molecular, cellular, and medical aspects* (pp. 745–759) (7th ed.). Elsevier.

Golde, T. E., Schneider, L. S., & Koo, E. H. (2011). Anti-abeta therapeutics in Alzheimer's disease: The need for a paradigm shift. *Neuron*, *69*, 203–213.

Gong, Y., Chang, L., Viola, K. L., Lacor, P. N., Lambert, M. P., Finch, C. E., et al. (2003). Alzheimer's disease-affected brain: Presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 10417–10422.

Gotz, J., Chen, F., Van Dorpe, J., & Nitsch, R. M. (2001). Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Abeta fibrils. *Science*, *293*, 1491–1495.

Gotz, J., & Ittner, L. M. (2008). Animal models of Alzheimer's disease and frontotemporal dementia. *Nature Reviews. Neuroscience*, *9*, 532–544.

Goutte, C., Tsunozaki, M., Hale, V. A., & Priess, J. R. (2002). APH-1 is a multipass membrane protein essential for the Notch signaling pathway in Caenorhabditis elegans embryos. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 775–779.

Haass, C. (2004). Take five-BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *The EMBO Journal*, *23*, 483–488.

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M. L., et al. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nature Genetics*, *41*, 1088–1093.

Heber, S., Herms, J., Gajic, V., Hainfellner, J., Aguzzi, A., Rulicke, T., et al. (2000). Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. *The Journal of Neuroscience*, *20*, 7951–7963.

Hsia, A. Y., Masliah, E., McConlogue, L., Yu, G. Q., Tatsuno, G., Hu, K., et al. (1999). Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proceedings of the National Academy of Sciences*, *96*, 3228–3233.

Iwatsubo, T. (2004). The gamma-secretase complex: Machinery for intramembrane proteolysis. *Current Opinion in Neurobiology*, *14*, 379–383.

Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., & Ihara, Y. (1994). Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: Evidence that an initially deposited species is A beta 42(43). *Neuron*, *13*, 45–53.

Jankowsky, J. L., Fadale, D. J., Anderson, J., Xu, G. M., Gonzales, V., Jenkins, N. A., et al. (2004). Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide *in vivo*: Evidence for augmentation of a 42-specific gamma secretase. *Human Molecular Genetics*, *13*, 159–170.

Jankowsky, J. L., Slunt, H. H., Gonzales, V., Savonenko, A. V., Wen, J. C., Jenkins, N. A., et al. (2005). Persistent amyloidosis following suppression of Abeta production in a transgenic model of Alzheimer disease. *PLoS Medicine*, *2*, e355.

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., et al. (2003). APP processing and synaptic function. *Neuron*, *37*, 925–937.

Khlistunova, I., Biernat, J., Wang, Y. P., Pickhardt, M., von Bergen, M., Gazova, Z., et al. (2006). Inducible expression of tau repeat domain in cell models of tauopathy—aggregation is toxic to cells but can be reversed by inhibitor drugs. *Journal of Biological Chemistry*, *281*, 1205–1214.

Kim, J., Basak, J. M., & Holtzman, D. M. (2009a). The role of apolipoprotein E in Alzheimer's disease. *Neuron*, *63*, 287–303.

Kim, J., Castellano, J. M., Jiang, H., Basak, J. M., Parsadanian, M., Pham, V., et al. (2009b). Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular A beta clearance. *Neuron*, *64*, 632–644.

Kistner, A., Gossen, M., Zimmermann, F., Jerecic, J., Ullmer, C., Lubbert, H., et al. (1996). Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 10933–10938.

Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomstrand, G., Holt, D. P., et al. (2004). Imaging brain amyloid in Alzheimer's disease using the novel positron emission tomography tracer, Pittsburgh Compund-B. *Annals of Neurology*, *55*, 1–14.

Koo, E. H., & Kopan, R. (2004). Potential role of presenilin-regulated signaling pathways in sporadic neurodegeneration. *Nature Medicine*, *10*(Suppl.), S26–S33.

Koo, E. H., Sisodia, S. S., Archer, D. R., Martin, L. J., Weidemann, A., Beyreuther, K. T., et al. (1990). Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. *Proceedings of the National Academy of Sciences*, *87*, 1561–1565.

Kotilinek, L. A., Bacskai, B. J., Westerman, M., Kawarabayashi, T., Younkin, L., Hyman, B. T., et al. (2002). Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *The Journal of Neuroscience*, *22*, 6331–6335.

Kounnas, M. Z., Danks, A. M., Cheng, S., Tyree, C., Ackerman, E., Zhang, X., et al. (2010). Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *Neuron*, *67*, 769–780.

Lee, V. M., Goedert, M., & Trojanowski, J. Q. (2001). Neurodegenerative tauopathies. *Annual Review of Neuroscience*, *24*, 1121–1159.

Lewis, J., Dickson, D. W., Lin, W. -L., Chisholm, L., Corral, A., Jones, G., et al. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutat tau and APP. *Science*, *293*, 1487–1491.

Li, R., Lindholm, K., Yang, L. B., Yue, X., Citron, M., Yan, R., et al. (2004). Amyloid beta peptide load is correlated with increased beta-secretase activity in sporadic Alzheimer's disease patients. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 3632–3637.

Li, T., Ma, G., Cai, H., Price, D. L., & Wong, P. C. (2003). Nicastrin is required for assembly of presenilin/gamma-secretase complexes to mediate Notch signaling and for processing and trafficking of beta-amyloid precursor protein in mammals. *The Journal of Neuroscience*, *23*, 3272–3277.

Li, Y. M., Xu, M., Lai, M. T., Huang, Q., Castro, J. L., DiMuzio-Mower, J., et al. (2000). Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature*, *405*, 689–694.

Liu, Y., Yoo, M. J., Savonenko, A., Stirling, W., Price, D. L., Borchelt, D. R., et al. (2008). Amyloid pathology is associated with progressive monoaminergic neurodegeneration in a transgenic mouse model of Alzheimer's disease. *The Journal of Neuroscience*, *28*, 13805–13814.

Lue, L. F., Kuo, Y. M., Roher, A. E., Brachova, L., Shen, Y., Sue, L., et al. (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *The American Journal of Pathology*, *155*, 853–862.

Luo, Y., Bolon, B., Kahn, S., Bennett, B. D., Babu-Khan, S., Denis, P., et al. (2001). Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. *Nature Neuroscience*, *4*, 231–232.

Ma, G., Li, T., Price, D. L., & Wong, P. C. (2005). APH-1a is the principal mammalian APH-1 isoform present in gamma-secretase complexes during embryonic development. *The Journal of Neuroscience*, *25*, 192–198.

Maeda, J., Ji, B., Irie, T., Tomiyama, T., Maruyama, M., Okauchi, T., et al. (2007). Longitudinal, quantitative assessment of amyloid, neuroinflammation, and anti-amyloid treatment in a living mouse model of Alzheimer's disease enabled by positron emission tomography. *The Journal of Neuroscience*, *27*, 10957–10968.

Marjaux, E., Hartmann, D., & De Strooper, B. (2004). Presenilins in memory, Alzheimer's disease, and therapy. *Neuron*, *42*, 189–192.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS–ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology*, *34*, 939–944.

Monsonego, A., & Weiner, H. L. (2003). Immunotherapeutic approaches to Alzheimer's disease. *Science*, *302*, 834–838.

Morgan, D., Diamond, D. M., Gottschall, P. E., Ugen, K. E., Dickey, C., Hardy, J., et al. (2000). Ab peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature*, *408*, 982–985.

Morris, J. C., & Price, J. L. (2001a). Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. *Journal of Molecular Neuroscience*, *17*, 101–118.

Morris, J. C., Storandt, M., Miller, J. P., McKeel, D. W., Price, J. L., Rubin, E. H., et al. (2001b). Mild cognitive impairment represents early-stage Alzheimer disease. *Archives of Neurology*, *58*, 397–405.

Naruse, S., Thinakaran, G., Luo, J. J., Kusiak, J. W., Tomita, T., Iwatsubo, T., et al. (1998). Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron*, *21*, 1213–1221.

Nestor, P. J., Scheltens, P., & Hodges, J. R. (2004). Advances in the early detection of Alzheimer's disease. *Nature Medicine*, *10*(Suppl.), S34–S41.

Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., et al. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Abeta and synaptic dysfunction. *Neuron*, *39*, 409–421.

Ohno, M., Sametsky, E. A., Younkin, L. H., Oakley, H., Younkin, S. G., Citron, M., et al. (2004). BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. *Neuron*, *41*, 27–33.

Perrin, R. J., Fagan, A. M., & Holtzman, D. M. (2009). Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature*, *461*, 916–922.

Petersen, R. C. (2003). Mild cognitive impairment clinical trials. *Nature Reviews. Drug Discovery*, *2*, 646–653.

Petersen, R. C., Doody, R., Kurz, A., Mohs, R. C., Morris, J. C., Rabins, P. V., et al. (2001). Current concepts in mild cognitive impairment. *Archives of Neurology*, *58*, 1985–1992.

Price, D. L., & Sisodia, S. S. (1998a). Mutant genes in familial Alzheimer's disease and transgenic models. *Annual Review of Neuroscience*, *21*, 479–505.

Price, D. L., Tanzi, R. E., Borchelt, D. R., & Sisodia, S. S. (1998b). Alzheimer's disease: Genetic studies and transgenic models. *Annual Review of Genetics*, *32*, 461–493.

Querfurth, H. W., & LaFerla, F. M. (2010). Alzheimer's disease. *The New England Journal of Medicine*, *362*, 329–344.

Reddy, M. M., Wilson, R., Wilson, J., Connell, S., Gocke, A., Hynan, L., et al. (2011). Identification of candidate IgG biomarkers for Alzheimer's disease via combinatorial library screening. *Cell*, *144*, 132–142.

Rogaeva, E., Meng, Y., Lee, J. H., Gu, Y., Kawarai, T., Zou, F., et al. (2007). The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nature Genetics*, *39*, 168–177.

Sagare, A., Deane, R., Bell, R. D., Johnson, B., Hamm, K., et al. (2011). Clearance of amyloid by circulating lipoprotein receptors. *Nature Medicine*, 1029–1031.

Savonenko, A. V., Melnikova, T., Laird, F. M., Stewart, K. A., Price, D. L., & Wong, P. C. (2008). Alteration of BACE1-dependent NRG1/

ErbB4 signaling and schizophrenia-like phenotypes in BACE1-null mice. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 5585–5590.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., et al. (1999). Immunization with amyloid-beta attenuates Alzheimer disease-like pathology in the PDAPP mouse. *Nature*, *400*, 173–177.

Selkoe, D., & Kopan, R. (2003). Notch and presenilin: Regulated intramembrane proteolysis links development and degeneration. *Annual Review of Neuroscience*, *26*, 565–597.

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science*, *298*, 789–791.

Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J., & Tonegawa, S. (1997). Skeletal and CNS defects in presenilin-1-deficient mice. *Cell*, *89*, 629–639.

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., et al. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, *375*, 754–760.

Sisodia, S. S., & George-Hyslop, P. H. (2002). gamma-Secretase, Notch, Abeta and Alzheimer's disease: Where do the presenilins fit in?. *Nature Reviews. Neuroscience*, *3*, 281–290.

Sisodia, S. S., Koo, E. H., Beyreuther, K. T., Unterbeck, A., & Price, D. L. (1990). Evidence that b-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science*, *248*, 492–495.

Sisodia, S. S., Koo, E. H., Hoffman, P. N., Perry, G., & Price, D. L. (1993). Identification and transport of full-length amyloid precursor proteins in rat peripheral nervous system. *The Journal of Neuroscience*, *13*, 3136–3142.

Sperling, R. A., Laviolette, P. S., O'Keefe, K., O'Brien, J., Rentz, D. M., Pihlajamaki, M., et al. (2009). Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron*, *63*, 178–188.

Steiner, H., Winkler, E., Edbauer, D., Prokop, S., Basset, G., Yamasaki, A., et al. (2002). PEN-2 is an integral component of the gamma-secretase complex required for coordinated expression of presenilin and nicastrin. *The Journal of Biological Chemistry*, *277*, 39062–39065.

Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K. H., Mistl, C., Rothacher, S., et al. (1997). Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proceedings of the National Academy of Sciences*, *94*, 13287–13292.

Sunderland, T., Linker, G., Mirza, N., Putnam, K. T., Friedman, D. L., Kimmel, L. H., et al. (2003). Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA: The Journal of the American Medical Association*, *289*, 2094–2103.

Takasugi, N., Tomita, T., Hayashi, I., Tsuruoka, M., Niimura, M., Takahashi, Y., et al. (2003). The role of presenilin cofactors in the gamma-secretase complex. *Nature*, *422*, 438–441.

Thinakaran, G., Harris, C. L., Ratovitski, T., Davenport, F., Slunt, H. H., Price, D. L., et al. (1997). Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *The Journal of Biological Chemistry*, *272*, 28415–28422.

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., et al. (1999). b-secretase cleavage of Alzheimer's amyloid precusor protein by the transmembrane aspartic protease BACE. *Science*, *286*, 735–741.

Vassar, R., Kovacs, D. M., Yan, R., & Wong, P. C. (2009). The beta-secretase enzyme BACE in health and Alzheimer's disease: Regulation, cell biology, function, and therapeutic potential. *The Journal of Neuroscience*, *29*, 12787–12794.

Walsh, D. M., & Selkoe, D. J. (2004). Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron*, *44*, 181–193.

Wang, Y., Garg, S., Mandelkow, E. M., & Mandelkow, E. (2010). Proteolytic processing of tau. *Biochemical Society Transactions*, *38*, 955–961.

Wang, Y. P., Biernat, J., Pickhardt, M., Mandelkow, E., & Mandelkow, E. M. (2007). Stepwise proteolysis liberates tau fragments that nucleate the Alzheimer-like aggregation of full-length tau in a neuronal cell model. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 10252–10257.

Wengenack, T. M., Reyes, D. A., Curran, G. L., Borowski, B. J., Lin, J., Preboske, G. M., et al. (2011). Regional differences in MRI detection of amyloid plaques in AD transgenic mouse brain. *Neuroimage*, *54*, 113–122.

Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T., & DeLong, M. R. (1981). Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Annals of Neurology*, *10*, 122–126.

Wolfe, M. S. (2002). Therapeutic strategies for Alzheimer's disease. *Nature Reviews. Drug Discovery*, *1*, 859–866.

Wong, P. C., Cai, H., Borchelt, D. R., & Price, D. L. (2002). Genetically engineered mouse models of neurodegenerative diseases. *Nature Neuroscience*, *5*, 633–639.

Wong, P. C., Price, D. L., & Cai, H. (2001). The brain's susceptibility to amyloid plaques. *Science*, *293*, 1434–1435.

Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E., et al. (1997). Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature*, *387*, 288–292.

Xia, P., Chen, H. S., Zhang, D., & Lipton, S. A. (2010). Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *The Journal of Neuroscience*, *30*, 11246–11250.

Yan, R., Bienkowski, M. J., Shuck, M. E., Miao, H., Tory, M. C., Pauley, A. M., et al. (1999). Membrane-anchored aspartyl protease with Alzheimer's disease b-secretase activity. *Nature*, *402*, 533–537.

Yang, L. B., Lindholm, K., Yan, R., Citron, M., Xia, W., Yang, X. L., et al. (2003). Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nature Medicine*, *9*, 3–4.

Zhang, D., & Raichle, M. E. (2010). Disease and the brain's dark energy. *Nature Reviews Neurology*, *6*, 15–28.