Proteases and Proteolysis in Alzheimer Disease: A Multifactorial View on the Disease Process

BART DE STROOPER

Center for Human Genetics, K.U.Leuven and Department for Molecular and Developmental Genetics, VIB, Leuven, Belgium

I. The Alzheimer Disease Puzzle 466
   A. The amyloid cascade hypothesis 466
   B. Moving towards more comprehensive theories for AD 466
   C. The importance of tau 467

II. Proteolysis Involved in Multiple Scenarios for Alzheimer Disease 467
   A. Proteolytic processing of the APP 467
   B. General disturbances of the endolysosomal-phagocytotic system in AD neurons 468

III. Heterogeneity of the Amyloid Aβ Peptide and the Toxic Oligomer Hypothesis 468
   A. Aβ toxicity in Alzheimer disease 468
   B. Heterogeneity of the Aβ peptide in vivo and potential contribution to overall toxicity 469

IV. α-Secretase and the Physiologically Most Relevant Proteolysis of APP 469
   A. α-Secretase activity and the generation of APPs, and p3 469
   B. Members of the ADAM family exert α-secretase activity 470
   C. Other α-secretases 471
   D. α-Secretase as a Drug Target for AD 471

V. β-Secretase 471
   A. BACE1 is the major β-secretase 471
   B. Molecular and cellular biology of BACE1 472
   C. Deregulation of BACE1 expression in AD 472
   D. BACE1 physiological functions 473
   E. BACE1 as a drug target for AD 474

VI. γ-Secretases 474
   A. The different γ-secretase complexes 474
   B. Molecular and cellular biology of the γ-secretase 475
   C. γ-Secretases proteolytic functions 476
   D. Role of γ-secretase in the generation of carboxy-terminal heterogeneity of Aβ 477
   E. γ-Secretase as a drug target for AD 478

VII. Neurofibrillar Tangles and Tau 478
   A. Tangles and the importance of nuclei formation 478
   B. Hyperphosphorylation of tau 479
   C. Proteolysis of tau and relevance for AD 480
   D. Tau as a drug target in AD 480

VIII. Proteolytic Degradation of the Amyloid Aβ Peptide 481
   A. Clearance of Aβ from the brain 481
   B. Neprilysin 482
   C. Endothelin converting enzymes 1 and 2 482
   D. Angiotensin converting enzyme 482
   E. Insulin degrading enzyme 482
   F. Matrix metalloproteases 483
   G. Plasmin 483
   H. Cathepsin B and cystatin C 483

IX. Conclusion 484
responsible for the generation and clearance of these fragments, and how disturbances in these pathways interact and provide a background for a novel understanding of Alzheimer disease as a multifactorial disorder. Recent insights evolve from the static view that the morphologically defined plaques and tangles are disease driving towards a more dynamic, biochemical view in which the intermediary soluble Aβ oligomers and soluble tau fragments are considered as the main mediators of neurotoxicity. The relevance of proteolytic pathways, centered on the generation and clearance of toxic Aβ, on the cleavage and nucleation of tau, and on the general proteostasis of the neurons, then becomes obvious. Blocking or stimulating these pathways provide, or have the potential to provide, interesting drug targets, which raises the hope that we will be able to provide a cure for this dreadful disorder.

I. THE ALZHEIMER DISEASE PUZZLE

Alzheimer disease (AD) is one of the major health problems in the world. The disease is characterized by the accumulation of proteins and protein fragments in the brain, progressive neuronal loss, inflammation, and the progressive and inexorable decline of memory and cognition. Major landmarks in AD research include the isolation and sequencing of the amyloid peptide (Aβ) from amyloid plaques and amyloid angiopathy in the brain and the demonstration of abnormal tau phosphorylation in neuronal tangles.

A. The Amyloid Cascade Hypothesis

The modern era of AD research began with the identification of missense mutations in the amyloid beta precursor protein (APP) (43) and presenilin (PS) genes (175, 246, 277), providing important clues in the advancement of the understanding of (rare) early-onset familial forms of AD. Mutations in the three genes affect the production and/or aggregation of the Aβ peptide (263). In 1998, it became clear that PS is an essential component of the proteolytic activity that cleaves APP to generate the Aβ peptide (58). Thus both mutations in the substrate (APP) and the protease (PS) responsible for generation of Aβ are sufficient to cause all of the symptoms of classical AD, and the protease (PS) responsible for generation of Aβ are sufficient to cause all of the symptoms of classical AD, including the neuronal tangle pathology. The initial version of the amyloid cascade hypothesis proposed that amyloid fibrils and plaques in the brain were the drivers of the disease, while more recent variations of the hypothesis focus on small soluble aggregates of Aβ peptide as the primary impetus of disease progression. Toxicity is also more specifically associated with certain variants of the Aβ peptide, which displays amino- and carboxy-terminal heterogeneity (discussed in section III) (99). Especially the Aβ1–42 is considered pathogenic because mutations causing AD in APP (296) or in PS (65, 263) increase the relative production of this peptide.1 This variant is also abundant in amyloid plaques of sporadic and familial AD patients (138, 190), and it provides a nidus for amyloid formation (141).

This novel version of the amyloid hypothesis accounts for several discrepancies in human and mouse studies, e.g., abundant amyloid deposition does not necessarily correlate with memory deficits or neuronal toxicity. However, the implications of the hypothesis remain the same: tangle formation and neurodegeneration are the consequence of abnormal Aβ metabolism, and a drug that inhibits production or facilitates removal of Aβ from the brain should significantly improve, cure, or prevent disease progression. Since such a drug is not available yet, the amyloid cascade hypothesis remains unproven. The amyloid cascade hypothesis provides, however, a very good explanation for the development of familial early-onset forms of AD. More than 30 mutations in APP and more than 180 mutations in the two PS genes have been identified (http://www.molgen.ua.ac.be/ADMutations). Nevertheless, these missense mutations account for <0.5% of all AD cases (37). The other, much more frequent forms of AD have been called somewhat paradoxically “sporadic” (as opposed to familial) AD and are caused by a combination of environmental and genetic factors.

B. Moving Towards More Comprehensive Theories for AD

Multifactorial diseases are very common in the elderly population, and treatment of these diseases usually requires a combination of different approaches. Indeed, treatment of Aβ alone would probably not suffice to effectively cure all forms of sporadic AD as likely part of the damage in the brain becomes irreversible (119). Thus we need to establish a more comprehensive, albeit more complex, view of the different factors that underlie the pathogenesis of AD. Decreased blood circulation and oxygenation of the brain, oxidative stress, or changes that affect housekeeping functions or survival mechanisms [proteostasis (11), the microRNA rheostat (109), and others] have to be taken into account to understand why the aging brain reacts differently from the young brain to Aβ peptides (87). These changes might not only increase levels of Aβ, but also the sensitivity of the brain to Aβ toxicity and compromise (via parallel pathways) tau func-

1 We use subscripts to indicate the number of amino acids present in the Aβ peptide. If not indicated otherwise, the peptide starts at amino acid residue Asp-1 and ends at Val-40. Aβ42 ends at Ala-42. Aβ1–42 starts at Glu-11 and ends at Ala-42 (see Fig. 1C for the amino acid sequence of Aβ).
tion, inflammation, synaptic connectivity, and/or neuronal survival. This sets the scene for a multifactorial view on AD and indicates how, depending on the relative contribution of these different causes to the disease, different therapeutic options will have to be considered. Abnormal proteolysis is an important part of the emerging multifactorial view on the pathogenesis of AD.

C. The Importance of Tau

Missense mutations in the tau gene are sufficient to cause dementia in frontotemporal dementia with Parkinsonism (FTDP-17) (88), demonstrating that tau phosphorylation and tangle formation is a self-propagating disease. Thus it is logical to assume that once tangle pathology is initiated in AD, further decline occurs independently of the presence of Aβ. Preliminary evidence from a follow-up study of one of the Aβ vaccination trials supports this possibility given that two patients, who were almost entirely devoid of Aβ deposition, displayed signs of severe and progressed tangle pathology (Braak stage VI). Importantly, the intellectual capacities of these patients were severely affected, and clinical decline was not significantly different from nontreated AD patients (119).

II. PROTEOLYSIS INVOLVED IN MULTIPLE SCENARIOS FOR ALZHEIMER DISEASE

Amyloid-centered research focuses on abnormal generation of the Aβ peptide and how changes in production or clearance affect assembly of toxic Aβ oligomers and neuronal toxicity. Alternative approaches focus on disturbances in lysosomal, autophagosomal (217), apoptotic (61, 348), ubiquitin-proteasome, or other proteolytic systems in the brain and the effect that disruption of these pathways has on the global health of neurons. In this field of study, Aβ production is usually considered to be a consequence or part of a feed-forward cycle, aggravating the underlying disease process, which results in AD.

A. Proteolytic Processing of the APP

The most prevalent area of research in AD studies the proteolytic generation of Aβ from APP. The β-secretase and γ-secretase cleave APP in the so-called amyloidogenic pathway (Fig. 1A). β-Secretase releases the ectodomain APPsβ, and the remaining APP carboxy-terminal fragment (APP-CTFβ) is subsequently cleaved by the γ-secretase liberating the (secreted) Aβ peptide(s) and the APP intracellular domain (AICD). The biological functions of APPsβ, Aβ, and the AICD remain rather elusive, although Aβ release is associated with synaptic activity, depressing excitatory synaptic transmission onto...
neurons (146). The AICD has been proposed to be a nuclear signaling molecule (4, 38, 52), but this remains controversial (112). A recent study suggests that APPs\textsubscript{\beta} is further processed by an unknown protease, generating a \textsim35-kDa amino-terminal domain fragment that serves as a ligand for the death receptor DR6. Binding to DR6 triggers activation of caspase-6 and mediates axonal pruning during embryogenesis (215). Although it would be intriguing to speculate that this pathway contributes to AD pathogenesis, no patient data are available to support this possibility.

The quantitatively and functionally most important proteolytic cleavage of APP is mediated by the \(\alpha\)-secretase, which releases the APP\textsubscript{\alpha} ectodomain. The resulting carboxy-terminal APP-CTF\textsubscript{\alpha} is further processed by the \(\gamma\)-secretase and generates a small \(\beta\) fragment and the AICD (Fig. 1A). APPs\textsubscript{\alpha} has been suggested to exhibit neuroprotective and synapse-promoting activities (13), but elucidation of a mechanism or identification of a receptor mediating these effects has not been achieved yet. Nevertheless, a study utilizing a knock-in of a stop codon in the mouse APP gene at the \(\alpha\)-secretase site elegantly demonstrated that mice that only produce APPs\textsubscript{\alpha} do not exhibit the various phenotypes caused by a full APP knockout, including disturbed LTP and memory function. Thus APPs\textsubscript{\alpha} probably mediates the majority of the functions of the APP holoprotein. (242). The structure-function of APP is reviewed elsewhere (238).

**B. General Disturbances of the Endolysosomal-Phagocytotic System in AD Neurons**

More general perturbations of the proteolytic degradation machinery in neurons have been implicated in the pathogenesis of AD and may occur downstream and even upstream of A\(\beta\) generation. A striking morphological alteration in neurons in AD brains is the accumulation of autophagosomes, autolysosomes, and lysosomal dense bodies in dystrophic neurites (30, 40, 216). Similar alterations are also observed in APP and/or PS transgenic AD mouse models (358), even before amyloid deposition is observed. However, these mice generate a large amount of the A\(\beta\) peptide. Thus (soluble) A\(\beta\) toxicity probably precedes the morphological alterations. Interestingly, PS loss-of-function mice also display disturbances in autophagy, suggesting a poorly understood role for PS in this process (72, 342). When autophagy is compromised in the brain of an APP overexpressing AD mouse model following inactivation of the Beclin gene (Beclin\textsuperscript{+/−} mice), enhanced A\(\beta\) deposition and synapse and neuronal loss are observed, which suggests that decreased autophagy could aggravate or independently contribute to the overall neurodegenerative process (232). Interestingly, expression of Beclin using lentiviral vectors attenuated the amyloid load in the injected mouse brain areas.

**III. HETEROGENEITY OF THE AMYLOID A\(\beta\) PEPTIDE AND THE TOXIC OLIGOMER HYPOTHESIS**

A. A\(\beta\) Toxicity in Alzheimer Disease

Several studies have suggested that A\(\beta\) or A\(\beta\) aggregates can induce tau phosphorylation and tangle formation or interfere with synaptic function as measured by decreased long-term potentiation (LTP), and can cause changes in dendritic spines and altered memory function in rats and mice. A\(\beta\) has been shown to kill neurons in cell culture and in the brain in vivo (164, 173, 329, 354, 355). Nevertheless, the nature and physiological relevance of A\(\beta\) toxicity has been criticized for a long time given the high micromolar concentrations of A\(\beta\) peptides required to observe an effect.

In AD mouse models and in the normal aging human brain, large amounts of amyloid plaques have been observed with minor neuronal alterations, indicating that the relationship between A\(\beta\) accumulation and A\(\beta\) toxicity is not straightforward (1, 74, 233). A\(\beta\) in its two extreme forms, i.e., soluble as a single monomer or insoluble when trapped in amyloid fibrils, displays an insignificant degree of toxicity (195). Over the last decade, the concept of "A\(\beta\)-derived diffusible ligands" (ADDL) (164) or "soluble toxic oligomers" (164, 173, 329) has advanced. Several different oligomeric assemblies of the A\(\beta\) peptide have been described, generated in vitro (45), or isolated from transfected CHO cells as stable dimers, trimers, and multimers (234) or from transgenic mouse brains as a 56-kDa oligomer (173). Various oligomeric species have also been isolated from the brains of AD patients; the smallest toxic isolate was reported to be comprised of a dimeric structure (275). It is quite likely that different oligomeric species are in dynamic equilibrium (Fig. 1B) with each other, single peptides, and inert fibrils (250), a balance which may be influenced by the presence of lipids (195). Currently, no consensus exists with regard to which toxic A\(\beta\) assembly is most relevant in vivo, and it is not unlikely that various conformations of toxic aggregates exist next to each other. Indeed, A\(\beta\) aggregates behave quite differently under different experimental conditions, explaining part of the controversy in the literature (115).

The second question is obviously what parameters determine the equilibrium between less toxic and more toxic assemblies of A\(\beta\). Apart from differences in the experimental conditions, a major parameter seems to be the biophysical nature of the peptide itself. A\(\beta\)\textsubscript{40} for instance appears to be much less toxic, and even protective compared with A\(\beta\)\textsubscript{42} (199). This might be explained by the
influence of Aβ42 on the relative equilibrium between toxic and inert assemblies of Aβ.

B. Heterogeneity of the Aβ Peptide In Vivo and Potential Contribution to Overall Toxicity

A large variety of Aβ peptides has been isolated from the brains of sporadic AD patients (Fig. 1C). This diversity can be explained by proteolysis and additional enzymatic modifications of Aβ, but also by chemical reactions that occur slowly during the many years that Aβ peptides reside in amyloid plaques in the brain. The former are of particular interest as they might provide targets for drug development.

Much of the carboxy-terminal heterogeneity of Aβ is readily demonstrated in cell culture, suggesting that this is an inherent part of the production of Aβ by the γ-secretase (see further discussion in the appropriate section). In contrast, Aβ peptides isolated from cell cultures contain a relatively homogeneous amino terminus that usually begins with an Asp-1 residue. In contrast, Aβ peptides isolated from AD brains display a variety of modifications at the amino terminus. The most prominent alterations are amino-terminal truncations, cyclized Glu residues, i.e., pyro-Glu-3 or pyro-Glu-11 (Fig. 1C) (205, 253), and isomerization of Asp (iso-Asp) residues (160, 248, 279). Such modifications make the peptide more resistant to proteolytic degradation and/or more hydrophobic as a result of the loss of the amino-terminal charge (251). Interestingly, both in vitro and in vivo evidence in mice indicates that pGlu derivatives are particularly neurotoxic (251, 266). Glutaminyl cyclase catalyzes the modification of Glu to pGlu (264) by apparently reacting with glutamate residues at low pH (265). Given that the majority of Aβ peptides are generated in the acidic endosomal pathway, modification of the Aβ peptide by glutaminyl cyclase may be physiologically relevant; however, it remains unclear whether glutaminyl cyclase can act intracellularly, or only later, when the Aβ peptide has been secreted. One of the issues of debate focuses on generation of the free amino-terminal Glu residue required for this reaction. Amino-terminal Glu-11 is an alternative cleavage product of authentic β-secretase activity (49, 170, 182, 319), but Glu-3 becomes only available after aminopeptidases (273) have removed Asp-1 and Ala-2. Alternatively, dipeptidyl peptidases or acyl amino acid releasing enzyme could remove iso-Asp-1 (27).

Additional factors that are linked to heterogeneity of the Aβ peptide include spontaneous reactions such as oxidation, hydrolysis, racemization, and disulfide bond or ketoamine formation. A spontaneous succinimide-mediated rearrangement of l-aspartate (l-Asp) (and l-asparagine) to D/L-isoAsp or D-Asp residues results in the incorporation of an extra methylene group in the Aβ polypeptide backbone. This reaction is part of the natural aging process of many proteins (240). As this reaction cannot occur on the amino-terminal Asp, the iso-Asp1 modification described in Aβ peptides isolated from brain must occur in the full-length APP protein (28). Interestingly, β-secretase cannot cleave sequences with an iso-Asp modification; however, cathepsin B has been shown to cleave this sequence in vitro. Thus cathepsin B is speculated to be an alternative β-secretase (123). It should be mentioned that the issue regarding the extent to which cathepsin B displays a quantitative contribution to (toxic) Aβ generation is highly controversial, particularly since cathepsin B is also involved in Aβ turnover as we will see below.

Both pGlu and iso-Asp modifications have profound effects on the biophysical properties of the Aβ peptide. These modifications are apparently a frequent event in AD (161, 231, 248, 253, 279), but only recent research has begun to indicate that these alternative pathways might provide valuable drug targets for the treatment of AD. For instance, compounds that specifically inhibit glutaminyl cyclase showed benefits in a transgenic AD mouse model by decreasing the amyloid plaque load. The mice also appeared to exhibit (weakly statistically significant) improvement in memory performance (266). Obviously, further work is urgently needed to evaluate the extent to which these modifications affect the equilibrium between monomeric, oligomeric, and fibrillized Aβ species, and to what extent these modifications affect the overall toxicity of the Aβ mixtures observed in AD patients.

IV. α-SECRETASE AND THE PHYSIOLOGICALLY MOST RELEVANT PROTEOLYSIS OF APP

A. α-Secretase Activity and the Generation of APPsα and p3

The major APP proteolytic process in nonneuronal cells actually prevents further generation of the Aβ peptide (285). This proteolytic activity is called α-secretase (95) and cleaves APP between Lys-16-Leu-17 in the Aβ sequence (Fig. 1C). The membrane-bound carboxy-terminal fragment (APP-CTFα) is further processed by the γ-secretase (95) (Fig. 1A). The p317-40 and p317-42 fragments have been identified in the brains of AD patients. These fragments could be considered to be part of the Aβ peptide family given that they are highly hydrophobic and tend to aggregate. They have been found in diffuse amyloid plaques in AD brains (93, 118) but seem a very minor component of the classical dense-cored plaques (139, 254). In fact, very little is known about the toxicity or function of these p3 fragments. Nevertheless, the majority of research suggests that the α-secretase-mediated cleavage of APP is the nonamyloidogenic pathway, and it is
generally believed that increasing α-secretase activity could be beneficial in AD (13), although proof for this is lacking.

B. Members of the ADAM Family Exert α-Secretase Activity

The protease(s) responsible for α-secretase activity are membrane-bound metalloproteases, and their activity is regulated by an intricate network of secondary messengers (13). These proteases are fairly promiscuous with regard to their cleavage specificity as demonstrated by extensive mutagenesis of the amino acid sequence around the cleavage site in APP (284). Several members of the “A disintegrin and metalloprotease” or ADAM family have been implicated as α-secretases. Downregulation or overexpression of ADAM9, -10, -17, and -19 in various cell lines leads to decreased or increased APPsα generation, respectively (9, 35, 154, 165, 301). Given the high structural and functional homology among the ADAMs (in particular the 8 other ADAM proteins that also contain the catalytic consensus sequence HEXXH, i.e., ADAM8, -12, -15, -20, -21, -28, -30, and -33), it is likely that additional APP α-secretases will be identified in specific cellular contexts. Interestingly, ADAM12 and -15 do not cleave APP directly but are involved in the processing of ADAM10, the major candidate α-secretase, potentially liberating a soluble form of the α-secretase (311) and indirectly regulating α-secretase activity. An emerging concept is that the ADAMs can substitute for each other in various shedding events (101, 168) as ADAMs in general appear to be fairly nonselective with regard to membrane topology and sequence specificity of their substrates.

Thus, while cellular assays may provide a hint with regard to the proteases involved, only in vivo experiments using loss of functions paradigms can really demonstrate the physiological relevance of cell culture observations. Three ADAM proteases have been intensively investigated for their role in APP processing.

1. ADAM10

Overexpression of ADAM10 in the brain lowers the amyloid plaque load and leads to an improvement of cognitive performance in a mouse AD model (236). Dominant negative ADAM10 or highly selective inhibitors of ADAM10 also block α-secretase cleavage of APP (165, 269), which suggests that endogenously expressed ADAM10 can cleave APP as well. ADAM10 has many other substrates and is crucial in several signaling pathways. Both N-cadherin (239) and E-cadherin (192) ectodomain shedding is mediated by ADAM10. Remarkably, this influences not only intercellular adhesion, but also the amount of β-catenin at the cell surface and in the nucleus, and thereby regulates to a certain extent β-catenin signaling (192, 239). ADAM10 is also responsible for the regulated ectodomain cleavage of Notch, initiating further processing by the γ-secretase (see below) and the formation of the Notch intracellular domain. The latter transmits Notch signals to the nucleus. Given that ADAM10 knock-out (KO) mice display an embryonic lethal phenotype, probably due to the predominant role of Notch in vascularization during embryogenesis (101), the ultimate genetic experiment, demonstrating that lack of ADAM10 expression blocks α-secretase processing of APP in the adult brain, has not been performed. Thus, based on overexpression and inhibitor studies, the current research indicates that ADAM10 is a major constitutive α-secretase in many cell types.

2. ADAM9

The ADAM9 knockout mouse display no major phenotypical alterations (340) but show decreased pathological retinal neovascularization after oxygen- or laser-induced retinopathy (94). In primary cultures of ADAM9−/− cells, a reduction in APP cleavage, as determined by p3 release, was not observed (340).

It is surprising that the knockout of ADAM9 displays such a minor phenotype in light of the widespread distribution of ADAM9 mRNA in embryonic and adult tissues. This contrasts with the severe phenotypes observed in ADAM10 (101) and ADAM17 knockout mice (230), which suggests that these two ADAMs could potentially compensate for the loss of ADAM9 in the brain with regard to α-secretase activity.

3. ADAM17

ADAM17 has a crucial role in the release of a series of membrane-bound proteins, including transforming growth factor-α (TGF-α), tumor necrosis factor-α (TNF-α), L-selectin, p75 tumor necrosis factor receptor (p75TNFR), and others (230). ADAM17 is also called TACE (TNF-α converting enzyme), and deficiency leads to perinatal and early postnatal lethality in mice with defects in diverse epithelial tissues, including skin, intestine, lung, and several glands (25). Given that the phenotype is clearly different from ADAM10 knockout mouse, these studies indicate that both ADAM proteases have different functions, although they can cleave similar substrates in cell culture (168). These differential effects are probably due to specific expression patterns and to different activation pathways that regulate the two proteases (168). The evidence to support a role for ADAM17 in α-secretase processing of APP is not conclusive given that experiments in ADAM17 brain or primary neurons have not been published thus far, for similar reasons as ADAM10. However, in fibroblasts derived from ADAM17 knockout mice, constitutive α-secretase activity is maintained, while induced α-secretase activity (by phorbol myristate acetate) was...
inactivated (35), suggesting that another \( \alpha \)-secretase activity mediates constitutive APP processing, e.g., ADAM10, and that ADAM17 is responsible for the regulated fraction of the cleavage process. Nevertheless, the extent to which this simple scheme holds true in the brain in vivo remains unclear. In this regard, conflicting data pertaining to the expression of ADAM17 in neuronal cells has been published (191, 286), whereas infusion of a highly specific TACE inhibitor in the mouse brain led to a significant reduction of APPs\(_{\beta}\), strongly arguing for the importance of TACE in \( \alpha \)-secretase processing in vivo in the brain (151).

C. Other \( \alpha \)-Secretases

Over the years, several other proteases have been proposed to contribute to \( \alpha \)-secretase processing of APP. Some positive, but also negative, reports have discussed the potential involvement of the prohormone convertase family members (57, 183, 184). This family of proteases, which are responsible for the removal of propeptides from hormones and proteolytic maturation of proteases and other proteins, indirectly influences \( \alpha \)-secretase activity (as they actually also do on \( \beta \)-secretase; Ref. 49) by catalyzing removal of the prodomain from certain ADAMs. Furin and other convertases have been implicated in the removal of the propeptide sequences of ADAM10 and ADAM17, rendering these protease catalytically active (3, 135, 184).

Interestingly, BACE2, a homolog of BACE1 (\( \beta \)-secretase see below), has also been suggested to act as an alternate \( \alpha \)-secretase because it cleaves between Phe-19 and Phe-20 (Fig. 1B), close to the classical \( \alpha \)-secretase site (14, 79, 81, 352). Selective knockdown of endogenous BACE2 in human embryonic kidney cells using siRNA elevates \( \beta \)\( \beta \) secretion (14), further arguing that BACE2 functions in the “nonamyloidogenic” pathway. However, results from experiments in cell lines are not always predictive for the in vivo situation. BACE2 is apparently not expressed in neurons, and knockdown of BACE2 in primary glia cell cultures paradoxically results in decreased \( \beta \)\( \beta \) generation, suggesting that BACE2 in glia cells might promote \( \beta \)\( \beta \) generation (62). Additional in vitro experiments have shown that BACE2 can cleave at the Asp-1 site in APP to a certain extent (79).

D. \( \alpha \)-Secretase as a Drug Target for AD

Numerous studies have suggested that the \( \alpha \)-secretase is an interesting alternative for the development of anti-amyloidogenic drugs (reviewed in Ref. 13). \( \alpha \)-Secretase activity appears to be regulated via protein kinase C, tyrosine kinase, mitogen-activated protein (MAP) kinase, and \( \text{Ca}^{2+} \)-mediated pathways, providing ample opportunities for drug development. Stimulating \( \alpha \)-secretase activity should decrease \( \beta \beta \) generation and increase \( \text{p3} \) and APPs\(_{\alpha}\) secretion, which has been proposed to have neuroprotective effects. However, the available data are insufficient to support these two assumptions: it remains unclear whether \( \text{p3} \) is indeed an innocent by-product of \( \alpha \)-secretase processing and whether the neuroprotective effect of APP has real clinical relevance.

In this regard, the ADAM10 protease has been studied most intensively. A transgene construct driving ADAM10 overexpression in brain was shown to have protective effects in an AD mouse model, lowering amyloid plaque load and improving several cognitive parameters (236). However, regulated \( \alpha \)-secretase activity is not mediated by ADAM10 but by ADAM17/TACE. Therefore, it remains unclear what therapeutic strategy can be followed to specifically stimulate ADAM10-mediated \( \alpha \)-secretase activity in the brain. One possibility is to target transcription of ADAM10 with retinoic acid derivatives (306).

Stimulating regulated \( \alpha \)-secretase activity via ADAM17 would provide an alternative strategy. However, ADAM17 is also involved in the release of the proinflammatory TNF-\( \alpha \), which might provoke inflammation in the AD brain. A recent report determined that specific inhibition of ADAM17 in the brain results in a decrease in \( \text{sAPP}_\alpha \) release; however, \( \beta \beta \) steady-state levels remain unaltered (151).

It is clear that further work is needed to fully understand the balance between the \( \alpha \)-secretase- and \( \beta \)-secretase-mediated pathways of APP processing and their relative importance for AD pathogenesis. Such studies should focus on the in vivo situation in adult brain and explain not only the relative contribution of both pathways to amyloid plaque generation but also address the biological relevance of these processing events, and whether APPs\(_{\alpha}\) and APPs\(_{\beta}\) have different functions in vivo. These questions will not be easy to address, given the various and not well understood phenotypes of the different genetic mouse models of APP and its homologs (108, 242).

V. \( \beta \)-SECRETASE

A. BACE1 is the Major \( \beta \)-Secretase

Cleavage of APP by \( \beta \)-site APP cleaving enzyme 1 (BACE1) is the rate-limiting step in the generation of the \( \beta \)\( \beta \) peptides. The protease was identified in 1999 by five different groups using different methodologies (133, 181, 283, 319, 351). BACE1 is a membrane-bound aspartyl protease that is optimally active at a slightly acidic pH. It primarily resides in the Golgi and in endosomes but briefly transits to the cell surface to locations where APP can also be found (156). BACE1 has a rather broad tissue distribution, but it is enriched in brain and in neuronal...
cells, in particular. Genetic inactivation of BACE1 results in a dose-dependent decrease in Aβ generation and is sufficient to block Aβ deposition in different APP-overexpressing mouse AD models (163, 185, 198, 222). Recently, a brain-penetrant BACE1 inhibitor was used to decrease Aβ levels in the cerebrospinal fluid of monkeys (256). Overall, there is little doubt that the Aβ peptides detected in cell culture and body fluids are generated by this protease, and therefore, BACE1 is regarded as the authentic β-secretase. Upon BACE1 overexpression, an additional cleavage at the β’-site, Tyr-10-Glu-11 in the Aβ sequence, is detected (Fig. 1C). This cleavage removes the aminoterminal part of the Aβ peptide and, when antibodies are used that only recognize this part of the peptide, less Aβ appears to be generated (49, 319). Interestingly, this alternative cleavage generates an Aβ peptide variant with an amino-terminal glutamate that can be converted to pyroglutamate by glutamate cyclase as discussed above.

B. Molecular and Cellular Biology of BACE1

BACE1 and its homolog BACE2 (see the section on α-secretase for further discussion of BACE2) are members of the pepsin-like family of aspartic proteases (cathepsin D and E, pepsin A and C, renin, napsin A). They display a typical bilobal structure with the catalytic site located at the interface between the amino- and the carboxy-terminal lobe (121, 226). BACE1 and -2 are anchored to the cell membrane via a transmembrane domain (Fig. 2), which, together with several unique amino acid stretches and the arrangement of the three disulfide bridges (98), sets BACE apart from the rest of the pepsin family and facilitates the generation of fairly specific inhibitors for BACE1 and -2. Moreover, the fact that BACE1 is membrane-anchored has been exploited to increase the efficacy of BACE1 inhibitors using a lipid anchor to target the inhibitor to the cell and endosomal membranes (237).

BACE1 and -2 are generated as prepropeptides (Fig. 2). BACE1 is N-glycosylated, sulfated, phosphorylated, and palmitoylated (21, 39, 49, 80, 131, 330), and the propeptide of BACE1 is removed by a member of the furin family (21, 49), generating a mature protease. A fraction of BACE1 is also shed from the cell surface after cleavage by a metalloprotease, probably ADAM10 (132), but the significance of shed BACE1 is unknown. Most BACE1 remains membrane-bound and is internalized from the cell surface to late endosomal vesicles, cycling further to the lysosomes or to the trans-Golgi network (TGN) (131, 330). BACE1 has a relatively long half-life (~16 h compared with ~3 h for its substrate APP) and a fast recycling rate, suggesting that BACE1 might undergo multiple cycles of transport from the endosomes to the cell surface and back.

A DxxLL dileucine motif in the carboxy terminus of BACE1 (Fig. 2) is a characteristic binding site for Golgi-localized γ-ear containing ARF binding (GGA) proteins (106). A similar acidic dileucine motif is also found in sortilin and the mannose-6-phosphate receptor. The GGA adaptor proteins are involved in sorting between the TGN and endosomes (31). Phosphorylation of Ser-498, next to this dileucine motif (Fig. 2), enhances the affinity of GGA proteins for BACE1 (278) and a redistribution of BACE1 in the cell. In contrast, deletion of the dileucine motif, overexpression of dominant forms of GGA1, or mutation of Ser-498 impair the transport of BACE1 from the endosomes to the TGN, resulting in accumulation of BACE1 in early endosomes (107, 324, 326). This accumulation is associated with increased Aβ generation, indicating that alterations in the subcellular localization of BACE1 have an effect on the amyloidogenic processing of APP. Some observations showed decreased GGA expression in the AD brain, suggesting that this interaction might be relevant for the disease (304, 326).

C. Deregulation of BACE1 Expression in AD

Several reports have documented increased BACE1 protein and activity in the brain of AD patients (83, 120, 177, 353). It should be noticed, however, that it is unclear whether BACE1 expression in healthy people is rate limiting for Aβ generation. Therefore, it remains uncertain whether the recorded changes are sufficient to affect Aβ generation in a quantitative significant way. BACE1 expression is affected by many stress factors associated with aging or neurodegenerative disease such as oxidative stress (300), energy inhibition (321), ischemia (337), hypoxia (360), and traumatic brain injury (26). Insight in these regulatory mechanisms would be important to un-
understand the potential contribution of environmental factors and aging to the development of sporadic AD.

BACE1-transcription is under complex regulation (Fig. 3) (249). Hypoxia induces several transcription factors, including hypoxia-inducible factor 1α, which binds to the hypoxia responsive elements (HRE) in the pro-moter region of BACE1 (294, 360). Hypoxia or other stresses might also activate cdk5/p25 which, acting via STAT3 activation, could induce BACE1 expression (338). Inflammation and anti-inflammatory drugs modulate BACE1 expression via NFκB (33) and peroxisome proliferator activating protein-γ (258). Further exploration of both pathways could yield viable drug targets for AD, not only to modulate BACE1 expression in a beneficial way, but also because of the potential neuroprotective and anti-inflammatory effects of this type of compounds (105, 258).

The BACE1 transcript contains long 5′- and 3′-untranslated regions (UTR). Alternative splicing generates BACE1 variants with low or no enzymatic activity (208). The long GC rich 5′-UTR of BACE1 mRNA acts as a translational repressor (53, 167). It displays extensive secondary structure and contains three upstream open reading frames (203, 247, 364). Interestingly, these properties are shared with other 5′-UTRs of transcripts that are translationally controlled by cellular stress. Impaired cerebral energy metabolism is a cardinal feature in AD, as exemplified by positron emission tomography (PET) imaging studies (207). Energy deprivation induces phosphorylation of the translation initiation factor eIF2α which may overcome the 5′-UTR-mediated repression of BACE1 translation and thereby increase BACE1 expression and possibly Aβ generation (221).

The long 3′-UTR of BACE contains several microRNA binding sites, and levels of protein expression are regulated by miRNA expression (29, 110, 331). miRNA is derived from noncoding RNAs that are encoded and transcribed like classical mRNA in the cell nucleus. Precursor miRNAs (pri-miRs) are sequentially cleaved by two RNase enzymes, Drosha and Dicer, into small functional ~22 nt oligonucleotides. The miRNAs are finally incorporated into a “RNA-induced silencing complex” (RISC), which suppresses translation and/or degrades target mRNA (157). miRNAs are abundantly expressed in the brain, and their absence results in severe neurodegeneration (109). Moreover, loss of miRNA 29a/b-1 correlates with increased BACE1 expression in a subgroup of sporadic AD patients (110).

Additional noncoding RNA is also involved in positive BACE1 regulation. A short (~2 kb) BACE1-antisense RNA is encoded by a gene located on the opposite strand of the BACE1 locus on chromosome 11q 23.3 (75). This antisense BACE1 stabilizes the BACE1 mRNA, and knockdown of the antisense RNA reduces BACE1 expression and Aβ generation. Increased expression of the antisense BACE1 RNA was observed in AD patients.

Finally, at the posttranslational (protein) level, BACE1 is regulated by recycling and degradation mechanisms and by its subcellular localization as discussed in the previous section. Tesco and co-workers (153, 304) found that the GGA proteins that control BACE1 localization in the endosomes are cleaved during apoptosis, resulting in increased BACE1 expression and Aβ generation. The authors suggest that GGA, in addition to their well-established role in protein trafficking between TGN and the endosomal compartment, are also involved in the transport of BACE1 to the lysosomes where BACE1 is degraded (153, 304).

D. BACE1 Physiological Functions

Interestingly, BACE1 is only found in vertebrates but not in Caenorhabditis elegans and Drosophila melanogaster. Therefore, the functions of BACE1 have been investigated in vertebrate-specific systems, e.g., the elaborate immune system and the brain. Studies that have attempted to identify the natural substrates of BACE1 have yielded the best clues to physiological function of BACE1. In addition to APP, the APP-like proteins 1 and 2 (176, 229), the sialyltransferase ST6Gal I (292), the P-
selectin glycoprotein ligand-1 PSGL-1 (179), β-subunits of voltage-gated sodium channels (346), and the low-density lipoprotein receptor-related protein (323) have been identified. The type I membrane protein PSGL-1 contributes to the rolling of leukocytes along the vessel wall before they transmigrate through the endothelium. The type II membrane protein ST6Gal I is involved in B-cell expansion and is required for the correct glycosylation of CD22 ligands. It is unclear whether BACE1 processing interferes with these functions.

BACE1 knockout animals display a subtle and complex phenotype that has been ignored for quite a while (62, 100). A variable number of mice die in the first weeks after birth, and this lethality is increased by combination with BACE2 deficiency. The surviving mice are smaller and present a hyperactive behavior. They display subtle alterations in the inactivation of the voltage-gated sodium channels. More recent work has demonstrated transient changes in the myelination process of peripheral and central nervous system neurons (130, 341). The changes manifest as thinner myelin sheets during the first few weeks after birth in mice when BACE1 expression is particular high in brain, but the defect is apparently restored later in development by unknown compensation mechanisms. Nevertheless, remyelination after a crush of stored later in development by unknown compensation mechanisms. Furthermore, the Bace1 deficient mice also display behavioral alterations such as prepulse inhibition, disturbed operational memory, and other symptoms that are associated with the schizophrenic phenotype in humans, and which could be restored with the antipsychotic drug clozapine (262). Interestingly, the Aph1b-γ-secretase knockout mice (see below) display a similar phenotype (59).

E. BACE1 as a Drug Target for AD

Knockout of Bace1 in mice drastically reduces Aβ production (36, 185, 244) and reduces amyloid plaque load and AD-related symptoms in AD mouse models (198, 222). A theoretical advantage of blocking BACE instead of γ-secretase is that this does not result in abnormal accumulation of the APP-CTFβ (Fig. 1A). The relative mild phenotypes in mice deficient for BACE1 (discussed above) compare quite favorably with the severe Notch-related phenotypes caused by broad spectrum γ-secretase inhibition (gastrointestinal bleedings, autoimmune phenotype, etc.). Moreover, it is not unlikely that part of the neuregulin-associated phenotypes, including the myelination and behavioral deficits, are developmental in nature (i.e., will not cause important side effects in adulthood) (257). Taking all of this into consideration, BACE1 is a primary drug target for AD. The catalytic site of BACE, although typical aspartyl protease like, is exceptionally long, and it has been very difficult to develop small compounds targeting BACE1 in an efficient way. The compounds should be small because they need to cross the blood brain barrier, and they should have a reasonable half-life to reduce the number of times older patients have to take the medication. A flurry of papers has described BACE inhibitors, but orally available highly efficient BACE1 inhibitors have only recently become available (256).

VI. γ-SECRETASES

A. The Different γ-Secretase Complexes

In 1993, γ-secretase was proposed as the generic name for the proteolytic activity that mediates cleavage of APP in the transmembrane domain, releasing the Aβ peptide (95). The proof that presenilin 1 (PS1) is a major constituent of this proteolytic activity was provided in 1998 in a study that showed that loss of PS1 in neurons leads to an almost complete loss of Aβ peptide generation (58). The remainder of the γ-secretase activity is mediated by presenilin 2 (PS2), a close homolog of PS1 (117, 361). Missense mutations in both the PS1 and PS2 genes are a major cause of familial AD (175, 246, 277). Interestingly, fluorescent life time microscopy studies indicate that these mutations affect the conformation of the protein (23).

Presenilin is an aspartyl protease (344) but requires the association of three additional proteins (subunits), nicastrin, aph-1, and pen-2 to become proteolytically active (70, 152, 299; reviewed in Ref. 54) (Fig. 4A). The stoichiometry derived from experiments using tagged overexpressed versions of the individual subunits is likely 1:1:1:1 (224, 261). Technical difficulties in purifying sufficient material make it difficult to analyze the structure-function of native complexes, and therefore, it remains uncertain whether larger multimers or different conformations of γ-secretase exist in situ. Several additional interacting proteins have been reported, and recently, an interactome of presenilin was published based on tandem-affinity purification of native complexes (327). These additional proteins are not in a stoichiometric relation to the four core proteins and are also not present in γ-secretase complexes purified using a biotinylated transition-state inhibitor (343), suggesting that they are not necessary for the activity of the protease per se. Some of these interacting proteins, like the tetraspanins, might however
be involved in the regulation or maturation of the activity of the complex (327).

In the human genome, two PS genes and two Aph1 genes (Aph1a and Aph1b) are present. With the use of specific antibodies for the different subunits, it was shown that either PS1 or PS2 and either Aph1a or Aph1b are incorporated into the mature \( \gamma \)-secretase complex. Thus a minimum of four different \( \gamma \)-secretase complexes exist. If alternative splicing of PS and Aph1 is considered, additional variants may have to be taken into account as well (Fig. 4A) (54, 111, 281). It should be noted that PS1 and PS2 differ \( \sim 33\% \), and Aph1a and Aph1b even \( \sim 43\% \) at the primary amino acid level. These different \( \gamma \)-secretase complexes coexist in cell lines in vitro (111), although they might display different tissue distribution patterns in vivo (59). Mouse knockout experiments have already demonstrated that the different complexes have divergent biological functions (see below) (59, 63, 116, 186, 271, 272).

B. Molecular and Cellular Biology of the \( \gamma \)-Secretase

The different tetrameric \( \gamma \)-secretase complexes are highly hydrophobic structures with 19 transmembrane domains. When the complex is purified from cell lines that overexpress the PS1/Aph1a/Pen2/Nicastrin variant, the estimated mass is \( \sim 230 \) kDa (224). A structure at 12-Å resolution was obtained by single-particle reconstitution and cryoelectron microscopy (224), revealing a globular structure with a smooth cytosolic side and a larger irregular extracellular surface. The structure displays three solvent accessible cavities, one accessible to the cytosolic side and two accessible to the extracellular side. It should be noted that, at this level of resolution, it would not be possible to definitively resolve the issue of whether these low-density cavities are really pores or merely unresolved loop structures. However, meticulous cysteine scanning studies of PS have indicated that the central core of the complex, consisting of the catalytic site and probably part of the substrate binding domain, is indeed water accessible (259, 260, 307, 309). Thus it appears that the \( \gamma \)-secretase, similar to other intramembrane cleaving proteases, sequesters its active site from the hydrophobic environment of the membrane in a structure that allows entrance of catalytic water molecules that are necessary for hydrolysis of its substrates. For further discussion and comparison with other intramembrane cleaving proteases, refer to a recent review (308).

The reason(s) why PS requires three additional proteins for its activity remains unclear, especially in light of the fact that other intramembrane cleaving proteases, including the related signal peptide peptidases (SPP), are apparently active as monomers or homodimers (220, 336). Nevertheless, the individual \( \gamma \)-secretase subunits strongly influence the stability of each other, and correct assembly is a prerequisite for transit to distinct subcellular compartments (cell surface, endosomes) where the \( \gamma \)-secretase is active. It has been proposed that the single transmembrane domain protein nicastrin is a “gate-keeper,” restricting access of substrates to the catalytic site. Nicastrin contains an aminopeptidase-like domain. The carboxylate side chain of a conserved glutamate residue (E333)
in this domain has been suggested to be crucial for the nicastrin-substrate interaction (274). However, a recent study has shown that this E333 substitution interferes with assembly of the γ-secretase complex, and the small amount of complexes that are still generated appear to be as active as the wild-type complexes on a per mole basis, which contradicts the "gate-keeper" hypothesis (44).

Less is known about the Aph1 and Pen2 subunits. Aph1 probably has a seven transmembrane domain structure with the carboxy terminus located in the cytoplasm (82). Aph1 is the most stable component of the complex and might serve as the initial scaffold for assembly. Aph1 forms a subcomplex with nicastrin before PS and finally Pen2 are incorporated. The two different Aph1 proteins alter the conformation of the catalytic subunit PS in the complex as assessed by fluorescent lifetime imaging microscopy (272), suggesting that they have a structural effect on the catalytic site of the γ-secretase complex. Pen2 is a small hairpinlike protein (50). Its incorporation into the complex is associated with a major conformational change of the complex (280), resulting in the (likely auto-) proteolytic cleavage of PS into amino- and carboxy-terminal fragments (299) and rendering γ-secretase a fully operational proteolytic machine.

An important clue to a true understanding of the diverse functions of the γ-secretase complex and cleavage of its many substrates (see below) could potentially be found in a more precise study of the exact subcellular localization of the different complexes relative to the appropriate substrates. This type of analysis has been very instructive for the signal peptide peptidases (SPP), which are PS-related multitransmembrane containing aspartyl proteases, and has shown the specific association of SPP/SPPL1 with the endoplasmic reticulum (ER) and SPPL2 with endosomes (158). The γ-secretases provide obviously a more complex situation: each enzyme consists of four different proteins, and PSs have additional functions beyond their role in the complex (328). An early study summarized this problem as "the spatial paradox." Indeed, PS, believed at that time to act as an independent protease, appeared to be mainly localized to the ER, whereas the γ-secretase is active in the late compartments of the secretory and endocytic pathways (5, 46, 51, 302). This spatial paradox has been resolved by current studies that indicate that PSs are present in the ER in a catalytically inactive conformation. This PS holoprotein or uncleaved PS forms Ca\(^{2+}\) channels that facilitate passive Ca\(^{2+}\) leakage across the ER membrane (213, 315).

Apart from more precise morphological localization studies, dynamic cell-free transport assays are necessary to investigate how the complex is progressively assembled and matures along the biosynthetic pathway. One of the first reports describing such results has made use of a cell-free ER budding assay and analyzed the presence of the γ-secretase subunits in COPII transport vesicles. The experiments confirmed that the complex proteins are not active in this early compartment (150). Interestingly, exit of γ-secretase from these early compartments is tightly regulated. "Retrieval to ER protein 1 (RER1p)" recognizes specific motifs both in Pen2 and nicastrin and continuously recycles these proteins from the ER-Golgi intermediate compartment (ERGIC) back to the ER provided that these proteins have not been incorporated into the complex (144, 287). In an elegant study, Annaert and colleagues (287) demonstrated that RER1p and Aph1 compete for binding to nicastrin, and only when the RER1p binding motif in nicastrin is covered by Aph1 can the assembled subcomplex leave the ER definitively. This quality control might be a way to control the total levels of active γ-secretases in the cell (287). Finally, not all assembled γ-secretase complexes in the cell are in active conformations as transition-state analog inhibitors apparently only bind a small subset of them in situ (18, 162). It is not unlikely that lipids play an important role in this regard (225) as in cell free assays, the lipid composition can affect activity severalfold, and in cells the complex appears to be associated at least partially with detergent-insoluble patches in the membrane (322, 327). The functional consequences of these associations remain to be fully explored.

C. γ-Secretases Proteolytic Functions

To date, most studies have considered the γ-secretase to be a homogeneous activity. In fibroblasts and other cell lines, this is probably a valid approach given that the Aph1a/PS1/Pen2/nicastrin combination is most abundant. In vivo, however, it appears that the different complexes have divergent tissue expression patterns and biological functions. The Aph1a/PS1 combination is crucial in notch signaling, both during embryogenesis and in adulthood as demonstrated by various knockout mouse studies (102, 174, 178, 186, 272, 276, 310, 345, 347; reviewed in Ref. 93). In contrast, PS2 knockout animals (116) only display mild signs of increased apoptosis in lung tissue. The Aph1b/PS1 (and PS2)/Pen2/nicastrin combinations display a more restricted expression profile. Deficiency of the Aph1b subunit results in a subtle behavioral phenotype with disturbed prepulse inhibition, defects in operational memory, and increased sensitivity to amphetamine, which are symptoms that have also been observed in other mouse models of neurodevelopmental disorders including schizophrenia (59). Interestingly, similar symptoms are observed in BACE1 knockout mice (262), and the symptoms in both animal models can be reversed with antipsychotic drugs. Evidence that BACE1 and the Aph1b-γ-secretase complex are both involved in proteolytic processing of (and likely signaling by) neuregulin provides a molecular basis for the understanding of this common phenotype.

Physiol Rev • VOL 90 • APRIL 2010 • www.prv.org
Currently, it is not completely clear what determines the specificity of the phenotypes in the γ-secretase knockout mice. Few in vitro experiments have been published that address the question of the biochemical properties and substrate specificities of the different complexes. These studies suggest that the different subunit combinations of the γ-secretase complex do not considerably affect the $K_m$ and $V_{max}$ relative to synthetic substrates (272) or secretion of Aβ peptides in cell lines (272, 282). However, these experiments revealed differences in the conformation of the PS1 catalytic subunit depending on the presence of the Aph1a or Aph1b subunit, and also differences in the spectrum of Aβ peptides generated in various in vitro assays (272). Earlier experiments in cell cultures suggested that PS2 is a much less efficient protease than PS1 with regard to APP cleavage and Aβ peptide generation (22, 162, 196). At the moment, the available in vitro assays are too rudimentary to permit reliable and conclusive analysis of the substrate preferences and critical kinetic parameters of the different γ-secretase complexes, but it is certainly important to pursue this avenue of research. Nevertheless, in vitro experiments, although crucial for further structure-function analysis and the development of specific inhibitors, have to be supplemented with in vivo experiments if one really wants to understand the physiological function of these particular proteases. Indeed, it is crucial to determine whether protease and substrate also meet in real life.

D. Role of γ-Secretase in the Generation of Carboxy-Terminal Heterogeneity of Aβ

The γ-secretase is responsible for endoproteolysis of APP and generation of different Aβ peptides (58). The major soluble Aβ peptides end at residue Val-40 and at Ala-42, and mutations of PS systematically increase the relative ratio of the “long” versus the “short” Aβ peptide species (Aβ42/Aβ40 ratio) (32, 263). The two cleavages occur at the “γ”-sites (Fig. 1C). Concomitantly, the intracellular cleavage peptides (APP intracellular domain or AICD) are released, which start at Val-51 or Met-52. These cleavages occur at the “ε”-sites (Fig. 1C). Similar dual cleavages have been identified for other γ-secretase substrates such as Notch (223) and CD44 (166). One model suggests that the γ-secretase initially cleaves APP at the ε-site and then progressively truncates the protein by about three carboxy-terminal acid residues (one α-helical turn) until the γ-sites are reached (145, 298). Although further cleavage is possible (generating Aβ39, and possibly even shorter peptides), the remaining peptide becomes increasingly hydrophilic and therefore is quite readily liberated from the interior of the γ-secretase complex (Fig. 5). In this model, Aβ49 is the precursor of Aβ46 (ζ-site), Aβ43 and Aβ40 (γ-site) (84, 145), while Aβ48 would give rise to Aβ45 and Aβ42 (362). The model remains descriptive and does not provide an explanation with regard to how substrates are progressively cleaved within the same catalytic site. It is possible that the putative α-helices formed by the transmembrane domains unwind after the initial ε-cleavage, which, in turn, advances the next peptide bond toward the catalytic site for further cleavage. The consecutive cleavage model of APP provides an explanation for the paradox that clinical mutations in PS decrease total Aβ generation but increase the amount of long Aβ peptide (22). Indeed, if these mutations render γ-secretase less efficient (i.e., cause a partial loss of function of PS), then a less complete digestion of Aβ will result in longer and more amyloidogenic Aβ (55).

More than 30 different substrates have been identified for the different γ-secretase complexes (Fig. 5) (for an exhaustive list, see Ref. 328). The γ-secretase cleaves the transmembrane domains of many proteins with type I topology (amino terminus oriented to the extracellular side of the membrane) and short (<50 amino acid) ectodomains with a remarkably relaxed sequence specificity (180, 290). Bulky ectodomains of 200 and more amino acid residues prevent γ-secretase cleavage and are usually removed by membrane-bound (metallo)proteases at the cell surface (see above for a discussion of the ADAM proteases and APP shedding). Additional sites in the substrates might contribute to cleavage efficiency and
specificity (113, 241, 303). Dimerization of APP (and maybe other substrates?) through the GxxxxG motif in its transmembrane domain might also potentially regulate processing by γ-secretase (211). Further work is clearly needed to more precisely delineate the substrate binding site in the γ-secretase and the constraints on this site that regulate access of substrates to the catalytic site buried in the interior of the protease.

E. γ-Secretase as a Drug Target for AD

Two observations, the essential role of PS in Aβ generation (58) and its role in notch signaling (56, 174, 291), have been a major part of the research agenda in the drug development for AD for at least a decade (Fig. 4B). Blocking Notch signaling in the crypts of the intestinal epithelium induces differentiation into goblet cells and interferes with the normal replacement of the epithelium, explaining the gastrointestinal toxicity of γ-secretase inhibitors (270, 318, 345). In addition, immune suppression or autoimmune disorders might be caused by notch deficiency in adulthood (97, 310). Several approaches to increase the therapeutic window, i.e., to find compounds that efficiently block Aβ generation without affecting notch signaling, have been explored. A rather unexpected turn in this drug development story is that γ-secretase inhibitors are now considered a viable therapeutic strategy for certain cancers in which notch signaling is activated, such as T-cell acute lymphoblastic leukemia (339), lung cancer (155), and precancerous adenoma (318).

Classical transition state compounds that target the catalytic site of the γ-secretase will block cleavage in the absence of substrate discrimination. A series of non-transition-state γ-secretase inhibitors have been described such as peptide-based inhibitors (DAPT), sulfonamides, and benzodiazepines (compound E). When these inhibitors are used at low concentrations, the ζ-site cleavage (Fig. 1C) is not affected, but the γ-site cleavages are potently inhibited and Aβ40 and Aβ42 levels decrease (349). Another class of inhibitors that has attracted much attention in the last years are the γ-secretase modulators (GSM). A subset of non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and sulindac sulfide, as well as enantiomers of flurbiprofen, are apparently able to shift the cleavage specificity of the γ-secretase towards the production of shorter Aβ38 peptides, without affecting Aβ40, AICD or, most importantly, notch cleavage (71, 297, 334, 335). The mechanism of action is not fully understood, but they could affect the conformation of γ-secretase (17), the dimerization of APP (211), or even directly bind to APP (159). The clinical usefulness of these different approaches needs to be further explored.

More recently, refined strategies that block APP processing and do not affect notch signaling have been proposed. G protein-coupled receptors (GPCR) have been implicated in the trafficking of the γ-secretase (214, 305) and appear to specifically regulate cleavage of APP versus notch, possibly by redistributing γ-secretase(s) to subcellular compartments where APP is present, but not notch. Indeed, downregulating GPR3 leads to a reduction in Aβ production without any notch side effect (305).

A second opportunity might be found in the heterogeneity of the γ-secretases. The Aph1b complex is expressed in the brain areas affected by AD and is not involved in notch signaling. Specific inhibition of this complex might certainly be a viable approach to block Aβ generation in a safe and effective way (272).

VII. NEUROFIBRILLAR TANGLES AND TAU

The microtubule-binding protein tau precipitates as tangles in AD and in a whole spectrum of neurodegenerative tauopathies including Pick’s disease, progressive supranuclear palsy, corticobasal degeneration, and others. Interestingly, the dementia symptoms in AD correlate to a greater extent with the gradual appearance and spread of tangles throughout the brain than with the deposition of Aβ in senile plaques (7, 91). The causal relationship between tau dysfunction and neurodegeneration has been unequivocally established by the identification of more than 40 mutations in the tau gene (http://www.molgen.ua.ac.be/ADMutations/) that cause familial frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (134, 235, 289). However, the mechanism by which tau causes neurodegeneration remains unclear. Both gain and loss of function mechanisms have been proposed (12). Mutations and posttranslational modifications decrease the affinity of tau for microtubules which will destabilize these structures and will lead to axonal transport problems. Gain of toxicity could be due to the aggregates themselves, which mechanistically block axonal transport or other neuronal functions and provide a trap for functionally important proteins, including tau itself. A third emerging concept is that small, soluble oligomeric aggregates of hyperphosphorylated tau could form highly toxic intermediates that interact with biological membranes or cause other disturbances and thereby affect the health of the neuron. This concept is not unlike the toxic Aβ oligomer concept discussed above (96).

A. Tangles and the Importance of Nuclei Formation

The longest version of the tau protein contains 441 amino acid residues. Alternative splicing of exons 2, 3, and 10 generates six major isoforms in the adult brain (Fig. 6). Exon 10 encodes one of the four repeat (R)
domains of tau which are involved in microtubule binding. Thus alternative splicing yields either three or four repeat motifs containing tau. The ratio of four repeats containing tau (4R) to 3R is 1:1 in adult brain, and changes in this ratio are characteristic for the different tauopathies (34, 88, 189). Tau is extensively posttranslationally modified by phosphorylation, glycosylation and glycation, ubiquitination, and proteolytic processing (92).

Most researchers will agree that the pathway converting soluble tau to filamentous insoluble tau is probably central to the pathogenesis of the tauopathies, including AD (12). Tau aggregation is a multistep process initiated by a rate-limiting nucleation step, which is followed by the progressive addition of tau proteins in an elongation process (Fig. 7). A first step seems to include the redistribution of microtubule-bound tau to the soluble cytoplasmic pool of tau as a consequence of hyperphosphorylation (218), mutations (103, 122), or other tau modifications. However, the mechanism by which increased cytoplasmic tau, which is highly soluble and intrinsically unfolded, clusters to generate the aggregation nuclei required for the further formation of tau fibrils remains unclear. Mutations (89, 212), posttranslational modifications like proteolysis, or additional factors like the presence of polyanionic structures (86, 90, 147) probably play a crucial rate-limiting role in this conversion and are therefore therapeutic targets. The canonical view on the tau cascade is centered on the abnormal phosphorylation status of tau, but as we will see, interesting evidence is emerging that abnormal proteolysis of tau plays a role in the transformation of tau to pathological species.

B. Hyperphosphorylation of Tau

Tau in tangles is characterized by a high degree of phosphorylation on its 45 serines, 35 threonine, and 5 tyrosine residues (189). Some of these sites are typically phosphorylated during embryogenesis, or under specific physiological conditions (hibernation in certain species), which might be related to synaptic remodeling, while other sites are highly pathological. Nevertheless, there is an ongoing debate with regard to the extent to which phosphorylation and which specific phosphorylation sites are crucial for tau toxicity or tangle formation. Phosphorylation affects tau’s affinity for the microtubule to variable extents, but unexpectedly, phosphorylation at sites that strongly interfere with microtubule binding (e.g., by the kinases Mark or protein kinase A) also strongly interfere with tangle formation (267). Soluble forms of abnormally phosphorylated tau have been found in mouse models overexpressing tau containing FTDP-17 mutations, which might suggest that they appear before tangle formation (2, 60). However, a causal relationship between abnormal phosphorylation and tangle formation has not been firmly demonstrated. The best evidence that increased phosphorylation might lead to tangle formation comes from crosses of mice that overexpress mutated tau with mice that express the activated kinase CDK5. These mice display increased phosphorylation of tau at multiple sites and a significant increase in tangle formation (218). Taking all of the evidence into consideration, the debate on the importance of tau phosphorylation in the pathogenesis of tauopathies, including AD, has not yet been resolved.
C. Proteolysis of Tau and Relevance for AD

The concept that tau proteolysis contributes to tangle formation in vivo has already been known for a long time. An important fraction of tau in fibrils is truncated at positions Glu-391 and Asp-421 (numbering for the longest splice form of tau) (15, 85, 201, 219). Removal of the carboxy-terminal part of tau enhances the nucleation-elongation reaction in vitro (24, 356), which might indicate that in sporadic AD truncated tau species play an important (maybe initiating) role. Interestingly, truncated tau might also be involved in neuronal toxicity or be responsible for the build up of intermediary toxic oligomeric species. Truncated tau is, when overexpressed in a rat transgenic model, indeed able to drive neurodegeneration and neurofibrillar pathology (127, 365).

Both amino- and carboxy-terminal parts of tau appear to have an inhibitory effect on the aggregation potential of tau. They fold back in a "hairpin" conformation (142) on the central domain which contains the microtubule binding repeats (R) (Fig. 8). In repeats R2 and R3, two hexapeptide motifs are present with a high propensity to form β-sheet structures (210, 325). A synthetic tau 4R construct truncated at both the amino and carboxy terminus has a high tendency to form intracellular tau aggregates (further enhanced by introducing the deltaK280 FTDP17 mutation) (333). Toxicity is clearly associated with the aggregation propensity of this construct in vitro (6, 149) and in vivo (206).

The protease generating the Glu-391 cleavage is unknown, but caspases are potentially involved in the Asp-421 cleavage. They are also involved in the removal of parts of the amino terminus (Fig. 6) by potentially generating a highly aggregation-prone tau fragment. Interestingly, exposure to Aβ can trigger caspase-mediated cleavage of tau in neurons in vitro. The truncated tau is readily phosphorylated at different sites, and an antibody specific for the Asp-421 site decorated the fibrillar pathology in the AD brain (85, 243). The link between apoptosis induced by Aβ, abnormal proteolysis of tau, and tau aggregation is, if confirmed, potentially very interesting given that it provides a link between the two major pathologies of AD and also suggests new therapeutic targets for the disease (48). Alternative mechanisms, e.g., activation of calpain by Aβ and generation of an amino-terminal neurotoxic proteolytic 17 kDa tau fragment (AA43-229), have been proposed to mediate tau-dependent Aβ toxicity as well (Fig. 6) (228). However, the importance of caspase activation and apoptosis in the progression of AD has still not been established (61).

One approach to clarify the role of (limited) proteolysis of tau in disease progression would obviously be to utilize in vivo modeling in various mouse models. However, the obtained results appear somewhat contradictory to date. While the rat model (365) supports the hypothesis that truncated tau can drive neurodegeneration, it is unclear whether truncation of tau is also necessary in the pathogenesis of AD in humans. For instance, mouse models overexpressing the tau-Pro-301 mutation generate abundant tangles and display early tau phosphorylation, whereas truncation at Asp-421 is only a minor occurrence and probably a late event (60, 359). It should be noted that rodent models come with their own experimental biases and assumptions concerning the pathogenic mechanisms and that any extrapolation to the human situation needs to be done with caution. For example, truncated tau is much more abundant in human brains than in these mouse models (359). Thus it should be taken into consideration that strong overexpression of pathological genes in mouse models strongly biases the models towards assumptions that were made when the model was generated.

Several caspase fragments of tau have been reported but have been insufficiently evaluated in further studies to draw conclusions with regard to their importance for AD. Horowitz and co-workers reported a caspase-6 cleavage at Asp-13 of tau. Amino-truncated tau has been detected in patients (126), and this amino-terminal domain of tau interacts with the carboxy-terminal domain to keep tau in its soluble state (125) (discussed in detail above). Corsetti et al. (47) report on an amino-terminal tau fragment (amino acids 26-230) that is generated during apoptosis and exerts NMDA-mediated neurotoxicity. Removal of the amino terminus has also been attributed to cleavage by aminopeptidases (148).

D. Tau as a Drug Target in AD

As mentioned above, it is possible that tangle pathology, once induced in AD, evolves independently of the Aβ...
pathology. The FTDP-17 mutations actually show that abnormal tau causes neurodegeneration and dementia. Unfortunately, no consensus exists with regard to the treatment of the tau pathology. Abnormal phosphorylation has been considered to be a potential drug target by many pharmaceutical companies (197), but as discussed above, proof of concept is clearly lacking. Other possibilities are to interfere with the tau aggregation process, to reduce tau expression (245), or to improve microtubule functions (268). Given the importance of nucleation in tangle formation, it might be worthwhile to explore the role of abnormal tau proteolysis in AD with more vigor in the future. Although proteases are reasonable drug targets, caspases might be difficult to block without major side effects.

VIII. PROTEOLYTIC DEGRADATION OF THE AMYLOID Aβ PEPTIDE

A. Clearance of Aβ From the Brain

If abnormal or increased production of Aβ peptide by genetic mutations causes familial AD, then any biochemical cause of increased Aβ, for instance deficient removal of Aβ peptide, has to be considered as a potential cause of sporadic AD (252). The normal Aβ fractional clearance rate is estimated to be ~8% per hour (16), and small chronic defects are theoretically sufficient to cause AD if the analogy with familial AD is maintained. Physiological parameters, like blood and cerebrospinal fluid flux in the brain, together with a series of clearance receptors, including LRP1 and VLDLR, are implicated in the removal of Aβ. Aβ can be taken up by smooth muscle cells delineating the arterioles or by endothelial cells that participate in the blood-brain barrier (19, 20, 64, 104, 366). A portion of Aβ might also be transcytosed and secreted into the bloodstream (187). Disturbances in these clearance processes appear particularly relevant for the accumulation of Aβ peptides in the blood vessel walls, causing the vascular component or cerebral amyloid angiopathy (CAA) of AD.

The proteolytic machinery in the brain also certainly contributes to the degradation of the Aβ peptide and the aggregates and fibrils that accumulate in AD. The diversity of the proteases implicated in this clearance process is rather perplexing, but in many cases, in vivo validation has been provided. A knockout experiment in mice or rats of a specific protease demonstrating increased steady-state levels of Aβ in brain is strong evidence that the particular protease is physiologically involved in Aβ turnover (summarized in Table 1). Ectopic overexpression using transgenes or viral vectors and demonstrating that the protease is able to lower Aβ levels or prevent amyloid plaque formation in the brain of AD mouse models is another piece of in vivo evidence, although the physiological relevance then has to be provided from additional experiments. Evidence of a genetic association with AD has been published for a few proteases; however, no consensus exists with regard to the importance or reproducibility of these associations in the general AD population. From a therapeutic point of view, it remains to be seen whether any of these proteases provides a viable AD drug target. Recent reports suggest even that some of the amyloid degrading proteases (insulin degrading enzyme, neprilysin, and angiotensin converting enzyme 2, see below) are actually upregulated anyhow in sporadic AD brain (204, 227). One has to consider the possible side effects of further upregulating the activity of these proteases in the brain for many years, as would be needed for

<table>
<thead>
<tr>
<th>Table 1. Aβ degrading proteases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Neprilysin (NEP)</td>
</tr>
<tr>
<td>Insulin degrading enzyme (IDE)</td>
</tr>
<tr>
<td>Angiotensin converting enzyme (ACE)</td>
</tr>
<tr>
<td>Endothelin converting enzyme (ECE1, -2)</td>
</tr>
<tr>
<td>Matrix metalloproteinases (MMP9, MMP2)</td>
</tr>
<tr>
<td>Plasmin</td>
</tr>
<tr>
<td>Cathepsin B</td>
</tr>
</tbody>
</table>

Most proteases involved in Aβ turnover are metalloproteases. It is indicated whether they degrade Aβ monomers (M), oligomers (O), or fibrils (F). If knock-out animal models have been described demonstrating increased Aβ levels, this is indicated by KO. See main text for further discussion.
a treatment of AD. Moreover, all of the implicated proteases have important functions in the turnover of biologically active peptides. Nevertheless, we will see how some approaches are currently explored to enhance Aβ proteolytic degradation in human brain.

B. Neprilysin

Neprilysin was identified as an important Aβ degrading enzyme using an unbiased approach that attempted to answer the fundamental question of Aβ degradation in the brain in vivo.

Saido and collaborators (137) injected multilabeled radioactive Aβ peptides into the rat brain and identified and characterized the proteolytic fragments generated over time. The profiles of the fragments and the sensitivity of the degradation pathway to inhibitors like thiorphan and phosphoramidon strongly supported a role for neprilysin in Aβ turnover in vivo. This was nicely confirmed in neprilysin knockout mice, which display a genetic dose-dependent increase in endogenous Aβ peptide accumulation in the brain (136). The Dutch, Flemish, Italian, and Arctic familial AD mutations in the APP gene appear to further hamper the degradation of Aβ by neprilysin in vitro, which argues that decreased turnover of these peptides in addition to their increased tendency to form fibrils could contribute to the pathogenesis of AD and CAA in patients (314).

Neprilysin, also referred to as enkephalinase or neu-tral endopeptidase, is a zinc-dependent metalloprotease of ~90 kDa. It is a type II membrane protein that hydrolyzes biologically active peptides, including enkephalin, cholecystokinin, substance P, neuropeptide Y, and others. Overexpression of neprilysin in the brain of AD mouse models using transgene expression from a calmodulin kinase II promoter (172) or viral vectors (194, 288) resulted in the clearance of a great deal of the amyloid-associated pathology, but as discussed, it remains unclear whether such an approach can be translated into a feasible therapy for humans. An alternate idea, to increase neprilysin levels using agonists of the somatostatin GPCRs, was elegantly proposed some years ago (255). Somatostatin levels decline during aging and in AD, and somatostatin deficiency in mice results in decreased neprilysin activity and increased Aβ levels, which might be reversed by such a treatment.

C. Endothelin Converting Enzymes 1 and 2

The endothelin-converting enzyme (ECE)-1 and -2 are related to neprilysin, displaying several similarities in sequence and domain structure. ECE-1 cleaves Aβ in vitro (67), and heterozygous knockout animals (68) show increased Aβ accumulation in brain. Interestingly, the combination of neprilysin and ECE-1 knockout further enhanced Aβ levels in brain, confirming that different proteases work together to turn over Aβ (66). ECE-1 exists as four isoforms that are localized to different regions in the cell and at the cell membrane. Conversely, ECE-2 displays a predominantly intracellular localization. Both are type II membrane-bound zinc metalloproteases that are primarily responsible for processing the vasoconstricting endothelins.

D. Angiotensin Converting Enzyme

Several antihypertensive drugs target angiotensin converting enzyme (ACE), although there is some concern that these drugs could also increase Aβ levels in brain. In this regard, ACE was shown to degrade Aβ in vitro (128), and polymorphisms in the ACE gene have been associated with increased or decreased genetic risk for AD. However, several investigations using genetic inactivation of ACE or inhibitors of ACE in vivo suggest that ACE does not have a major role in Aβ turnover in the brains of mice (66, 114, 137). In contrast, one study reported a significant effect on Aβ levels in mice treated with the ACE inhibitor captopril, an antihypertensive drug (367). Thus the evidence that implicates ACE as an Aβ degrading enzyme is somewhat conflicting.

E. Insulin Degrading Enzyme

Insulin degrading enzyme (IDE) is a 110-kDa zinc protease that cleaves physiologically active peptides like insulin, glucagon, atrial natriuretic factor, and others. The subcellular localization of IDE is somewhat controversial: it acts certainly in the cytoplasm where it degrades, for instance, the AICD once it is released by γ-secretase (see above) (69). On the other hand, IDE is also found at the cell surface and secreted into the medium via the “unconventional secretory pathway” (363).

IDE degrades Aβ in the conditioned medium of cell cultures, including neurons (320). Both a rat and a mouse model deficient in IDE have been studied (77, 78), and increased levels of Aβ in neuronal cultures, and in brain samples, were discovered. Deficiencies in IDE also lead to increased insulin levels in the blood and might provide a clue toward a possible link between diabetes and AD, although this remains to be further investigated. Overexpression of IDE in brain, similar to neprilysin, improves pathological aspects in an AD mouse model (172).

IDE together with neprilysin and a third protease implicated in Aβ degradation, the mitochondrial precursor peptidase or PreP (76), all have a characteristic catalytic chamber that can encapsulate a variety of peptides of 70 amino acid residues or less. The chamber plays...
a key role in the selectivity of these proteases for their substrates, including Aβ (188).

F. Matrix Metalloproteases

Matrix metalloproteases (MMPs) are secreted zinc- and calcium-dependent endopeptidases. They are expressed at low levels in the brain, but their expression can be induced in astrocytes by stress, e.g., by the presence of Aβ peptide itself. MMP-9 can cleave Aβ, but unlike most other Aβ degrading enzymes, it can also degrade fibrillar Aβ (350). Knockout of MMP2 results in increased steady-state levels of Aβ1-40 and Aβ1-42 in the soluble fraction of hippocampus and cortex, while infusion of a broad-spectrum metalloprotease inhibitor in the brain also increased Aβ levels (357), suggesting the potential in vivo relevance of these (and other metalloproteases) in Aβ turnover. It remains unclear whether the expression of MMPs is really altered in the AD brain (10). However, MMP9 is an important factor in ischemic brain insult, and genetic inactivation is protective (8, 332).

G. Plasmin

Plasmin is generated from plasminogen by the urokinase-type or tissue-type plasminogen activator (uPA and tPA, respectively). Plasmin is involved in degradation of fibrin aggregates in the blood. In the brain, plasminogen is synthesized in neurons, and uPA and tPA can be induced during ischemia and excitotoxicity. The subsequent plasmin generation and degradation of extracellular matrix have been implicated in neuronal loss, and tPA-deficient mice are resistant to this type of insult (312, 313).

The three proteases are serine proteases, but only plasmin has been directly implicated in Aβ degradation (169, 316). Interestingly, fibrillar Aβ, similar to fibrin, can stimulate tPA-mediated plasmin activation, and plasmin is able to degrade fibrillar Aβ (316). In the brain of AD patients, plasmin activity was found to be decreased (73, 169).

Steady-state Aβ in the brain or plasma does not appear to be affected by plasminogen deficiency, suggesting that under normal steady-state conditions plasmin contributes little to Aβ turnover (317). However, when radioactively labeled Aβ was injected into the brain of plasminogen-deficient mice, a slower turnover was observed (200), indicating that under certain experimental conditions plasmin becomes sufficiently activated to significantly contribute to Aβ turnover. As mentioned, fibrillar Aβ might activate tPA-mediated plasminogen activation (171, 316). Recently, inhibition of plasmin by neuroserpin (73) was implicated in AD, while the reverse, activation of plasminogen activator inhibitor-1 (PAI-1) (140), was proposed as a potential approach to increase plasmin-mediated Aβ degradation in AD patients. An orally active, brain-penetrable inhibitor of PAI-1 lowered Aβ levels in both plasma and in the brain of an AD transgenic mouse model and improved memory deficits in these animals. The possibility that such treatments would lead to increased bleeding has to be balanced against the potential benefits. However, partial PAI-1 inhibition might be possible without major side effects, as discussed by Jacobsen et al. (140).

H. Cathepsin B and Cystatin C

Cathepsin B (CatB) is a cysteine protease located in the endolysosomal system where it degrades peptides. CatB is also secreted through exocytosis and is involved in matrix degradation in cancer and arthritis. CatB has been implicated in APP and Aβ processing for a long time (40, 41) and has been proposed as an alternative β-secretase, see also above (124). However, good experimental evidence supports a contrasting hypothesis that suggests that CatB is involved in Aβ-monomer and, importantly, Aβ-fibril degradation. Genetic ablation of the gene aggregated, while overexpression of the gene using lentiviral transfer improved the pathology in an AD mouse model (209). CatB truncates Aβ peptides at the carboxy terminus, reducing the relative amount of the longer Aβ42 peptide. No evidence exists as to whether genetic ablation of CatB also affects Aβ levels at the endogenous level of APP expression, which would further strengthen the hypothesis that this protease is indeed part of the natural degradation machinery of Aβ. The contribution of CatB toward Aβ turnover may strongly depend on its expression levels, which are induced by inflammation and other factors.

This is also probably the only way to understand the discrepancies in the results that have been obtained with cystatin C, a cysteine protease inhibitor of CatB (293). Genetic ablation of cystatin C resulted in decreased Aβ peptide accumulation in mice and primary neurons and improved cognition. The beneficial effects of cystatin C reduction were not observed on a CatB-null background, providing strong genetic evidence for a cystatin C/CatB-regulated pathway for Aβ degradation (293). In contrast, several studies suggest that cystatin C binds directly to Aβ or Aβ fibrils and is responsible for the clearance of the peptide. Genetic inactivation of cystatin C did not affect Aβ load in another AD mouse model (143), while overexpression of cystatin C in the mouse brain of several AD models actually reduced plaque load (143, 202). Moreover, the clinical reports are conflicting: both increases and decreases in cystatin C activity have been associated with AD (42, 295). The point might be that cystatin C has dual roles, i.e., a proamyloidogenic effect based on its inhibition of CatB, and an antiamyloidogenic effect by
binding the A\beta peptide in fibrils and clearing it from the brain. The net result in a given experimental paradigm will depend on the levels of CatB expression in the given conditions and on where in the brain cystatin C is expressed.

IX. CONCLUSION

Alzheimer disease research is coming of age, and its focus is expanding toward the more complicated pathogenesis of sporadic AD. A multifactorial hypothesis is probably the best way to integrate the many bits and pieces of evidence that link multiple molecular pathways to sporadic AD. Proteases and proteolytic fragments play a crucial role in this thinking. Proteases are reasonable good drug targets, and many of the described avenues might open new perspectives for additional therapies or possibilities for new approaches to drug development in AD. We anticipate that future research will capitalize on the emerging knowledge on AD, and we hope for the day that AD becomes a curable or preventable disease.

ACKNOWLEDGMENTS

I thank Dr. A. Thathiah for proofreading the manuscript.

Address for reprint requests and other correspondence: B. De Strooper, Center for Human Genetics, VIB and K.U.Leuven, O&N1 Herestraat 49, Box 602, 3000 Leuven, Belgium (e-mail: Bart.Destrooper@cme.vib-kuleuven.be).

GRANTS

Work in my laboratory was supported by a Pioneer Award from the Alzheimer’s Association; the Fund for Scientific Research Flanders; KULeuven (GOA); Federal Office for Scientific Onderzoek (SAO).

DISCLOSURES

I am a paid consultant for several pharmaceutical and biotech companies.

REFERENCES


Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoconals: evidence that an initially deposited species of Aβ42(43) is degraded in diffuse plaques. *Am J Pathol* 149: 1823–1830, 1996.


Physiol Rev Vol 90 April 2010 www.prv.org


Tousseyn TA, Thathiah A, Jorissen E, Raemaekers T, Koni- 
311.
310.
311.

Tesco G, Ginestroni A, Hiltunen M, Kim M, Dolios G, Hyman 
Tippmann F, Hundt J, Schneider A, Endres K, Fahrenholz F. 
Tesco G, Koh YH, Kang EL, Cameron AN, Das S, Sena-Esteves 
302.
311.
311.

Tucker HM, Simpson J, Kihiko-Ehmann M, Younkin LH, 
Van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, 
319.
319.
319.

Tucker HM, Kihiko M, Caldwell JN, Wright S, Kawarabayashi 
308.
308.
308.

Tolias A, Horre K, De Strooper B. 
305.
305.
305.

Tolias A, Spittaels K, Hoffmann M, Staes M, Cohen A, 
Horre K, Vanbrabant M, Coun F, Baekelandaet Y, Delacourte A, 
Fischer DF, Pollet D, De Strooper B, Merchiers P. The orphan 
G protein-coupled receptor 3 modules amyloid-beta peptide 

Tolias A, Chavez-Gutierrez L, De Strooper B. Contribution of 
presenilin transmembrane domains 6 and 7 to a water-containing 

Tolias A, De Strooper B. Structure and function of y-secretase. 

Tolias A, Horre K, De Strooper B. Transmembrane domain 9 of 
presenilin determines the dynamic conformation of the catalytic 

Tournoy J, Bossuyt X, Snellinx A, Regent M, Garmyn M, 
Serneels L, Saftig P, Craessaerts K, De Strooper B, Hartmann 
D. Partial loss of presenilins causes seborrheic keratosis and au-

notch and other proteins, is processed itself by ADAMS 9, -15, and 
-19 and other proteins. ADAMS 9, -15, and -19 are processed by 

Tsuiki SE, Blitzer RD, Cullen WK, Anwyl R, Wolfe MS, 
Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid 

Takayashu J, Cho Y, Yang SN, Kim EJ, Lin HY, Hyman BT. 
The low density lipoprotein receptor-related protein (LRP) is a novel 

Takayashu J, Cho Y, Yang SN, Kim EJ, Lin HY, Hyman BT. 
The low density lipoprotein receptor-related protein (LRP) is a novel 

Takayashu J, Cho Y, Yang SN, Kim EJ, Lin HY, Hyman BT. 
The low density lipoprotein receptor-related protein (LRP) is a novel 

Takayashu J, Cho Y, Yang SN, Kim EJ, Lin HY, Hyman BT. 
The low density lipoprotein receptor-related protein (LRP) is a novel 


