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Closing in on the Amyloid Cascade

Recent Insights into the Cell Biology of Alzheimer's Disease

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Abstract

Accumulation of the amyloid-β (Aβ) peptide in the central nervous system (CNS) is considered by many to be the crucial pathological insult that ultimately leads to the development of Alzheimer's disease (AD). Regulating the production and/or aggregation of Aβ could therefore be of considerable benefit to patients afflicted with AD. It has long been known that Aβ is derived from the proteolytic processing of the amyloid precursor protein (APP) by two enzymatic activities, β-secretase and γ-secretase. Recent breakthroughs have led to the identification of the aspartyl protease BACE (β-site APP-cleaving enzyme) as β-secretase and the probable identification of the presenilin proteins as γ-secretases. This review discusses what is know about BACE and the presenilins, focusing on their capacity as secretases, as well as the options for therapeutic advancement the careful characterization of these proteins will provide. These findings are presented in the context of the "amyloid cascade hypothesis" and its physiological relevance in AD pathogenesis.

Index Entries: Alzheimer's disease; amyloid-β; β-secretase; γ-secretase; BACE; PS1; PS2.

Introduction: Amyloid and APP

From the moment of its discovery in the early 1900s, Alzheimer's disease (AD) has been inseparably linked with its two hallmark pathological lesions, amyloid plaques and neurofibrillary tangles. Indeed, efforts to elucidate how these insoluble aggregates form in brain tissue have driven the vast majority of AD-related research to this day. Recently, two classes of enzymes believed to be crucial players in the pathogenesis of AD have been identified and characterized providing excellent options for tangible therapeutic advances. This article will review these developments and dis-

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cuss their significance in the context of AD progression and potential strategies for treatment.

Advances in the understanding of AD pathology have been accompanied by numerous hypotheses as to whether and how amyloid plaques and neurofibrillary tangles eventually lead to clinical dementia. The most well-documented and supported of these involves the notion of a pathogenic "cascade" beginning with the deposition of amyloid protein in the brain parenchyma. This initial biochemical insult is then presumably followed by a variety of neurotoxic sequelae including microglial activation, astrocytosis, free-radical damage, hyperphosphorylation of tau protein, and neurofibrillary-tangle formation that ultimately result in neuronal death and the accompanying cognitive decline *(1).* Amyloid plaques themselves are extracellular lesions composed primarily of aggregated amyloid β (Aβ) protein *(2).* Aβ is an amphipathic peptide whose length typically varies between 39 and 43 amino acids, although shorter versions with a variety of post-translational modifications are quite common *(3).* Its two most prevalent forms end at either amino acid 40 (Aβ40) or amino acid 42 (Aβ42) and investigations into this C-terminal heterogeneity have provided considerable insight into the potential role of Aβ in AD pathogenesis. Although Aβ40 is the more abundantly produced of the two by roughly a factor of 10, Aβ42 is the major species found in parenchymal amyloid deposits *(4).* In addition, Aβ42 has been shown to aggregate more readily in biochemical and biophysical studies and exhibits a greater degree of neurotoxicity than Aβ40 *(5–7).* Thus, a large body of evidence suggests that Aβ42 is the crucial pathological factor responsible for the initiation and maintenance of amyloid deposition.

The Aβ peptide is derived from the proteolytic processing of the larger amyloid precursor protein (APP) *(8)* (*see* Fig. 1). APP is a ubiquitously expressed type 1 membrane glycoprotein that, depending on alternative splicing, can exist as an isoform of either 770, 751, or 695 amino acids in length (APP770, APP751, APP695) *(9).* While the normal physiological function of APP remains unclear, it has been implicated in cell-substrate adhesion *(10–14),* the maintenance of neuronal viability and neurite outgrowth *(15–17),* and the regulation of intracellular calcium levels *(18).* Studies have also indicated that the protein may act as a growth factor for fibroblasts and epithelial cells *(19,20).* APP-null mice exhibit a mild phenotype that includes reactive astrogliosis and subtle locomotor abnormalities *(21).* In addition, APP770 and APP751, both of which contain a so-called KPI (Kunitz-type protease inhibitor) domain, have been shown to inhibit a variety of serine proteases in vitro, implying yet another potential function *(22–24).*

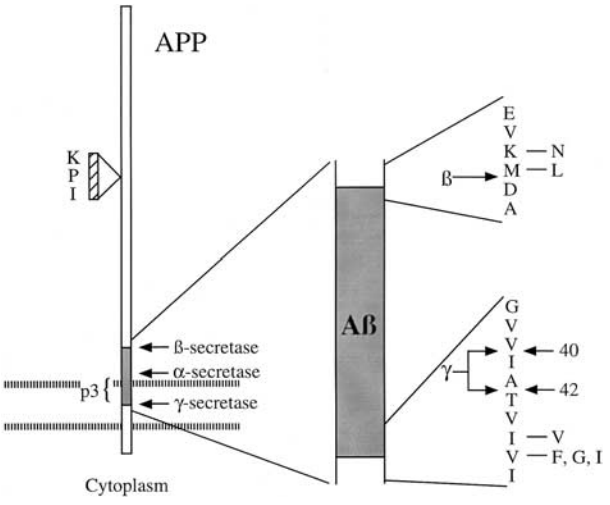

Fig. 1. APP processing. Cleavage of APP by β-secretase and γ-secretase releases the Aβ peptide. The nonamyloidogenic α-secretase cleavage pathway generating p3 is also indicated. Regions surrounding the secretase cleavage sites are enlarged and the C-termini of Aβ40 and Aβ42 are indicated *(40,42).* Missense mutations associated with FAD are also shown. KPI denotes the Kunitz-type protease inhibitor domain.

In the central nervous system (CNS), cleavage of APP by two protease activities, β-secretase and γ-secretase, releases the Aβ peptide (*see* Fig. 1). β-secretase is believed to act on APP first, generating a secreted APP derivative

(APPβ) and a membrane-anchored C-terminal fragment (C99). Cleavage of C99 within its transmembrane domain by γ-secretase then yields the Aβ peptide. Aβ production and secretion has been found to occur readily both in tissue culture *(25,26)*, and in the CNS as evidenced by the presence of Aβ in human as well as rodent cerebral spinal fluid (CSF) *(26,27)*. In contrast to Aβ depositions in amyloid plaques, the level of Aβ in human CSF does not appear to correlate directly with the progression of AD *(26)*. On a cellular level, Aβ appears to be generated in a variety of membranous compartments including the endosomal/lysosomal system *(28,29)*, the trans-Golgi network (TGN) *(30–32)*, and the endoplasmic reticulum/intermediate compartment (ER/IC) *(33–36)*. Alternatively, in a pathway that precludes Aβ formation, processing of APP by so-called α-secretase activity results in a different secreted APP variant (APPα) and a shorter C-terminal fragment (C83) whose subsequent cleavage by γ-secretase generates a small peptide known as p3 *(37)* (*see* Fig. 1). α-secretase exists as a mixture of constitutive and regulated activities, the latter of which has been shown to compete with β-secretase for APP substrate in the secretory pathway *(38)*. A group of membrane-bound metalloproteinases including tumor necrosis factor-α (TNF-α) converting enzyme (TACE) and ADAM-10 (*a d*isintegrin *a*nd *m*etalloproteinase-10) appears to be at least partially responsible for α-secretase cleavage *(39,40)*.

APP Genetics: A Case for the Importance of Aβ

The initial localization of APP to chromosome 21 *(41)* provided immediate support for the amyloid cascade hypothesis as a viable mechanism for AD. Individuals with an extra copy of chromosome 21 (Down's syndrome) have been shown to develop diffuse amyloid plaques as early as their teens and twenties, eventually leading to complete AD pathology in the ensuing decades *(42–44)*. These patients exhibit APP expression levels that exceed even what would be predicted by their augmented gene dosage *(45)*. Accelerated disease progression can therefore be correlated with abnormally high levels of APP expression and Aβ production in the human CNS.

The characterization of a variety of heritable forms of AD has yielded perhaps the best evidence that Aβ, specifically Aβ42, is the crucial causative agent in AD. Familial Alzheimer's disease (FAD) is a collection of autosomal-dominant syndromes characterized by aggressive early-onset AD accompanied by the typical pathological lesions. Although FAD accounts for only ~10% of AD cases *(46)*, its pathological similarity to sporadic AD suggests a common underlying mechanism that is simply accelerated in the context of certain genetic polymorphisms. FAD mutations are found most commonly in the AD-associated presenilin proteins (discussed more thoroughly in a later section). However, several FAD-associated mutations have been localized to the APP gene and their study has served to more clearly define the important role of Aβ in AD pathogenesis (for review, *see 47*). FAD mutations in APP, for the most part, tend to cluster around the N- and C- termini of the Aβ peptide (*see* Fig. 1) and their presence appears to significantly impact the efficiency of either β- or γ-secretase mediated cleavage. For instance, the K670M671 → N670L671 double mutation (KM→NL), located directly upstream of the putative β-secretase cleavage site, appears to enhance the β-site proteolysis of APP leading to an increase in all forms of Aβ *(48)*. In addition, a number of mutations at residues 716 and 717 *(49–51)*, near the γ-secretase proteolysis site, have been found to affect the specificity of γ-secretase mediated cleavage resulting in a selective increase in the production of Aβ42 *(52)*.

The fact that Aβ42 levels are increased in the context of mutations associated with early-onset AD underscores the physiological relevance of the amyloid cascade. Strategies to regulate the generation and clearance of Aβ42, therefore, could be of considerable therapeutic

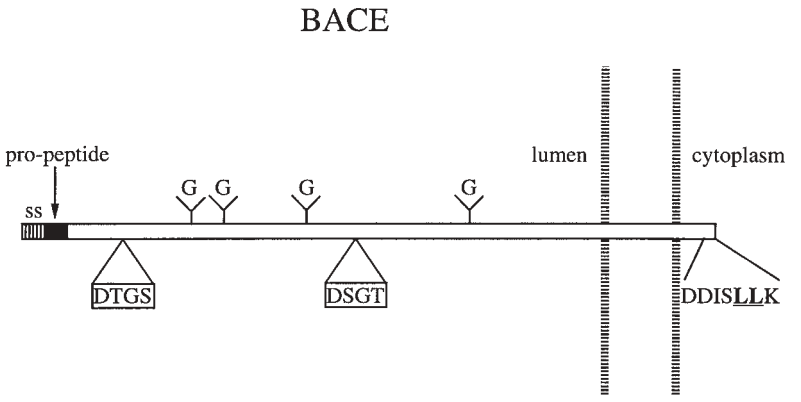

Fig. 2. Schematic showing BACE. Sites for potential N-linked glycosylation are indicated (G) along with the signal sequence (ss) and pro-peptide domain. Aspartyl protease signature sequences are boxed and their relative locations in the polypeptide are shown. The extreme C-terminus of the molecule is also enlarged to demonstrate the dileucine motif that has been implicated in the endosomal targeting of the protein.

benefit to patients afflicted with AD. The secretases themselves provide obvious targets for drug design and, with the recent cloning and characterization of β-secretase coupled with the probable identification of γ-secretase, AD-related research has entered an exciting new phase with a variety of rational, mechanistically grounded therapies seemingly close at hand.

Unmasking the β-Secretase

The characterization of β-secretase began long before its identification as the unusual aspartyl protease BACE (β-site *A*PP-Cleaving *E*nzyme) *(53–57).* Parenchymally deposited Aβ was initially found to have heterogeneous, "ragged" N-termini making precise determination of the β-secretase cleavage site(s) difficult *(58–60).* However, subsequent investigations demonstrated that β-secretase activity is responsible for proteolysis primarily at positions Asp^{1} and Glu^{11} *(25,61),* while cleavage at other sites is most likely the work of alternative enzymes *(62).* β-secretase activity was found to be more pronounced in mixed brain cultures than in peripheral cells, implying higher expression levels in the CNS than in other tissues *(63).* In addition, cell biological studies revealed an acidic pH optimum for β-secretase *(64,65),* and its activity was detected in the endosomal/lysosomal system *(28,29),* the TGN *(30),* and the ER *(33).* The efficiency of β-site cleavage was found to be enhanced in the context of the FAD-associated KM→NL double mutation *(48).* However, other amino-acid substitutions in or around the presumed APP proteolysis site appeared to decrease β-secretase activity, demonstrating a significant degree of sequence specificity *(66).*

BACE (also called Asp2, and memapsin 2) is a 501 amino-acid type 1 membrane glycoprotein initially implicated as a β-secretase candidate by expression cloning *(55),* direct purification from human brain *(54),* and bioinformatics *(53,56,57)* (*see* Fig. 2). It completely satisfies all of the criteria for classification as a β-secretase. Expression of BACE in tissue culture increases β-secretase activity *(53–57)* with appropriate proteolysis site specificity to Asp^{1} and Glu^{11} *(55).* In addition, purified full-length BACE as well as a BACE-IgG fusion protein effectively cleave synthetic APP peptide substrates, with enhanced activity toward those harboring the KM→NL mutation *(55,56).* BACE is expressed more robustly in brain than in most peripheral tissues *(55–57),* though high

levels of BACE mRNA are also observed in the pancreas, suggesting an important role for the protein in normal gastrointestinal physiology *(55–57).* That being said, however, β-secretase activity in pancreatic tissue has been demonstrated to be quite low *(54).* BACE exhibits a mildly acidic pH optimum *(55,57)* and has been localized to both the endosomal system and the TGN *(53,55,67,68).* The extracellular/lumenal domain of BACE contains two signature sequences (DT/SGT/S) typically associated with aspartyl proteases, and mutagenesis of the crucial aspartic acid residue in either site completely abrogates β-secretase activity *(53).* Thus, BACE appears to represent the defining member of a new class of membrane anchored aspartyl proteases.

BACE Trafficking and Structure: Implications for Therapy

The normal cellular trafficking of BACE has been elucidated with considerable detail. During translation the ER, BACE undergoes core glycosylation at 3 or 4 N-linked sites before export to the secretory pathway *(68,69).* The subsequent maturation of BACE in the Golgi apparatus occurs rapidly and efficiently with the modification of the protein's oligosaccharide side chains and the removal of its 24-amino acid propeptide domain *(67–69).* The serine protease furin or another member of the proprotein convertase (PC) family appears to be responsible for cleaving the BACE propeptide *(70).* Mature BACE is ultimately targeted from the secretory pathway to endocytic vesicles from where the protein cycles to and from the cell surface and possibly the TGN *(68).* The normal endosomal trafficking of BACE is mediated in part by a dileucine motif on the cytoplasmic tail of the protein (*see* Fig. 2). In the absence of this targeting signal, BACE accumulates on the plasma membrane and does not exhibit its normal cycling pattern between the cell surface and endocytic compartments *(68).* Pulse-chase experiments have demonstrated the BACE protein to have an unusually long $t_{1/2}$, anywhere from 12–16 h *(68,69).* Given this stability, BACE must cycle from the endosomal system to the cell surface and TGN several times through the course of its life-span.

Identifying the cellular compartments where BACE normally resides serves the important purpose of helping to elucidate where BACE exerts its pathological effect on APP. The fact that BACE appears to be actively targeted to endocytic vesicles coupled with its mildly acidic pH optimum strongly suggests that the protein is most biologically active in the endosomal system. APP internalization from the cell surface to endosomes has been demonstrated *(28)* implying no shortage of substrate in this location. Indeed, a number of studies have shown both β-secretase activity and Aβ production to occur readily in endocytic compartments *(28,29,64,65).* BACE mediated β-site cleavage most likely occurs in the secretory pathway as well. As stated previously, β secretase activity has been localized to both the ER and TGN *(30,33).* BACE exists in an itinerant fashion in both of these compartments and may also repetitively cycle to and from the TGN after its initial targeting to the endosomal system. The more neutral pH of the secretory pathway most likely reduces the efficiency of BACE-mediated proteolysis. However, the availability of substrate along with an abundance of γ-secretase makes the secretory pathway perhaps the intracellular site most primed for the generation of Aβ.

Detailed structural information on the BACE protein has recently emerged from X-ray crystallographic studies. The extracellular/lumenal domain of BACE adopts a bilobal folding pattern typical of aspartyl proteases with the catalytic aspartate residues located on either side of a central substrate-binding cleft *(71).* Insertions and deletions in the BACE amino acid sequence, relative to standard aspartyl proteases like pepsin, account for most of the unique topological qualities of the protein's extracellular domain and appear to significantly impact substrate-binding specificity *(71).* Information provided by the

crystal structure of BACE in complex with the peptide inhibitor OM99-2 has allowed for the careful dissection of the BACE active site. The S4 and S2 binding subsites (those responsible for binding substrate residues P4 and P2), for instance, are more spacious and hydrophilic than the corresponding subsites in pepsin. In addition, the hydrophobic S3 and S1 subsites of BACE are shaped differently than in pepsin due to the absence of a characteristic α-helix *(71).* A crucial active site arginine residue (Arg296) appears to be intimately involved with substrate positioning. Molecular modeling suggests that Arg296, which normally makes significant electrostatic contacts only with the P1′ aspartic acid residue on APP, forms an additional salt bridge with the P2 asparagine in the context of the APPKM→NL mutation *(72).* This altered binding configuration may contribute to the increased efficiency of β-secretase processing that has been well-documented in the presence of the KM→NL mutation. The singular aspects of the BACE active site may prove invaluable in the design of specific BACE small-molecule inhibitors.

The recent identification of the BACE homolog BACE2 (Asp1, memapsin 1) has underscored the possibility that β-secretase cleavage may be the result of multiple enzymatic activities *(56,73–75).* BACE2 is a 518 amino acid, type one membrane glycoprotein whose 64% homology with BACE includes the two defining DT/SGT/S aspartyl protease signature sequences. Recombinant BACE2, in a similar fashion to BACE, cleaves both APP and APP-derived peptides at the Asp^1 position of Aβ *(76).* These facts, along with the localization of the BACE2 gene to the Down's syndrome critical region of chromosome 21 make BACE2 an intriguing β-secretase candidate. Expression analysis of BACE2, however, argues against this possibility. Northern-blot and *in situ* hybridization studies show very low levels of BACE2 message in both fetal and adult brain. By contrast, the protein appears to be transcribed quite highly in other tissues such as placenta, kidney, colon, prostate, pancreas, thyroid, and stomach *(74).* This type of expression pattern is inconsistent with BACE2 having a major role in the β-site processing of APP in the CNS.

The physiological function of BACE remains unclear although its high level of expression in the pancreas implies a potential role in the processing of zymogens and hormones. In the CNS, BACE may serve a similar purpose acting on neuropeptides and other types of secreted proteins. Analysis of BACE endosomal trafficking suggests that the protein may interact with its substrate(s) while cycling to and from the cell surface. Knockout mice and other in vivo models should provide a clearer understanding of what are the normal substrates for both BACE and BACE2. The defining position held by BACE in pathological APP processing makes the protein an obvious target for therapeutic intervention. By contrast, BACE2 appears to have a relatively benign role in the grand scheme of AD pathogenesis. Thus, designing inhibitors directed specifically toward BACE and not BACE2 may be an effective way to target Aβ production without dramatically affecting the other important biological functions mediated by the BACE class of proteases.

The Presenilins: Cell Biology and Functionality

Although the presenilins have been implicated in AD for some time, their exact role in the progression of the disorder remains elusive. Presenilin 1 and its close homolog presenilin 2 (PS1 and PS2) define their own class of ubiquitously expressed polytopic membrane proteins *(77–79).* While structural models for the presenilins vary considerably, most evidence supports a topology with eight membrane-spanning regions and a cytoplasmic orientation for both the N-and C-termini *(80–85)* (*see* Fig. 3). The 463 amino acid PS1 and the 448 amino acid PS2 are 67% homologous, with the areas of highest variability being in the N-terminal region and the large cytoplasmic loop between transmembrane domains 6 and 7 *(78).*

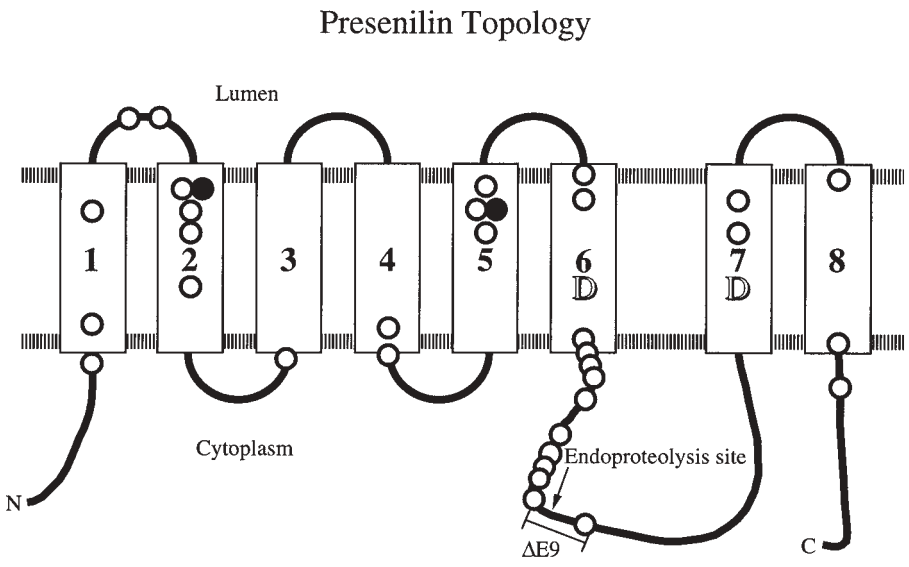

Fig. 3. Topological map of presenilin 1 and 2. Eight transmembrane domains are shown along with the large cytoplasmic loop, N- and C-terminal extensions. Circles indicate FAD-associated mutations in presenilin (white-PS1, black-PS2). In addition, a deletion mutation eliminating exon nine in PS1 is shown (ΔE9). The locations of the presumed catalytic aspartate residues are indicated (D).

After translation in the ER, both PS1 and PS2 are endoproteolized by an unknown "presenilinase" to generate N- and C-terminal fragments, which then appear to incorporate into a high molecular-weight (150–250 kD) complex containing β-catenin *(86–89).* The endoproteolysis of the presenilins along with their assembly into this large oligomeric structure is tightly regulated, presumably by limited cellular factors *(90).* This is exemplified by the fact that overexpression of human PS1 or PS2 leads to a downregulation of endogenous murine presenilin fragments in both N2a cells and transgenic mice *(90).*

The N- and C-terminal fragments of the presenilins are thought by most to be the biologically active forms of the proteins. Endogenously expressed presenilin fragments can be readily detected both in cultured cells and in tissue samples, whereas the holoproteins typically cannot *(86).* In addition, PS1 and PS2 holoproteins are degraded quite rapidly by the proteosome ($t_{1/2}$ ~ 1.5 h), whereas their cleavage products exhibit a high degree of stability ($t_{1/2}$ ~ 24 h) *(87,91,92).* Most studies have localized the presenilins to the ER and the Golgi apparatus *(82,93–95),* although the proteins have also been detected to varying degrees in the endosomal system *(96),* the nucleus *(97),* and on the cell surface *(83,98–100).* However, whether this reflects the native distribution of the presenilins or is a consequence of overexpression in vitro is not known.

The presenilins have been linked to several physiological processes, with considerable evidence pointing to a role in the Notch signaling pathway. The presenilins share 50% homology with sel-12, a protein functioning downstream of a Notch family receptor in *Caenorhabditis elegans* (LIN-12) *(101).* In addition, PS1 null mice (PS1–/–) exhibit severe skeletal malformations suggestive of gross defects in the development of the paraxial mesoderm *(102,103).* A similar, although more extreme phenotype has been observed in Notch knockouts, implying that both proteins operate in the same pathway *(104,105).* While

PS2 knockouts show a comparatively mild pulmonary defect *(106,107)*, mice null for both PS1 and PS2 exhibit a phenotype more dramatic than that of PS1–/– mice and highly reminiscent of Notch knockouts *(106,107)*. This suggests not only that the presenilins are essential mediators in the Notch signaling pathway, but that they can, to a certain extent, effectively substitute for one another on a functional level.

The presenilins appear to have a role in apoptosis although the precise nature of their involvement remains unclear. C-terminal fragments of PS2 have been found to protect both human T-cells and HeLa cells from Fas-induced apoptosis *(108,109)*. By contrast, full-length presenilins have been shown to enhance apoptosis under a variety of conditions, especially when harboring FAD-associated mutations *(110–113)*. It has also been hypothesized that the presenilins somehow mediate the trafficking of proteins within the secretory pathway. However, conflicting data has been obtained on this subject *(114,115)*. Finally, PS1 and PS2 have been found to bind a surprisingly diverse group of proteins involved in biological processes ranging from cell adhesion to G-protein mediated signaling *(116–123)*. It remains to be seen, however, which of these interactions will uncover important, physiologically relevant events.

The Presenilins as γ-Secretases

The role of the presenilins in APP processing is perhaps their most intriguing functional aspect, especially in the context of AD. As mentioned previously, the majority of documented FAD cases to date have resulted from one of several mutations in the presenilin genes (for review, *see 47*). Presenilins harboring FAD mutations either specifically increase the production of Aβ42 or increase the ratio of Aβ42/Aβ40 in both cultured cells and mouse models *(124–133)*. These same presenilin variants also increase the levels of Aβ42 detectable in human plasma *(127)*. Thus, in a similar fashion to FAD mutations located C-terminal to the Aβ region on APP, FAD mutations in the presenilins appear to modulate γ-secretase activity. These same mutations, however, do not seriously hamper the ability of presenilin constructs to rescue PS1–/– mice from their lethal phenotype *(134,135)*, implying a gain of function with regard to APP processing.

Recent studies have suggested that the presenilins are in fact γ-secretases. Cells derived from mice deficient in the presenilins do not produce detectable quantities of Aβ and accumulate APP C-terminal fragments *(114,136,137)*, implying a direct link between the presenilins and γ-secretase. In addition, two highly conserved aspartic acid residues in transmembrane domains six and seven of the presenilins appear to be necessary for normal γ-secretase activity (*see* Fig. 3). When either or both of these residues are mutated in PS1, Aβ and p3 production is greatly reduced with a concomitant increase in the levels of APP C-terminal fragments *(138)*, and when both PS1 and PS2 are mutated in this way, Aβ production is essentially eliminated *(139)*.

Several other lines of evidence support the identification of the presenilins as γ-secretases. Immunoprecipitation of PS1 leads to the quantitative recovery of detergent solublized γ-secretase activity extracted from cell lysates *(140)*. In addition, detectable amounts of APP C-terminal fragments have been shown to co-immunoprecipitate with PS1 and PS2 from Golgi-rich membrane fractions *(141)*. γ-secretase has been demonstrated to have an acidic pH optimum, consistent with the notion that the presenilins are intramembranous aspartyl proteases *(138)*. Finally, and perhaps most importantly, several γ-secretase inhibitors have been shown to bind directly to the presenilins *(142,143)*. Both the presenilin N- and C-terminal fragments appear to interact specifically with these inhibitors implying that portions of each cleavage product contribute to the molecule's active site. This is consistent with the fact that the presenilin N- and C-terminal fragments each contain one of the two ostensibly catalytic aspartic-acid residues. Thus, a number of studies indicate

that the presenilins directly mediate γ-secretase activity, or are very intimately involved with the process as obligate cofactors. In either case, PS1 and PS2 constitute valuable therapeutic targets for AD.

Proteolytic activity directly mediated by the presenilins would be consistent with the role played by the proteins in Notch signaling. In response to ligand, the intracellular domain of the Notch receptor (NICD) is released from the cell membrane, whereupon it enters the nucleus, presumably to mediate its signaling functions *(144–146).* Multiple studies have shown that the intramembranous proteolytic event resulting in the liberation of the NICD requires presenilin *(137,147–149)* and can be blocked by γ-secretase inhibitors *(147).* These data demonstrate that presenilin, acting in its capacity as a γ-secretase, mediates the intramembranous cleavage of Notch. It is interesting to note that the efficiency of presenilin-mediated Notch cleavage is reduced in the context of FAD mutations *(148,150),* in sharp contrast to the effect these same mutations have on APP processing, that being increased Aβ42 production. This finding suggests that FAD mutations may function in part by shifting the substrate specificity of PS1 and PS2 toward APP.

The γ-secretase activity of the presenilins has also been implicated in the unfolded protein response (UPR). In situations of ER stress, signaling by the transmembrane kinase/endoribonuclease IRE1 results in the transcription of numerous chaperones including BiP, GRP94, and protein disulfide isomerase (PDI) *(151).* This signaling pathway appears to require the intramembranous proteolysis of activated, phosphorylated IRE1, an event which allows the cytoplasmic domain of the protein to migrate into the nucleus where it processes the mRNA of the Hac1 transcription factor *(152).* IRE1 cleavage is markedly reduced in the absence of PS1 resulting in a compromised UPR *(152).* This finding adds IRE1 to the list of candidate substrates for PS1 mediated γ-secretase cleavage. Interestingly, FAD mutations in PS1 lead to an impairment of the IRE1 signaling implying that the inability of cells to effectively respond to ER stress may be a contributing factor to the pathogenesis of heritable forms of AD *(153).*

The evidence linking PS1 and PS2 to intramembranous proteolysis is quite compelling. However, certain studies suggest that γ-secretase itself must be a much more complex entity than presenilin alone. γ-secretase has been shown to be comprised of multiple, pharmacologically distinct activities *(154)* implying the involvement of several enzymes. In addition, presenilin aspartate mutants do not appear to be incorporated into the same high molecular-weight complex that typically contains functional wild-type presenilin *(155).* This finding suggests that other components of the oligomer are essential for normal γ-secretase activity and that the inability of presenilin aspartate mutants to effectively interact with these proteins might explain their impaired effects on APP processing. Consistent with the notion of an oligomeric γ-secretase is the recent isolation of nicastrin, a 709 amino acid type-one membrane glycoprotein, from co-immunoprecipitation with PS1 *(156).* Nicastrin interacts strongly with the presenilins and appears to be required for normal notch signaling in *C. elegans.* It can also be co-immunoprecipitated with APP C-terminal fragments as well as the full-length protein. Interestingly, more APP C-terminal fragments co-immunoprecipitate with nicastrin in the context of presenilin FAD mutations, and the deletion of specific regions within the nicastrin molecule leads to significantly reduced Aβ production *(156).* Nicastrin appears to have a role, therefore, in the binding of γ-secretase substrates and may even be involved in the regulation of the enzymatic activity itself. Regardless its exact function, the discovery of nicastrin provides additional support for the idea of a large, multi-component protein complex, similar in many ways to the proteosome, mediating γ-secretase activity. This complex has aptly been named the "secretasome" *(156).*

Interrupting the Amyloid Cascade

Exciting developments over the last few years have identified what may be the two crucial enzymes responsible for Aβ production in the human CNS. Effective therapies targeting BACE and the presenilins may represent the defining step in the path toward a cure for AD. Combating amyloid pathology, however, does not necessitate the inhibition enzymatic activities. Recently, simple immunization with Aβ42 has been shown to inhibit the deposition of amyloid and clear plaques already present in mice overexpressing FAD mutant APP *(157).* The mechanism for this remarkable effect appears to be Fc-receptor mediated phagocytosis of aggregated Aβ by microglia, consistent with the additional finding that peripherally administered antibodies directed against Aβ42 yield similar results *(157,158).* Whether this kind of vaccine-based strategy will work in humans remains unclear. The mice used in this series of studies exhibited no obvious immunological side effects and the crucial Aβ-recognizing antibodies, whether endogenously developed or peripherally administered, appeared to effectively cross the blood-brain barrier (BBB). Nevertheless, clearing plaques in mice and reversing the course of AD in humans are two very different problems. As stated previously, the direct connection between amyloid deposition, neurodegeneration, and cognitive decline in AD has yet to be definitively demonstrated. However, the recent identification of β-secretase, the continuing insights into the biological characteristics of γ-secretase, and the prospects of an amyloid vaccine have brought the AD field tantalizingly close to the stage where fundamental questions regarding the importance of amyloid can finally be addressed in human subjects.

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