A Portrait of Alzheimer Secretases—New Features and Familiar Faces

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The amyloid β-peptide (Aβ) is a principal component of the cerebral plaques found in the brains of patients with Alzheimer's disease (AD). This insoluble 40– to 42–amino acid peptide is formed by the cleavage of the Aβ precursor protein (APP). The three proteases that cleave APP, α-, β-, and γ-secretases, have been implicated in the etiology of AD. β-Secretase is a membrane-anchored protein with clear homology to soluble aspartyl proteases, and α-secretase displays characteristics of certain membrane-tethered metalloproteases. γ-Secretase is apparently an oligomeric complex that includes the presenilins, which may be the catalytic component of this protease. Identification of the α-, β-, and γ-secretases provides potential targets for designing new drugs to treat AD.

We are still far from the time when people will understand the curious relationship between one fragment of nature and another, which all the same explain each other and enhance each other.

—Vincent van Gogh

More than a century later, van Gogh's assertion is still rich in meaning and yet rings only half true, for we are now in an age when we can truly appreciate molecular relations between entities and processes that at first glance appear unconnected. Indeed, recent discoveries in such seemingly disparate areas of inquiry as neurodegenerative disease, developmental biology, and lipid biochemistry have coalesced to paint a portrait of nature more intricate than we could have imagined, each aspect explaining and enhancing the other. At the same time, these discoveries have illuminated important therapeutic targets for Alzheimer's disease (AD). This disease is characterized pathologically by cerebral plaques containing the amyloid β-peptide (Aβ), a proteolytic product derived from the Aβ precursor protein (APP) (Fig. 1A). The search for the proteases responsible for processing APP has unexpectedly revealed proteins that are also involved in a signaling pathway essential for proper cell differentiation during embryonic development. And one of these proteins appears to be a member of an emerging class of polytopic membrane proteases that includes an unusual metalloprotease involved in cholesterol biosynthesis.

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APP Processing and the Amyloid Hypothesis

First described by Alois Alzheimer in 1906, the disease that bears his name largely remained an enigma until the twilight of the 20th century. Along with descriptions of progressive loss of memory and general cognitive decline, Alzheimer noted the presence of intraneuronal tangles and extracellular "amyloid" plaques in the diseased-damaged brain, but he could not decipher whether the tangles or plaques were causative or merely markers of the disease. In 1991, the search for genetic linkages yielded a major clue: Missense mutations in APP caused autosomal dominant, early-onset (familial) AD, and these mutations occurred in and around the Aβ region of the precursor protein (*1–3*). These findings, together with observations that Aβ readily forms neurotoxic, threadlike structures called fibrils (*4–7*), bolstered the view that the accumulation and deposition of Aβ in the brain over decades leads to neuronal dysfunction and eventually clinical manifestation of the disease (the amyloid hypothesis) (*8*).

The APP is an integral membrane protein processed by several different proteases called secretases (see Fig. 1A). β-Secretase generates the NH_2-terminus of Aβ, cleaving APP to produce a soluble version of APP (β-APP_s) and a 99-residue COOH-terminal fragment (C99) that remains membrane-bound. In contrast, α-secretase cuts within the Aβ region to produce α-APP_s and an 83-residue COOH-terminal fragment (C83). Both C99 and C83 are substrates for γ-secretase, which performs an unusual proteolysis in the middle of the transmembrane domain to produce the 4-kD Aβ from C99 and a 3-kD peptide called p3 from C83. Proteolysis by γ-secretase is heterogeneous: Most of the full-length Aβ species produced is a 40-residue peptide ($Aβ_{40}$), whereas a small proportion is a 42-residue COOH-terminal variant ($Aβ_{42}$). The longer and more hydrophobic $Aβ_{42}$ is much more prone to fibril formation than is $Aβ_{40}$ (*5*), and even though $Aβ_{42}$ is a minor form of Aβ, it is the major Aβ species found in cerebral plaques (*8*). Moreover, AD-causing mutations in APP near the β- and γ-secretase cleavage sites all increase $Aβ_{42}$—those near the β-secretase cleavage site augment β-site proteolysis, leading to elevation of both $Aβ_{40}$ and $Aβ_{42}$ (*9*, *10*), whereas those near the γ-site specifically increase production of $Aβ_{42}$ (*11*). Taken together, these findings implicated $Aβ_{42}$ in the pathogenesis of AD and spurred AD researchers to identify the Aβ-releasing proteases.

β-Secretase: A Family Resemblance to Aspartyl Proteases

Two years ago, β-secretase was identified as a protein with homology to the pepsin family of aspartyl proteases (*12–16*). β-Secretase contains a single transmembrane domain near the COOH-terminus, a signal sequence and propeptide region at the NH_2-terminus, and two aspartates in its ectodomain, Asp^{93} and Asp^{289}, that are required for activity. Mutation of either aspartate does not affect removal of the propeptide region, indicating that β-secretase does not proteolytically cleave itself. Instead, the responsible protease appears to be a furinlike protease or may even be furin itself (*17*). β-Secretase mRNA is highly expressed in the brain and is also found in a variety of human tissues (*12*, *14*, *16*), consistent with the finding that Aβ is normally produced by many cell types (*18–21*). The β-secretase protein is expressed primarily in the Golgi and in endosomes, although the enzyme can be detected at the plasma membrane as well. The gene for β-secretase (also referred to as beta-site APP-cleaving enzyme, or *BACE*) is located on chromosome 11, but no AD-causing mutation in this gene has been identified so far (*22*). However, a β-secretase homolog, *BACE2*, maps to chromosome 21, raising the possibility that this protease contributes to AD associated with Down syndrome. Down syndrome patients carry an extra copy of chromosome 21, secrete more Aβ from birth, and invariably develop AD by age 50 (*22*). Although BACE2 cleaves APP and short model

peptides in a β-secretase–like manner (*23*), there is very little of this protease in the brain, suggesting that it may play little, if any, role in the formation of cerebral plaques seen in AD. Instead, the AD associated with Down syndrome is probably due to the presence of an extra copy of the *APP* gene, which is also located on chromosome 21.

Several inhibitors of β-secretase activity have been designed from the β-site in APP and contain a moiety that mimics the transition state of aspartyl protease catalysis (*13*, *24*). The bilobal crystal structure of β-secretase bound to one of these compounds at 1.9 Å resolution (*25*) displays the conserved general folding of aspartyl proteases. The inhibitor is located in the substrate-binding cleft between the lobes, with the transition-state mimicking moiety interacting with the two active site aspartates. As with other aspartyl proteases, β-secretase has a "flap" that partially covers the cleft, and the backbone of the inhibitor is mostly in an extended conformation. Moreover, most of the hydrogen bond interactions between the enzyme and the backbone of the inhibitor are highly conserved among eukaryotic and HIV aspartyl proteases. But β-secretase does display some structural differences with other aspartyl proteases. The β-secretase active site is more accessible than that of pepsin; in particular, the S2 and S4 subsites are relatively hydrophilic and open to solvent. The hydrophilic character of these subsites is not conserved in the corresponding subsites of other human aspartyl proteases, suggesting that these differences could be exploited for the design of selective inhibitors. In contrast, the P3′ and P4′ inhibitor side chains point toward the molecular surface and have little interaction with the protease, and the backbone of residues P2′ to P4′ deviates from the regular extended conformation, with a kink at P2′. This is also an unusual feature for an aspartyl protease and might be turned to advantage in designing β-secretase inhibitors.

β-Secretase appears to be an optimal therapeutic target for the prevention and treatment of AD: The protease catalyzes the initial step in Aβ production, Aβ is strongly implicated in the pathogenesis of the disease, and the recently solved structure of the enzyme-inhibitor complex allows structure-based design. Still, significant hurdles remain before the development of useful therapies. For agents to work effectively in vivo, the compounds must not only cross the blood-brain barrier, but they must also be taken up by cells. As they must work inside the cell, these agents should be highly selective: Interference with other intracellular proteases and critical signaling pathways must be minimized. Another concern is that β-secretase may process substrates in addition to APP. Given that other membrane secretases, such as tumor necrosis factor–α (TNF-α) converting enzyme or TACE (*26*), have multiple substrates, β-secretase may likewise cleave other membrane proteins. The development of β-secretase knockout mice has so far not answered this question, because these mice have no phenotype except for a dramatic reduction in Aβ levels (*27*, *28*). The lack of phenotype in the *BACE* knockout mice suggests that blocking β-secretase pharmacologically should effectively lower Aβ with minimal side effects. But even if it does turn out that β-secretase is an important player in normal adult physiology, only partial inhibition may be needed for a therapeutic effect. Another key unknown is the function of BACE2. This enzyme is strongly expressed in heart, kidney, and placenta, suggesting that it may be important in highly vascularized systemic tissues (*23*). If so, it will be critical to develop drugs that selectively block BACE but not BACE2. Mice deficient in BACE2 should provide critical clues to this important question.

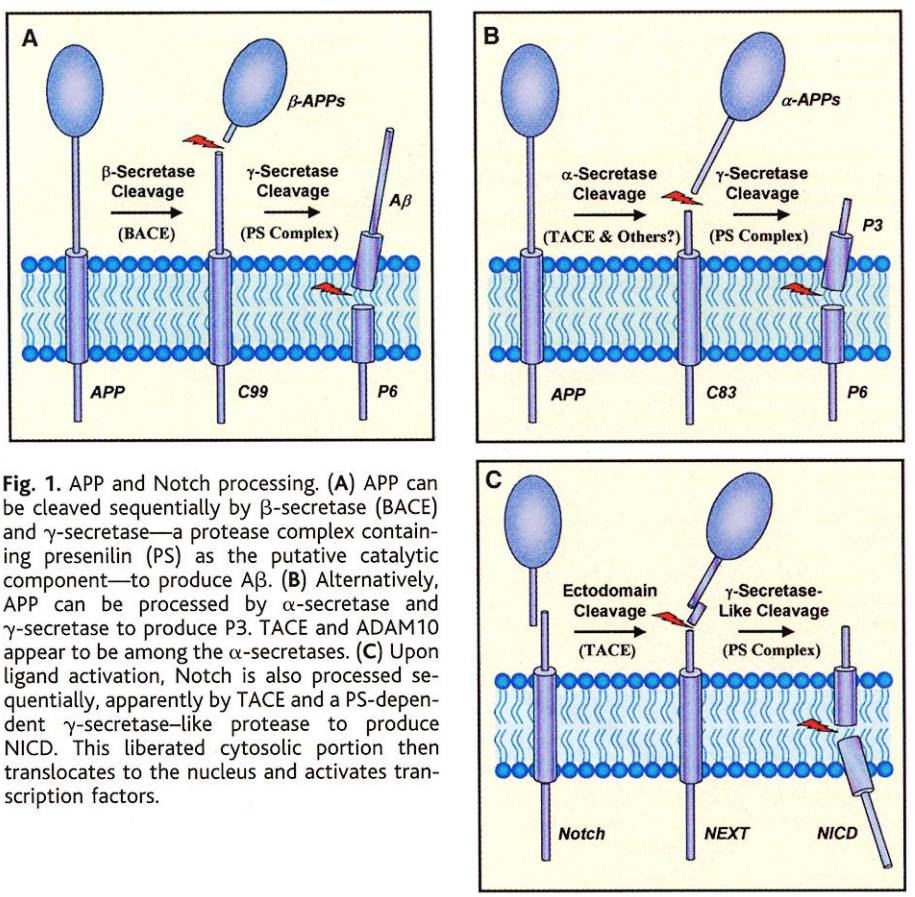

Fig. 1. APP and Notch processing. (**A**) APP can be cleaved sequentially by β-secretase (BACE) and γ-secretase—a protease complex containing presenilin (PS) as the putative catalytic component—to produce Aβ. (**B**) Alternatively, APP can be processed by α-secretase and γ-secretase to produce P3. TACE and ADAM10 appear to be among the α-secretases. (**C**) Upon ligand activation, Notch is also processed sequentially, apparently by TACE and a PS-dependent γ-secretase–like protease to produce NICD. This liberated cytosolic portion then translocates to the nucleus and activates transcription factors.

α-Secretase: A Familiar Metalloprotease Intersects with APP and Notch

Alternative processing of APP by α-secretase precludes Aβ production, as this enzyme cleaves within the Aβ sequence (*29*, *30*). Although cells contain a certain level of basal α-secretase activity, proteolysis by this enzyme can be increased by activators of protein kinase C (PKC), such as phorbol esters (*31*–*34*). Moreover, activation of receptors that work through PKC can augment α-secretase cleavage of APP with concomitant reduction in β-secretase processing. For instance, agonists of the metabotropic glutamate receptors can lower Aβ by shunting APP toward the α-secretase pathway (*35*). Muscarinic agonists (M1 and M3) can likewise decrease Aβ production, and this effect has been observed in vitro as well as in vivo (*31*, *36*–*39*). Because of this effect on Aβ production, M1 and M3 agonists might be useful agents for treating AD. In addition, experimental evidence suggests that APP_s may have a neuroprotective effect and may enhance learning and cognition (*40*); thus, augmenting α-secretase processing of APP to release APP_s might be beneficial in treating AD.

The principal determinants of APP cleavage by α-secretase appear to be the distance of the hydrolyzed bond from the membrane (12 or 13 residues) and a local helical conformation (*41*). Pharmacologic studies initially suggested that α-secretase might be a zinc-dependent metalloprotease, because its activity can be blocked by peptide hydroxamates

(*42*). Indeed, in mice lacking the metalloprotease TACE, there is elimination of inducible α-secretase activity, indicating that TACE is associated with α-secretase processing of APP (*43*). Moreover, TACE (also called ADAM17) correctly cleaved peptide substrates based on the α-secretase cleavage site in APP, suggesting that TACE itself may be one of the α-secretases. TACE apparently processes a spectrum of type I membrane glycoproteins, including TNF-α, the p75 TNF receptor, L-selectin adhesion molecule, and transforming growth factor–α (TGF-α). Mice lacking TACE die in utero, emphasizing the importance of ectodomain shedding during embryonic development (*26*).

The metalloprotease TACE also appears to process the Notch receptor. After translation in the endoplasmic reticulum, Notch is processed by a furinlike protease, resulting in a heterodimeric receptor that is shuttled to the cell surface (*44*) (see Fig. 1C). Upon interaction with a cognate ligand, the extracellular domain of Notch is shed by a metalloprotease similar or identical to TACE (*45*, *46*). The membrane-associated COOH-terminus is then cut within the postulated transmembrane domain to release the Notch intracellular domain (NICD), which then translocates to the nucleus, where it interacts with and activates the CSL family of transcription factors (where CSL stands for CBF1, Su(H), and Lag-1) (*47*). Such signaling is essential for cell fate determinations and tissue patterning during embryonic development. Another metalloprotease, ADAM10, also appears to process APP in an α-secretase–like manner (*48*). Overexpression of ADAM10 in human cells increased both basal and PKC-inducible α-secretase activity severalfold. Moreover, endogenous α-secretase activity was inhibited by a dominant-negative form of ADAM10 with a point mutation in the zinc-binding site, and a peptide substrate based on the α-secretase cleavage site in APP was processed by ADAM10 in a manner consistent with α-secretase. It is interesting that ADAM10 is also implicated in the Notch signaling pathway (*49*). Thus, TACE and ADAM 10 appear to have very similar roles with respect to APP and Notch processing. Definitive proof that TACE and ADAM10 are α-secretases and whether other proteases also contribute to this APP processing activity remain to be determined.

The Elusive γ-Secretase: New Features in the Face of Proteolysis

After either α- or β-secretase release the bulk of APP, the remaining COOH-terminal fragments, C83 and C99, are clipped in the middle of their transmembrane regions by γ-secretase. It has long been suspected that γ-secretase is central to AD pathogenesis, because of its importance in determining the ratio of $A\beta_{40}$ to $A\beta_{42}$. Despite intense efforts, the identity of γ-secretase evaded scientists for over a decade and still remains to some extent a mystery. This is in large part due to its unusual properties, the most peculiar being its ability to cut in the middle of the transmembrane region of its substrate. How hydrolysis takes place in what is otherwise a water-excluded environment is unclear. Further complicating matters, γ-secretase appears to be a multiprotein complex (see below), making its identification through strategies such as expression cloning unlikely to succeed. Nevertheless, substrate mutagenesis and the development of substrate-based inhibitors have allowed indirect characterization of γ-secretase. The protease has remarkably loose sequence specificity for its substrate, because a number of natural and engineered mutations in APP near the γ-secretase cleavage site still allow Aβ production in transfected cells (*50*–*53*). Moreover, γ-secretase displays pharmacologic characteristics of an aspartyl protease: Substrate-based compounds that mimic the transition state of aspartyl protease catalysis block γ-secretase activity in cell culture (*54*). This finding provided an important clue, because all members of this class of protease contain two active aspartates that catalyze hydrolysis of the peptide bond. These substrate-based inhibitors have also served as important molecular tools for identifying the catalytic component of γ-secretase through affinity labeling.

During the search for genes on chromosomes 14 and 1 responsible for most cases of familial AD, it was thought that the encoded proteins would reveal at least one, if not both, of the proteases involved in Aβ production. When the search identified the presenilins-1 and -2 (PS1 and PS2) in 1995 (*55*–*57*), it was far from clear what the normal function of these multipass membrane proteins might be and how mutant forms might lead to AD. These proteins also undergo proteolytic processing. Cleavage within the large cytosolic loop between transmembrane domain (TM) 6 and TM 7 results in the formation of a stable, heterodimeric PS complex (*58*, *59*) (see Fig. 2C). Remarkably, the presenilins are the sites of dozens of AD-causing missense mutations (*60*): More than 70 such mutations have now been identified, with all but six occurring in PS1 (*61*). Intriguingly, these AD-causing mutations result in specific increases in $A\beta_{42}$ production in transfected cells, in transgenic mice, and in plasma and brain from human carriers (*62*–*66*). Thus, these mutant presenilins somehow modulate γ-secretase activity to enhance production of $A\beta_{42}$.

A major advance toward understanding the function of presenilins came from *PS1* knockout mice. Although deletion of PS1 in these mice was lethal in utero or shortly after birth (*67*, *68*), primary neurons from PS1-deficient embryos could be cultured, and transfection of these cells with APP revealed a marked reduction in γ-secretase activity (*69*). The absence of PS1 did not affect the maturation and distribution of APP, nor did it alter α- or β-APP_s release. However, γ-secretase substrates C83 and C99 were dramatically elevated, and Aβ production was lowered to roughly 20% of levels seen in primary neuronal cultures from wild-type littermates, together indicating that PS1 is somehow involved in γ-secretase activity. Cells from *PS1*/*PS2* double-knockout mice were found to be completely devoid of γ-secretase activity (*70*, *71*), demonstrating the absolute requirement of presenilins for the γ-secretase cleavage of APP.

How do the presenilins mediate γ-secretase activity? γ-Secretase displays the pharmacologic profile of an aspartyl protease, catalyzes the transmembrane cleavage of substrates, and requires presenilins for activity. Moreover, presenilin forms complexes with APP and with Notch in cultured cells (*72*, *73*). Given these characteristics of γ-secretase and presenilin, could presenilin actually be the protease? Presenilins contain two completely conserved transmembrane aspartates (see Fig. 2C), one found in TM6 and one in TM7. These aspartates are predicted to lie at the same distance within the membrane (that is, they could interact with each other) and are roughly aligned with the γ-secretase cleavage site in APP such that they might work together to cut C99 and C83. Mutation of either TM aspartate did not affect the expression or subcellular distribution of APP (*74*). However, the mutant presenilins were completely incapable of undergoing endoproteolysis to PS1 heterodimers and acted as dominant-negatives with respect to γ-secretase processing of APP. Subsequent reports confirmed these observations for PS1 as well as for PS2 (*75*–*79*). The aspartates are critical for γ-secretase activity independent of their role in presenilin endoproteolysis: A natural PS1 splice variant that lacks the endoproteolysis site and is not cleaved to heterodimers is a functional presenilin, but expression of an aspartate mutant of this variant still blocked γ-secretase activity (*74*). Together, these results suggest that presenilins might be the catalytic component of γ-secretase: Upon interaction with as-yet-unidentified limiting cellular factors (see below or vide infra), presenilin undergoes autoproteolysis via the two aspartates, and the two presenilin subunits remain together, each contributing one aspartate to the active site of γ-secretase. In this model, the presenilin cleavage site is part of a pro domain that blocks the protease active site and keeps the enzyme in a dormant state. Cleavage, deletion, or mutation of this pro domain would allow formation of functional γ-secretase (*74*).

Some observations seem at odds with the notion that presenilins are proteases. Most notable is the "spatial paradox": The subcellular localization of endogenous presenilin is primarily in the endoplasmic reticulum and Golgi (*80*), whereas most Aβ production apparently requires endocytosis, which takes place at the cell surface (*81*). Small amounts of endogenous heterodimeric presenilin, however, have been detected at the cell surface (*82*), and a recent study found most presenilin to be in endosomes (*83*). Also, β-secretase is principally found in endosome membranes (*84*) and catalyzes the first of the two cuts leading to Aβ formation from APP; therefore, this protease is expected to be the primary determinant of the subcellular localization of Aβ production. To determine the subcellular distribution of γ-secretase, the activity of this presenilin-dependent protease must first be decoupled somehow from β-secretase activity (for example, by expressing γ-secretase substrate C99 exogenously). Another problem with the "presenilin as protease" hypothesis is a report suggesting that one of the conserved aspartates is not absolutely required for γ-secretase cleavage of APP: Mutation of this conserved aspartate still allowed Aβ production (*85*). Nevertheless, APP γ-secretase substrates are elevated in the presence of these mutant presenilins (*85*), indicating a certain degree of protease inhibition. Testing the ability of these mutant presenilins to rescue Aβ production in *PS1/PS2* double-knockout cells (*70*, *71*) should provide a definitive answer to this question. Still another problem with the hypothesis, in particular the corollary that presenilins are autoactivated, is that attempts to block presenilin endoproteolysis with γ-secretase inhibitors have so far failed. If presenilins undergo autoproteolysis (that is, the active sites for "presenilinase" and γ-secretase are the same), then shouldn't γ-secretase inhibitors also prevent the conversion of presenilin to heterodimers? However, the lack of effect on presenilin endoproteolysis may be the difference between the ability to block an intermolecular interaction (substrate and enzyme) and the ability to block an intramolecular interaction (enzyme and pro domain)—that is, the pro domain may sterically prevent access of the inhibitor. More rigorous tests of presenilin autoproteolysis will have to await identification of other members of the γ-secretase complex and subsequent reconstitution or identification of a separate presenilinase enzyme.

These unresolved issues notwithstanding, recent biochemical evidence provides strong support for the unusual but unifying hypothesis that presenilin is the business end of γ-secretase. The development of cell-free assays for γ-secretase led to the discovery that this activity copurifies with PS heterodimers after subcellular fractionation of microsomes (*86*) and even after chromatographic separation of detergent-solubilized microsomes (*87*). Moreover, precipitation with PS-specific antibodies under conditions that keep PS heterodimers together depletes γ-secretase activity from detergent-solubilized preparations, and the activity can be recovered in the precipitate (*87*). The γ-secretase substrates C83 and C99 form stable complexes with PS heterodimers, and mutating one of the key PS aspartates enhances the amounts of these γ-secretase substrates brought down with PS antibodies (*86*). Tagging γ-secretase with small molecule inhibitors targeted to the active site (for example, transition-state analogs) has provided direct biochemical evidence in support of the "presenilin as protease" hypothesis. Parenthetically, similar strategies using peptide analogs directed to the active site led to the successful identification of both BACE (β-secretase) and TACE (a putative α-secretase) (see Fig. 2) (*13*, *88*). The γ-secretase inhibitors were modified to contain a reactive cross-linking group and a molecular handle suitable for detection as part of an unbiased search for γ-secretase candidates. Two independent reports using this approach confirmed that presenilin heterodimers are the molecular target of transition-state analog γ-secretase inhibitors (*89*, *90*). Because the designed affinity reagents each contain a transition-state analog and are thus expected to interact directly with the catalytic machinery of γ-secretase, these findings provide compelling evidence that PS heterodimers contain the active site of γ-secretase.

The discovery that presenilins are probably the proteins that catalyze the last step in the generation of Aβ provides a linchpin for the amyloid hypothesis of AD: All known forms of early-onset familial AD are apparently caused either by mutations near the cleavage sites of the substrate precursor of Aβ (APP) or by mutations in a protease that generates Aβ (presenilin/γ-secretase). However, it seems clear that presenilins do not work alone, that they are part of a larger γ-secretase complex. PS heterodimers, the biologically active form of PS, are only produced in limited amounts even upon overexpression of the holoprotein (*58*, *91–93*), indicating competition for limiting cellular factors needed for stabilization and endoproteolysis. PS heterodimers migrate through density gradients as part of a high-molecular-mass complex (~250 kD) (*94*), and detergent-solubilized γ-secretase activity eluted from a size exclusion column with an estimated molecular mass of ≥2 megadaltons (*87*). Because other members of the γ-secretase complex might themselves be targets for therapeutic intervention in AD, the identification of these proteins and reconstitution of the γ-secretase complex are of paramount importance. The first potential γ-secretase cofactor, a new single-pass membrane protein dubbed nicastrin, was identified last year by precipitating it with PS antibodies under conditions that keep PS complexes together (*95*). Because overexpression of nicastrin does not alter levels of PS heterodimers, other members of this complex remain to be discovered.

The presenilins are not only involved in the proteolytic processing of APP; they are also critical for processing Notch (*96*). The parallels between APP and Notch processing are striking. Not only are both apparently cleaved by TACE, but also the transmembrane regions of both proteins are processed by a γ-secretase–like protease that requires presenilins. Deficiency of PS1 or of both PS1 and PS2 in mice is lethal to embryoes, with a phenotype similar to that observed upon knockout of *Notch1* (*67*, *68*, *97*, *98*). Deletion of *PS1* dramatically reduces NICD formation (*99*), and the complete absence of presenilins results in total abolition of NICD production (*70*, *71*). Similarly, treatment of cells with γ-secretase inhibitors or mutation of the putative catalytic aspartates in presenilin likewise blocks NICD production and nuclear translocation and reduces Notch signaling (*75*, *82*, *85*, *99*, *100*). Thus, if presenilins are the catalytic components of the γ-secretases that process APP, they are also likely to be the catalytic components of the related proteases that clip the transmembrane region of Notch. These similarities between the processing of Notch and APP, although intriguing, raise the serious concern that γ-secretase inhibitors under development for the treatment of AD might cause severe problems due to interference with Notch signaling. In vivo studies using γ-secretase inhibitors with good pharmaceutical properties should address this issue.

These remarkable parallels also raise the question of whether APP processing is part of an undiscovered cell signaling pathway similar to the Notch signaling pathway. Indeed, a recent study demonstrates that release of the APP cytosolic tail from the membrane allows an APP-interacting protein called Fe65 to activate transcription (*101*). In the nucleus, these proteins form a complex with Tip90, a histone deacetylase, and activate transcription through heterologous DNA binding domains. Thus, the APP and Notch signaling pathways seem to be as distinct as their interacting proteins (Fe65 and CSL, respectively) are different. However, both signaling pathways may involve transcriptional activation by cytosolic tails released from the membrane by proteolysis. It will be important to determine what regulates APP processing, whether APP, like Notch, is activated by specific ligands, and which

genes are regulated by APP signaling.

Despite the accumulating evidence that presenilins are aspartyl proteases, they actually bear little or no sequence homology to known members of this protease family. The same, though, can be said for a number of other polytopic membrane proteins that are apparently proteases. The site 2 protease (S2P) responsible for processing a transcription factor involved in cholesterol metabolism provides a case in point (*102*). This protein is essential for the final proteolysis of the sterol regulatory element–binding protein within its first transmembrane domain (*103*), allowing this transcription factor to translocate to the nucleus, where it activates the expression of genes needed for cholesterol biosynthesis. S2P has an essential short motif, HEIGH, that resembles the zinc-coordinating active site of metalloproteases (*104*). Otherwise, this multipass membrane protein is not homologous to any known metalloproteases. An entire family of proteins has now been identified, including numerous bacterial members, that have certain signature motifs for polytopic membrane metalloproteases (*105*). Another family of polytopic membrane proteases is the bacterial type 4 prepilin peptidases (TFPP) (*106*). These proteins contain eight TM domains and two completely conserved aspartates essential for protease function (although conservative mutation of one of these aspartates to glutamate retains activity). Although both aspartates of TFPP apparently lie outside of TM regions, an interesting sequence similarity around the critical aspartates has been noted between TFPP and presenilins (*107*). The yeast Rce1 and Ste24 proteases are likewise multi-TM proteins, and they clip prenylated proteins with CAAX termini, although their mechanism of action is unclear (*108*). Thus, a family of polytopic membrane proteases is emerging, and for several members of this family, their mechanisms of action appear remarkably similar to those of soluble and membrane-tethered proteases.

Conclusions

The search for proteases that process APP was inspired by the tremendous medical challenge presented by AD. The recent unmasking of these proteases has not only identified potential targets for drugs to treat this disease, but has also provided important insights into other fields of investigation. These other research fields, including Notch signaling and cholesterol metabolism, likewise have informed AD research. The APP cleaving β- and α-secretases are membrane-anchored versions of known protease families, and it is likely that these membrane-tethered forms and their soluble relatives are derived from common ancestor proteins. The catalytic component of γ-secretase may be the multipass membrane protein presenilin, part of a growing family of polytopic membrane proteases. Although this new family of proteases seems mechanistically similar to the known classes of soluble proteases, by any other criterion they do not even

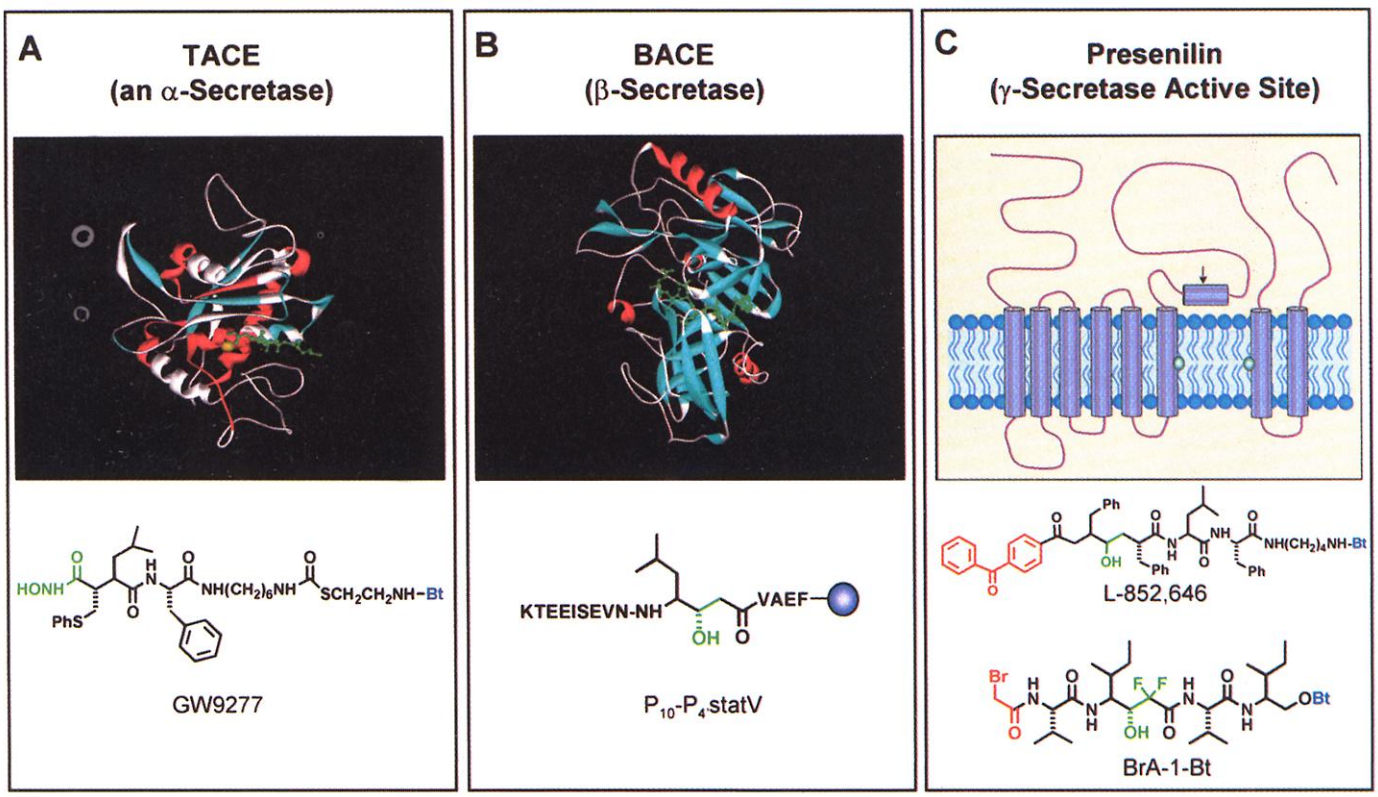

Fig. 2. The structure and identification of Alzheimer secretases. Proteins believed to be responsible for α-, β-, and γ-secretase activity are shown along with the affinity reagents that were used to identify them. (**A**) The cocrystal structure of TACE (red and blue) complexed to an active-site-directed peptidomimetic hydroxamate inhibitor (green) shows this compound coordinating with the catalytic zinc atom (yellow) (*109*). A biotinylated hydroxamate inhibitor (GW9277) used to identify the enzyme (*110*), is depicted below, with the zinc-coordinating hydroxamate group colored green and the biotin tag colored blue. TACE is thought to be among the APP-cleaving α-secretases. (**B**) The cocrystal structure of BACE (red and blue) complexed to a substrate-based transition-state analog inhibitor (green) is shown (*25*). The transition-state mimicking moiety of this inhibitor coordinates with the two active-site aspartates. The transition-state analog inhibitor shown below (P_{10}-P_4statV) was covalently attached to Sepharose beads (blue sphere) to create an affinity column to isolate the enzyme (*13*). The transition-state mimicking moiety is colored green. BACE displays all the properties expected of β-secretase. (**C**) The predicted topology of presenilin is shown with the two putative catalytic aspartates depicted as green spheres. Endoproteolysis within the hydrophobic portion of the large loop (arrow) leads to the formation of a stable PS heterodimer. The structure of two transition-state analog inhibitors used to identify PS as a candidate for the catalytic component of γ-secretase (*89, 90*) are shown below. For both compounds, the transition-state mimicking moiety is green, the reactive cross-linking group is red, and the biotin tag is blue.

qualify as distant cousins. The polytopic membrane proteases may have originated from ancestral membrane proteins that over time acquired residues that could carry out hydrolysis. Such issues, although esoteric, interface with the more pressing practical concern of solving a major human health problem. The portrait of these proteases continues to be a work in progress. Like an Impressionist painting, this portrait displays elements that seem unrelated, but upon stepping back, we see images come into focus with surprising results.

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111. We gratefully acknowledge C. Das for rendering the crystal structures in Fig. 2, and thank D. Selkoe, P. Lansbury, and T. Kimberly for helpful comments on the manuscript. This work was funded by grants from the NIH (M.S.W.) and the Alzheimer's Association (W.P.E.).