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The γ-Secretase Complex: Membrane-Embedded Proteolytic Ensemble

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ABSTRACT: γ-Secretase is responsible for the proteolytic processing of a variety of membrane-associated fragments derived from type I integral membrane proteins, including the amyloid β-protein precursor and the Notch receptor. This enzyme is composed of four different integral membrane proteins: presenilin, nicastrin, Aph-1, and Pen-2. During assembly and maturation of the protease complex, presenilin is endoproteolyzed into two subunits, each of which contributes one aspartate to the active site of an aspartyl protease. Substrate apparently interacts with an initial docking site before passing in whole or in part between the two presenilin subunits to the internal water-containing active site. The ectodomain of nicastrin also interacts with the N-terminus of the substrate as an essential step in substrate recognition and processing. Sites for allosteric regulation on the protease complex allow selective inhibition or modulation of APP processing without interfering with Notch signaling, and such selective agents may represent promising leads for the development of Alzheimer's disease therapeutics. Elucidation of detailed structural features of γ-secretase and other membrane-embedded proteases is the next frontier in understanding how these enzymes carry out hydrolysis within the lipid bilayer.

In the game of baseball, it is often stated as if it were axiomic that pitching is more important that hitting. One player, however, memorably quipped, "Good pitching will beat good hitting every time. And vice versa." In the debate over the importance of basic versus applied research, it can likewise be said that basic biology informs medical science and vice versa. The discovery of the protease complex called γ-secretase provides a perfect illustration of this interplay, in which the hunt for genes and proteins involved in the pathogenesis of Alzheimer's disease dovetailed in a most surprising way with parallel studies of essential signaling pathways in developmental biology. In the process, our understanding of protease biochemistry has been broadened to include a fundamentally new type of membrane-embedded hydrolytic enzyme.

Within the cerebral cortex and limbic system of the Alzheimer brain are found deposits, or plaques, primarily composed of the 4 kDa amyloid β-protein (Aβ)[1] (*1*). This protein is clipped out of a 110−120 kDa type I integral membrane protein called the amyloid β-protein precursor (APP) by the sequential action of two proteases, β- and γ-secretases (Figure 1) (*2*). The discovery of genetic mutations in the APP genes that cause early-onset hereditary Alzheimer's disease strongly suggested that Aβ was a key pathogenic player: these mutations were found within the region encoded by Aβ or immediately adjacent to β- and γ-secretase cleavage sites (*3*). Indeed, these mutations alter either the properties of Aβ or how much and what type of Aβ is produced. Those mutations near the N-terminus of the Aβ region of APP increase the extent of cleavage by β-secretase, resulting in an increased level of Aβ (*4*, *5*). Those near the

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[1] Abbreviations: Aβ, amyloid β-protein; APP, amyloid β-protein precursor; AICD, APP intracellular domain; CTF, C-terminal fragment; DDM, dodecyl β-D-maltoside; EM, electron microscopy; NSAID, nonsteroidal anti-inflammatory drug; NTF, N-terminal fragment; PS1, presenilin-1; PS2, presenilin-2; TMD, transmembrane domain.

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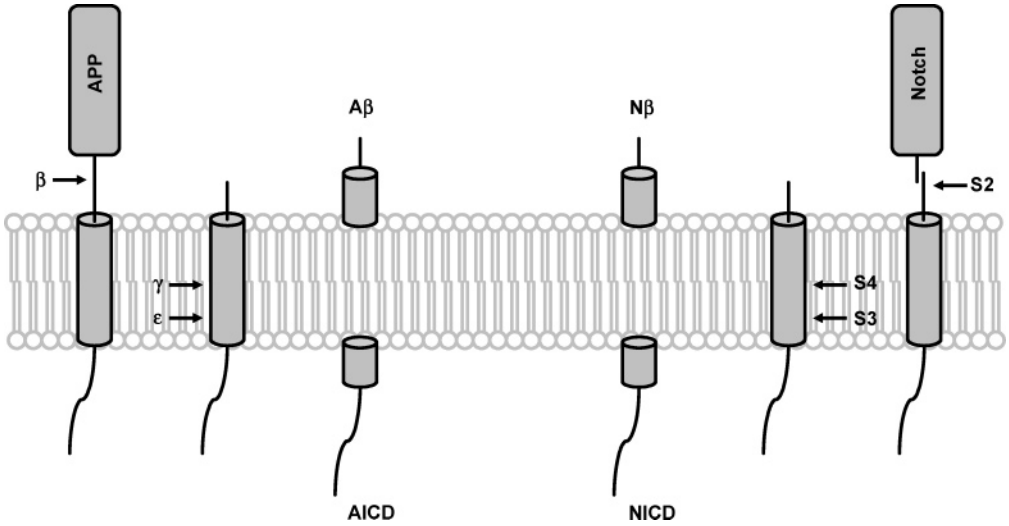

FIGURE 1: Proteolytic processing of APP and Notch. The ectodomain of APP is first shed by β-secretase. Alternatively, APP can be cut within the Aβ region by α-secretases (not shown). The remaining membrane-associated stub is cleaved at least twice in the transmembrane region, at the γ site to produce Aβ and at the ϵ site to produce the intracellular domain (AICD). The Notch receptor is first processed in the secretory pathway at the S1 site by furin (not shown) to produce a heterodimer. Upon activation by ligand, this heterodimer is first cleaved at the S2 site by metalloproteases to shed the ectodomain, and the remaining membrane-associated stub is cleaved within the transmembrane domain at the S3 and S4 sites. The transmembrane cleavage events are carried out by the presenilin-containing γ-secretase complex.

γ-secretase site, however, have a more subtle effect, changing the proportion of 40- to 42-residue forms of Aβ (*6*). Although the latter is a minor Aβ variant, it is highly prone to aggregation and represents the majority of Aβ found in Alzheimer plaques (*7*, *8*).

Another major clue to Alzheimer pathogenesis came with the discovery of two related genes, presenilin-1 (PS1) and presenilin-2 (PS2), likewise associated with early-onset disease (*9*−*11*). The presenilin genes encode ∼50 kDa multipass membrane proteins. At the time, the only gene that is even distantly homologous to those of the presenilins was found in worms and only known to play a role in spermatogenesis (*12*). The connection to Alzheimer's disease could not have been more obscure. Nevertheless, it became clear that the Alzheimer mutations in the presenilins alter Aβ production (*13*−*16*). More than 100 such missense mutations have been identified so far, and the vast majority of those examined in detail skew the proportion of Aβ toward the more aggregation-prone 42-residue form (*17*). Thus, the presenilin mutations change the cleavage site specificity of γ-secretase. The subsequent discovery that knockout of presenilin-1 dramatically reduced the level of γ-secretase cleavage of APP suggested that presenilin mediates this transmembrane proteolytic event (*18*).

Concurrently, the Notch signaling pathway in developmental biology was being deciphered. The Notch family members are type I integral membrane proteins, and activation of these receptors is essential for critical cell differentiation steps in all metazoans, both during embryogenesis and in adulthood (*19*). On the heels of the discovery of presenilins as Alzheimer genes came the identification of a close homologue in *Caenorhabditis elegans* that facilitates Notch signaling (*20*), and it soon became clear that proteolysis of Notch was essential for signaling from this receptor. Upon contact with its cognate ligand, the Notch receptor undergoes ectodomain shedding followed by proteolysis of the membrane-associated stub within its transmembrane region (*21*). The released intracellular domain then translocates to the nucleus, interacting with certain transcription factors and coactivators to regulate gene expression critical for determining cell fate. Intriguingly, knockout of presenilin-1 in mice resulted not in neurodegeneration but in embryonic lethality and a phenotype remarkably similar to that seen upon knockout of Notch1 (*22*, *23*). Culturing of cells from these knockout embryos revealed that presenilin is not only needed for the γ-secretase cleavage of APP, but also for proteolysis of the Notch transmembrane domain (*24*). Still, the biochemical function of presenilin remained unknown, as did the identity of γ-secretase.

Presenilin: Membrane-Embedded Aspartyl Protease

Membrane topology experiments on presenilin gave some conflicting results, with suggestions of six, seven, or eight transmembrane domains, and with the N-terminus being either lumenal/extracellular or cytosolic (*25*). However, the strongest evidence originally favored eight transmembranes, with the N-terminus, large cytosolic loop, and C-terminus all on the cytosolic side (*26*−*28*). Most recently, this topology has been updated and refined, with nine-transmembrane domains (*29*, *30*) and the C-terminus on the lumenal/extracellular side but folded into the protein or its partners (see below). A biochemical clue about the function of presenilin came with the observation that the protein is endoproteolyzed into two pieces (Figure 2), an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that remain associated, have a long biological half-life together, and are tightly regulated by limiting cellular factors (*31*−*33*). These findings suggested that the NTF−CTF heterodimer is the mature, active form of presenilin, whatever its biochemical function might be. Meanwhile, the design of substrate-based peptidomimetic inhibitors for γ-secretase suggested that the enzyme is an aspartyl protease: hydroxyl-containing transition-state mimics could block APP processing at the γ-secretase level in cells (*34*, *35*).

Given evidence that γ-secretase is an aspartyl protease and that presenilin is critical for γ-secretase activity, presenilin

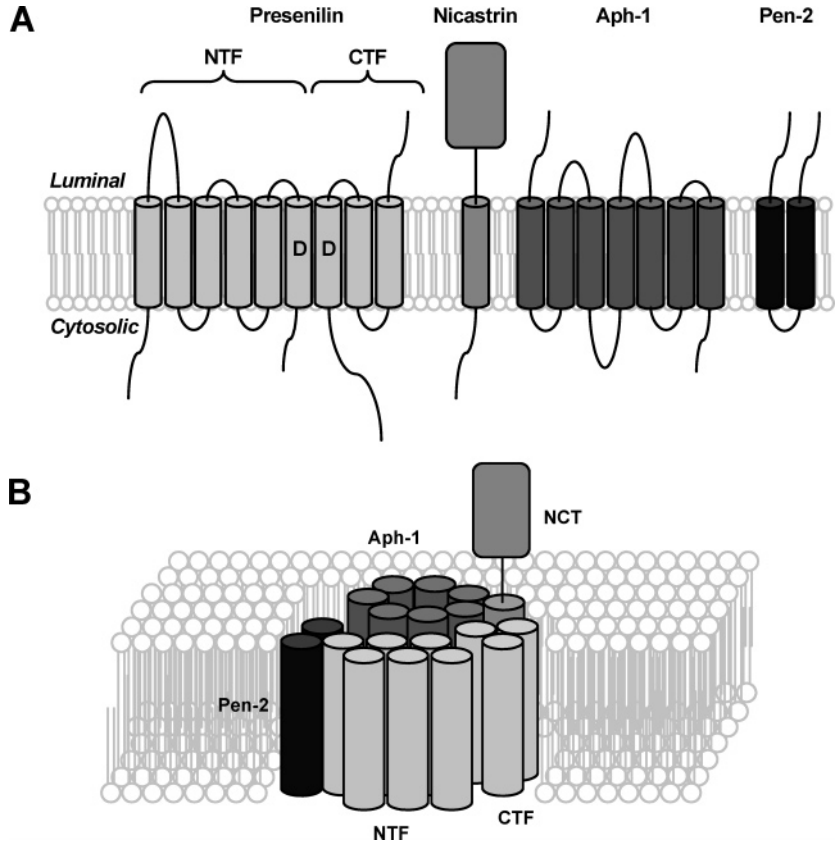

FIGURE 2: Components and assembly of the γ-secretase complex. (A) γ-Secretase is composed of four different integral membrane proteins: presenilin, nicastrin, Aph-1, and Pen-2. Presenilin undergoes endoproteolysis into an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that remain associated. Two conserved aspartates within adjacent transmembrane domains are essential for both presenilin endoproteolysis and γ-secretase activity. (B) Model for how the components of γ-secretase are arranged within the active protease complex [modified from Fraering et al. (*76*)].

was examined for the presence of two aspartates that might be part of a novel protease. Two completely conserved transmembrane aspartates, one in the NTF domain and one in the CTF domain, were identified and found to be independently essential for both presenilin endoproteolysis and γ-secretase cleavage of APP (*36*, *37*). This discovery suggested that presenilin was indeed a novel aspartyl protease, and one activated by autoproteolysis. Upon maturation into NTF and CTF, the active site of γ-secretase was proposed to lie at the interface between these two subunits, each of which contributed one of the catalytic aspartates. Further validation for this hypothesis came from affinity reagents designed from transition-state analogue γ-secretase inhibitors, which directly bound to presenilin NTF and CTF (*38*, *39*). These affinity labeling reagents, directed to the γ-secretase active site, tagged heterodimeric presenilin, identifying this interface as the protease catalytic site.

Presenilin alone, however, was clearly not the whole of γ-secretase. Consistent with the need for limiting cellular factors to gate presenilin endoproteolysis, the protein and its fragments were found to enter into high-molecular weight complexes (*40*, *41*). Moreover, presenilin alone did not cleave itself, and a mutant presenilin that does not require endoproteolysis to support γ-secretase activity in cells did not display proteolytic activity on its own either (i.e., limiting cellular factors were necessary). However, key support for presenilin as a protease came with the discovery of signal peptide peptidase (SPP). This enzyme, which clips remnant signal peptides in the membrane after their release by signal peptidase, was discovered using affinity labeling with a transition-state analogue inhibitor. The responsible protein was clearly similar to presenilin, including two highly conserved transmembrane motifs, each containing an aspartate (*42*). Unlike presenilin, SPP apparently supported proteolytic activity on its own, without the need for limiting cellular factors or endoproteolysis. Thus, if a presenilin-like protein such as SPP is a protease, presenilin itself is most likely the catalytic component of γ-secretase. Nevertheless, in addition to its role in γ-secretase, presenilin has been shown to have important functions independent of γ-secretase activity (e.g., refs *43*−*45*).

Given the proteolytic function of presenilin, the pathogenic effects of the Alzheimer-associated missense mutations are thought to be due to the alteration of Aβ production, especially with mutations in the APP gene itself likewise causing the disease. The disease-associated presenilin mutations tend to inhibit overall proteolytic function (*46*), even producing *less* Aβ in some cases, while increasing the ratio of 42- to 40-residue Aβ (*47*). This increased proportion of the more aggregation-prone Aβ42 is thought to initiate the disease process. However, γ-secretase also cuts further along the transmembrane domain, at the so-called ϵ site (*48*), in producing the APP intracellular domain (AICD) (Figure 1), and Alzheimer-associated presenilin mutations modulate the position of this ϵ cleavage as well (*49*). The possibility remains that alteration of the N-terminus of the released intracellular domains, especially those involved in cell signaling, could contribute to the disease process.

Partners for Presenilin

With presenilin identified as the catalytic component, the search to identify other members of what would become known as the γ-secretase complex had begun. The first to be discovered was nicastrin, a presenilin-interacting protein found by co-isolation upon immunoaffinity purification (*50*). Nicastrin is a highly glycosylated 120−140 kDa type I integral membrane protein, with almost the entire protein being lumenal/extracellular (Figure 2A). RNAi knockdown experiments demonstrated that nicastrin was essential for the γ-secretase cleavage of both APP and Notch (*50*). Nicastrin was also shown to be required for presenilin endoproteolysis, suggesting that it was at least one of the "limiting cellular factors" gating presenilin subunit formation (*50*). Consistent with these observations, isolation of γ-secretase using an immobilized transition-state analogue inhibitor resulted in copurification of nicastrin, suggesting that nicastrin was indeed a bona vide member of the γ-secretase complex (*51*). Nicastrin is found primarily as two bands by SDS−PAGE, the upper band being composed of a more highly glycosylated form that is especially associated with presenilin NTF and CTF subunits and active γ-secretase (*52*−*56*). As with presenilin, knockout of nicastrin in different species results in lethal phenotypes resembling those seen with Notch deficiencies (*57*, *58*).

However, overexpression of presenilin and nicastrin still did not result in increased presenilin endoproteolysis or γ-secretase activity, the implication being that other associated proteins were yet to be discovered. Genetic studies in *C. elegans* to identify new Notch modifiers revealed two novel genes, Aph-1 and Pen-2, which encode proteins of seven and two predicted transmembrane domains, respectively (Figure 2A) (*59*, *104*). RNAi knockdown of these genes, as with nicastrin and presenilin, blocked γ-secretase cleavage of APP and Notch. Follow-up studies demonstrated that overexpression of all four proteins together (presenilin, nicastrin, Aph-1, and Pen-2) resulted in increased levels of presenilin NTF and CTF, mature nicastrin, and γ-secretase activity (*60*, *61*). One study even showed this to be true in *Saccharomyces cerevisiae* (*62*), the genome of which does not encode for any homologues of these four proteins. Expression of all four proteins is required for these effects. In addition, co-immunoprecipitation of any one of the five proteins (PS1 NTF and CTF being separately examined) brought down all the others, indicating that they interact with each other. Partial purification of γ-secretase through several steps resulted in isolation of all five proteins (*61*). Several groups have now reported purification to near homogeneity (*63*−*65*), providing definitive proof that these proteins form a single complex and are the essential components of γ-secretase. Purification has so far allowed low-resolution structural elucidation by electron microscopy (EM) coupled to single-particle image analysis, which suggested that the complex contains a cylindrical 20−40 Å cavity and 20 Å pores at the top and bottom that might serve as exit ports for products (*66*).

Despite full identification, purification, and determination of a low-resolution structure, the stoichiometry of the protease complex remains unclear. Particularly vexing has been the issue of whether the complex contains two presenilin molecules at its catalytic core. Although some evidence supports this idea (*67*−*69*), including the finding that the presenilin homologue SPP forms a SDS-stable dimer (*70*), confirmation from other laboratories has not been forthcoming, and even the EM structure cannot discern this clearly. Another complication is that six variants of the γ-secretase complex apparently exist, due to different combinations of the two presenilins and three different Aph-1 proteins. Perhaps these different complexes have different affinities for the various γ-secretase substrates, which besides APP and Notch1 also include APP-like proteins APLP-1 and -2, Notch2−4, ErbB4, E- and N-cadherins, and the CD44 receptor (*71*). Biochemical differences between these complexes no doubt exist; indeed, PS2-containing complexes display lower proteolytic activity than PS1-containing complexes (*72*).

Assembly of the γ-secretase complex begins in the endoplasmic reticulum soon after translation and membrane insertion. Nicastrin and Aph-1 assemble into a subcomplex, with nicastrin remaining in an immature, hypoglycosylated form (*73*). Presenilin and Pen-2 are added afterward. Whether presenilin and Pen-2 interact with each other first before assembly with the nicastrin−Aph-1 subcomplex is unclear, but knockdown of Pen-2 does lead to a nicastrin−Aph-1−presenilin subcomplex in which presenilin remains a holoprotein (*60*, *74*, *75*). The addition of Pen-2 leads to presenilin NTF−CTF formation, maturation of nicastrin, and active γ-secretase. Partial dissociation of the γ-secretase complex using the nonionic detergent dodecyl β-D-maltoside (DDM) followed by two-dimensional PAGE analysis revealed how the γ-secretase components are arranged in the active protease complex (*76*). As expected, nicastrin interacts with Aph-1; however, PS1 NTF was found to interact with Pen-2 as well as with PS1 CTF, and nicastrin and Aph-1 together can also interact with PS1 CTF. Other studies have confirmed the PS1 NTF−Pen-2 interaction and pinpointed transmembrane domain 4 of PS1 as the site of contact with Pen-2 (*77*, *78*). These findings have led to the model for the γ-secretase complex shown in Figure 2B.

The transmembrane domain (TMD) of nicastrin is required for complex assembly. Swapping in a different TMD prevents incorporation of nicastrin, with the N-terminal region of the TMD being especially important (*79*). The function, if any, of the very short cytosolic tail of nicastrin is unclear, but the large ectodomain has been elegantly and rigorously shown to play an essential role in substrate recognition (see below) (*64*). The specific biochemical role of the small hairpin Pen-2 protein is unknown, but the seven-transmembrane Aph-1 is thought to be a scaffolding protein for the rest of the complex (*60*). The reported topologies of Aph-1 and Pen-2 (*80*, *81*) are as shown in Figure 2. As mentioned above, the topology of presenilin has recently been reinvestigated and shown to contain nine transmembrane domains (*29*). The final three transmembrane segments had been

the difficult ones to confirm, one reason apparently being their interdependence. TMD7 is a rather short hydrophobic domain, with one of the conserved aspartates in the middle. Incorporation of this TMD has been shown to require TMD8 (*30*). This concept of more hydrophobic regions pulling in less hydrophobic regions is emerging as a theme in membrane protein insertion and folding (*82*). Another study suggested that presenilin forms a ringlike structure (*83*), and

this notion has received recent support. First, mutagenesis showed that certain residues in TMD1 are critical for γ-secretase activity (*84*), and second, a cysteine in TMD1 of the PS1 NTF can be chemically cross-linked to either of two cysteines found in TMD8 of the PS1 CTF (*85*). Most of the large lumenal/extracellular loop between TMD6 and TMD7, a region that is poorly conserved, is not essential for presenilin's proteolytic function (*86*). However, a highly conserved hydrophobic region in this loop includes the site of presenilin endoproteolysis (*87*) and harbors a domain (including Tyr-288) that is critical for overproduction of Aβ42 (*88*). The C-terminus of presenilin is also essential for function (*89*). This region has been shown to contain an ER-retention sequence; once assembled with other γ-secretase members, this region apparently becomes folded into the complex, allowing transport to the Golgi and beyond (*90*). Evidence specifically suggests the presenilin C-terminus may interact with the nicastrin transmembrane domain (*90*).

Substrate Recognition

Among the more intriguing questions about the entire emerging family of intramembrane-cleaving proteases is how they handle substrates and cleave their TMDs in at least two locations (γ and ϵ; see Figure 1). Because it presumably contains water and uses hydrophilic residues, the membrane-embedded active site should be sequestered from the hydrophobic environment of the surrounding lipid tails. Thus, the active site might be envisioned to be part of a pore or channel that could allow the entry of water (*37*). However, the substrate passes through the membrane and cannot enter such a pore or channel directly; docking on the outer surface of the protease, with lateral gating to bring the substrate into the internal active site, might be required (*37*). Initial evidence for such a mechanism came from isolation of the γ-secretase complex with an immobilized transition-state analogue inhibitor (*51*). Detergent-solubilized membranes from human HeLa cells were passed through this affinity matrix, resulting in copurification of γ-secretase complex members and an endogenous membrane-bound APP stub found in HeLa cells. This stub results from alternative processing of APP by α-secretases, and like the stub produced by β-secretase, it is also a γ-secretase substrate. Thus, an endogenous substrate was copurified with the γ-secretase complex, while the protease active site was blocked by the immobilized transition-state analogue inhibitor, suggesting the existence of a separate substrate binding site. Substrate bound to this special type of exosite, dubbed the "docking site", could be copurified without being subject to proteolysis.

Designed peptides based on the transmembrane domain of APP and constrained in a helical conformation can potently inhibit γ-secretase, apparently by interacting with this docking site (*91*). Conversion of these helical peptide inhibitors into affinity labeling reagents led to the localization of the substrate docking site to the presenilin NTF−CTF interface (*92*). Transition-state analogue inhibitors also bind directly to the NTF−CTF interface, but at a site distinct from that of helical peptide inhibitors. These findings suggest a pathway for γ-secretase substrate from the docking site to active site: upon binding to the outer surface of presenilin at the NTF−CTF interface, the substrate can pass, either in whole or in part, between these two presenilin subunits to access the internal active site (Figure 3). Interestingly, extension of a 10-residue helical peptide inhibitor by just three additional residues resulted in a potent inhibitor (*93*) apparently capable of binding both the docking site and the active site (*92*), suggesting that these two substrate binding sites are relatively close to each other.

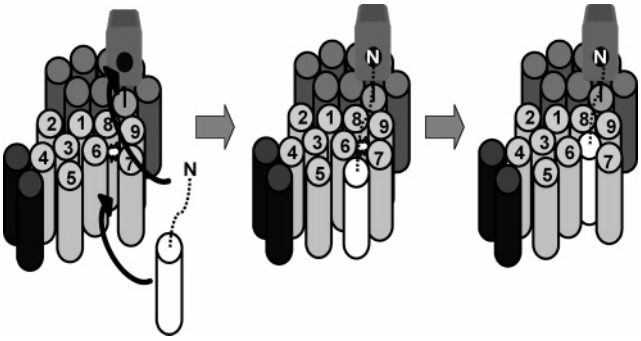

FIGURE 3: Model for the γ-secretase complex and its interaction with substrate. The transmembrane region of the substrate initially docks at the presenilin NTF−CTF interface, while the N-terminus of the substrate interacts with the nicastrin ectodomain. The substrate, either in whole (as depicted) or in part, then accesses the internal active site, which contains water and two aspartates. Interaction of the substrate N-terminus with the nicastrin ectodomain may facilitate binding of the substrate transmembrane domain to the docking site and/or movement into the active site. The γ-secretase complex is drawn to take into account the following: (1) dissociation into partial complexes using the detergent DDM, (2) the initial formation of nicastrin and Aph-1 as a subcomplex, (3) the interaction of the C-terminus of presenilin with the nicastrin TMD, (4) the interaction between Pen-2 and the presenilin TMD4, and (5) the proximity between presenilin TMD1 and TMD8.

Until recently, all the action seemed to be taking place on presenilin. However, an elegant study has demonstrated that nicastrin also plays a critical role in substrate recognition (*64*). The ectodomain of nicastrin bears a resemblance in sequence to aminopeptidases, although certain catalytic residues are not conserved. Nevertheless, nicastrin recognizes the N-terminus of γ-secretase substrates derived from APP and Notch, and mutation of the aminopeptidase domain prevents this interaction. One conserved glutamate is especially important, probably because this residue forms an ion pair with the amino terminus of the substrate. The sequence of the substrate N-terminus is apparently not critical for the interaction, but a free amino group is. Indeed, simple formylation of the substrate N-terminus is enough to prevent effective substrate interaction and proteolytic processing. Thus, nicastrin can be considered a kind of gatekeeper for the γ-secretase complex: type I membrane proteins that have not shed their ectodomains cannot interact properly with nicastrin and do not gain access to the active site (Figure 3).

Allosteric Modulation

Although γ-secretase has in many ways been an attractive target for Alzheimer's disease therapeutics, interference with Notch processing and signaling may lead to toxicities that preclude clinical use of inhibitors of this protease. Long-term treatment with γ-secretase inhibitors causes severe gastrointestinal toxicity and interferes with the maturation of B- and T-lympocytes in mice, effects that are indeed due to inhibition of Notch processing and signaling (*94*, *95*). However, compounds that can modulate the enzyme to alter or block Aβ production with little or no effect on Notch

would bypass this potential roadblock to therapeutics. Recent studies suggest that the protease complex contains allosteric binding sites that can alter substrate selectivity and the sites of substrate proteolysis. Certain nonsteroidal anti-inflammatory drugs (NSAIDs, e.g., ibuprofen, indomethacin, and sulindac sulfide) can reduce the production of the highly aggregation prone Aβ42 peptide and increase the level of a 38-residue form of Aβ, a pharmacological property independent of inhibition of cyclooxygenase (*96*). The alteration of the proteolytic cleavage site is observed with isolated or purified γ-secretase (*63*, *97*), indicating that the compounds can interact directly with the protease complex to exert these effects. Enzyme kinetic studies and displacement experiments suggest the selective NSAIDs can be noncompetitive with respective to APP substrate and to a transition-state analogue inhibitor, suggesting interaction with a site distinct from the active site (*98*). The site of cleavage within the Notch transmembrane domain is similarly affected, but this subtle change does not inhibit the release of the intracellular domain and thus does not affect Notch signaling (*99*). For this reason, these agents may be safer as Alzheimer's disease therapeutics than inhibitors that block the active site or the docking site. Indeed, one compound, (*R*)-flurbiprofen, has recently advanced to Phase III clinical trials. Surprisingly, the site of proteolytic cleavage by SPP can also be modulated by the same NSAIDs that affect γ-secretase, suggesting that presenilin is the site of NSAID binding within the γ-secretase complex and that SPP and presenilin share a conserved drug binding site for allosteric modulation of substrate cleavage sites (*100*).

Another type of allosteric modulator includes compounds that resemble kinase inhibitors and interact with a nucleotide binding site on the γ-secretase complex. The discovery that ATP can increase Aβ production in membrane preparations prompted the testing of a variety of compounds that interact with ATP binding sites on other proteins (*101*). In this focused screen, the Abl kinase inhibitor Gleevec emerged as a selective inhibitor of Aβ production in cells without affecting the proteolysis of Notch. In light of these findings, ATP and other nucleotides were tested for effects on purified γ-secretase preparations and found to selectively enhance the proteolytic processing of a purified recombinant APP-based substrate without affecting the proteolysis of a Notch counterpart (*102*). Furthermore, certain compounds known to interact with ATP binding sites were found to selectively inhibit APP processing vis-à-vis Notch in purified protease preparations. The γ-secretase complex could be pulled down with beads containing immobilized ATP, and the presenilin-1 CTF was specifically photolabeled by 8-azido-ATP. This labeling was not blocked by a transition-state analogue inhibitor or by the recombinant APP- and Notch-based substrates; however, the APP-selective inhibitors could prevent photolabeling by 8-azido-ATP. Taken together, these results suggest that the γ-secretase complex contains a nucleotide binding site, to which the presenilin-1 CTF is at least a contributor, and that this site allows allosteric regulation of γ-secretase processing of APP with respect to Notch. Whether this regulation is physiologically important is unclear, but the pharmacological relevance is profound and may lead to new therapeutic candidates for Alzheimer's disease. This hope is tempered by the fact that γ-secretase cleaves numerous other type I membrane protein stubs that result from ectodomain shedding. Agents selective for APP versus Notch may reveal new long-term toxicities due to blocking proteolysis of these other substrates, toxicities masked by the severe Notch-related effects with nonselective inhibitors.

Perspective

γ-Secretase is a founding member of a new class of membrane-embedded proteases that process the transmembrane domains of their substrates (*103*). These enzymes also include (1) the site 2 protease (S2P) family, putative metalloproteases responsible for cholesterol and fatty acid biosynthesis in metazoans and mating factor signaling in bacteria, (2) the rhomboid family, serine proteases involved in growth factor signaling, mitochondrial matrix remodeling, and parasite invasion, and (3) the SPP family of presenilin homologues, exemplified by signal peptide peptidase, which processes remnant signal peptides produced by signal peptidase and plays a role in immune surveillance and maturation of certain hepatitis C core proteins. Discovery of membrane proteins responsible for these proteolytic activities has led to some degree of understanding of their mechanisms and how these proteases interact with substrates. However, intimate understanding of enzymatic mechanisms, including direct evidence for the involvement of the putative catalytic residues, will likely require detailed structural information. Indeed, this is considered by many to be the next major goal in this fascinating field of investigation. No doubt high-resolution structures will provide mechanistic insight vis-à-vis previous biochemical studies. And vice versa.

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