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γ-Secretase

Substrates and Inhibitors

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Abstract

The amyloid β-protein (Aβ) deposited in Alzheimer's disease (AD), the most common form of dementia in the elderly, is a secreted proteolytic product of the amyloid β-protein precursor (APP). Generation of Aβ from the APP requires two sequential proteolytic events, β-secretase cleavage to generate the amino terminus, followed by γ-secretase cleavage to generate the carboxyl terminus. Because this process is a central event in the pathogenesis of AD, γ-secretase is believed to be an excellent therapeutic target. γ-Secretase activity has been demonstrated to be membrane-associated, with the cleavage site primarily determined by the location of the substrate with respect to the membrane. It has also been shown that this unusual proteolytic activity not only occurs for APP, but also for proteins involved in morphogenic processes or cell proliferation and differentiation such as Notch and ErbB4. Thus far, all γ-secretase substrates are involved in some form of nuclear signaling. These recent findings have important implications for the development of pharmacological interventions that target γ-secretase.

Index Entries: Alzheimer's disease; γ-secretase; presenilin; amyloid β-peptide; amyloid β-protein precursor; Notch; ErbB4;γ-secretase inhibitors.

Introduction

Alzheimer's disease (AD) is the major neurodegenerative disorder affecting the elderly, and, as the population of the developed world ages, is becoming an ever greater social and economic burden. As the disease progresses, patients commonly show a cumulative loss of memory as well as other behavioral alterations including paranoia, delusions, and a decline in language function. The most common form of AD is "sporadic," with no clear etiology, and occurs late in life. Much less common are cases of inherited, autosomal dominant familial AD (FAD), which almost invariably occur at a younger age. Apart from the age of onset, the neuropathology and clinical course of AD and

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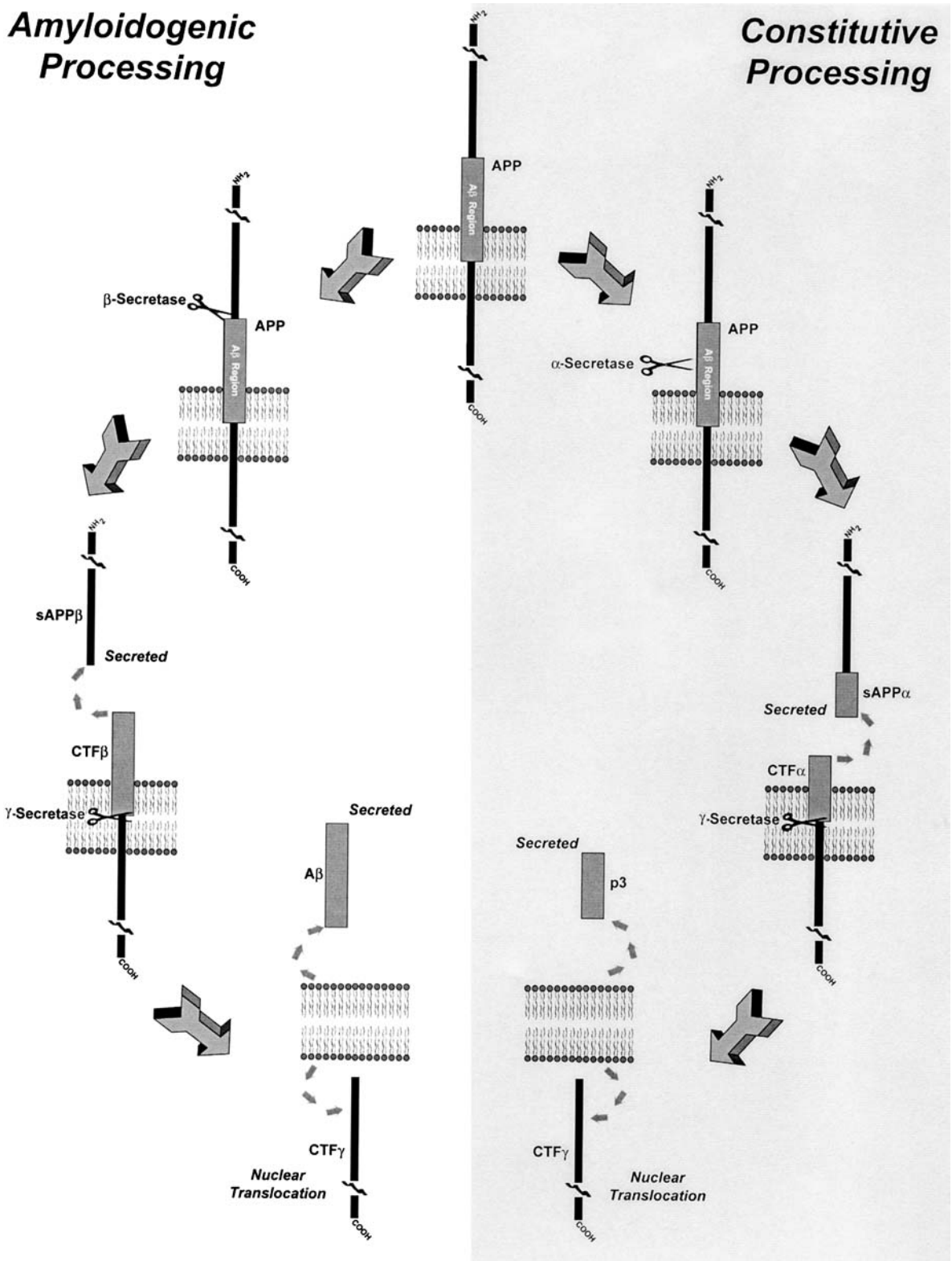
Amyloidogenic Processing
Constitutive Processing
APP
Aβ Region
β-Secretase
α-Secretase
sAPPβ
Secreted
sAPPα
CTFβ
CTFα
γ-Secretase
Aβ
p3
CTFγ
Nuclear Translocation
COOH
NH2

Fig. 1. Amyloid β-Protein Precursor Processing. APP is processed by two distinct pathways. The left pathway, also called amyloidogenic processing, starts with the β-secretase (BACE) cleavage of APP to release sAPPβ, leaving behind a membrane associated carboxyl terminal fragment (CTF) of 99 amino acids (CTFβ) which is then cleaved by γ-secretase; this cleavage leads to Aβ secretion. The right pathway (constitutive processing) starts with the α-secretase (TACE) cleavage of APP, causing the release of sAPPα. The 83 amino acid CTFα remains associated with the membrane and, like CTFβ, is later cleaved by γ-secretase. In this case, the peptide secreted is a smaller, ~3 kDa fragment called p3. Constitutive processing is ~20 fold more active than the amyloidogenic pathway. In both cases, the resulting CTFγ (also known as the AICD; see text) translocates to the nucleus where it may function as a transcriptional coactivator. Adapted with permission from ref. *(113).*

FAD are, with rare exception, essentially identical. For this reason, the study of the genes mutated in FAD have been highly informative in elucidating the molecular basis of the disease process *(1,2).*

A defining neuropathology of AD are the extracellular deposits of amyloid that occur principally in a filamentous, compact form, usually referred to as plaques. The principal protein deposited in plaques is the amyloid β-peptide (Aβ) *(3),* although many additional components have also been identified *(4).* In addition, neurofibrillary tangles composed of hyperphosphorylated tau protein can also be observed intracellularly. These two classical lesions can occur independent of each other, and develop in characteristic patterns within the afflicted brain *(5).* The matter by which these lesions precedes the other has been a subject of (often heated) debate within the AD research community since the disease was first described. In recent years, what has become known as the "amyloid cascade hypothesis" has become dominant *(1,6).* Indeed, it has been the improved understanding of the molecular pathogenesis and genetics of AD that have lead to its widespread acceptance.

Aβ is a secreted, proteolytic product derived from the amyloid β protein precursor (APP) *(2,7).* In fact, numerous different Aβ species exist; Aβ1–40 (Aβ40) is the major peptide produced (~90%), followed by Aβ42 (~5–10%). It is this latter form that is the more fibrillogenic and is the initial species deposited in the AD brain. β-secretase first cleaves APP to release a large secreted derivative, sAPPβ *(8).* This process also results in the generation of a membrane-bound C-terminal fragment, CTFβ (Fig. 1); this fragment is a γ-secretase substrate and the direct precursor of Aβ.

Beginning in the early 1990s, APP mutations were identified in several FAD families that flanked either the β-*(9)* or γ-secretase cleavage sites within APP *(10–16).* These mutations were later shown to increase the amount of total Aβ *(17),* the proportion of the more amyloidogenic Aβ42 *(12,18),* or affect the rate of amyloid protofibril formation *(19).* Overexpression of mutant APPs in the brains of transgenic mice leads to Aβ deposition and plaque formation *(20,21).* As predicted by the amyloid cascade hypothesis, the production of Aβ in these mice accelerates the formation of tangle pathology and neuronal loss when they are crossed with mice that also overexpress a mutant form of the human tau protein *(22).* Similarly, direct brain injection of Aβ42 in another strain of tau mutant mouse accelerates the development of neurofibrillary pathology *(23).* Together, these data place Aβ production upstream of the development of neurofibrillary tangles, and strongly support its role as the major causative factor in AD pathogenesis. For these reasons, the enzymatic activities responsible for Aβ production, β- and γ-secretase (Fig. 1), are considered to be prime targets for the development of pharmacological interventions in the disease process.

β- and γ-Secretase: Targets for Therapy?

The first enzyme in Aβ generation, β-secretase (or BACE1) is a membrane-bound aspartyl protease *(24).* BACE1 is ubiquitously expressed,

including at high levels in the brain. BACE1 overexpression causes all of the expected β-secretase cleavage products (including Aβ secretion) to increase, and its inhibition causes Aβ levels to decrease *(24).* Studies performed in BACE1 knockout mice showed significantly reduced levels of Aβ and CTFβ in brain, also arguing in favor of BACE1 being the entity responsible for β-secretase activity *(25,26).* Importantly, BACE1 knockout mice appear to be perfectly healthy, arguing strongly in favor of BACE1 as a therapeutic target *(26,27).*

γ-Secretase is the enzymatic activity responsible for the generation of the carboxyl terminus of the Aβ peptide. γ-Secretase is part of a high molecular-weight complex containing presenilin 1 and/or 2 (PS1 or PS2; PSs), Nicastrin and, possibly, β-catenin *(28,29).* There is now a large body of evidence supporting the PSs as the likely active site of the enzyme. (This is a complex issue, and well beyond the scope of this review; *see (30–33.)* Presenilin 1 and presenilin 2 are polytopic membrane proteins encoded on chromosomes 14 and 1, respectively *(34–36).* Mutations in the PS genes are responsible for most cases of early-onset FAD, with the mechanism being via a selective increase in the production of Aβ42 *(37).* Also, PS1 knockout *(38)* or the expression of dominant-negative forms of PS1 *(39–41)* reduce γ-secretase activity by approx 80%. Further, the combined knockout of PS1+PS2 results in no γ-secretase activity *(42,43)* (however, *see* ref. *[44]).* These results therefore suggest that targeting PSs/γ-secretase might be another viable strategy for treating AD. However, in contrast with BACE1 deficient mice, knockouts of PS1 or PS1+PS2 are lethal, producing a phenotype essentially identical to Notch deficiency *(45–48).*

γ-Secretase Substrates

The finding that the knockout of PS1/γ-secretase activity and embryonic lethality go hand in hand suggests by itself that γ-secretase might not be an easy target for the development of an AD therapy. Although the lethal effects of PS1 knockout are mediated through deficits in Notch cleavage, other likely γ-secretase substrates are emerging. Presenilin has numerous binding partners *(49).* Some proteins, such as Ire1 and ATF6, which were once considered to be possible γ-secretase substrates *(50–52),* may have been excluded by more recent studies *(53,54).* However, others, such as E-cadherin *(55,56),* remain candidates. It is clear that with each new substrate discovered, the spectrum of possible deleterious side effects from γ-secretase inhibitor therapy widens. Although the nature of these side effects are speculative at present, it is known that inhibition of Notch signaling in a mature organism may result in immune system dysfunction due to faulty haematopoiesis *(57,58).*

Amyloid β-Protein Precursor (APP)

As outlined earlier, APP is the most important substrate of γ-secretase purely from the perspective of the development of an effective AD therapy. APP is a ubiquitously expressed type 1 membrane protein of ~120–140 kDa, with the heterogeneity arising from alternatively spliced mRNA transcripts, the major forms of which are 695, 751, and 770 amino acids in length *(59,60).* APP is post-translationally modified by sulfonation, phosphorylation, and through the addition of N- and O-linked sugars *(61).* The APP holoprotein is constitutively cleaved at or near the cell surface by the metalloproteases ADAM10 or ADAM17 *(62,63).* The latter is usually referred to as tumor necrosis factor α converting enzyme (TACE), and is primarily involved in the regulated, protein kinase C (PKC)-dependent α-secretase activity *(63,64).* Alternatively, full-length APP can be cleaved by the aspartyl protease BACE *(24).* In both cases, a membrane-bound carboxyl terminal fragment (CTF) remains (Fig. 1).

After more than 15 years of study, it is remarkable that there is still no complete consensus as to the function of APP. The APP has been hypothesized to act as an autocrine or

neuroprotective factor, or possibly to play a role in cell contact or adhesion *(65–67).* APP deletion in mice shows a lack of vital consequences; however, combined knockout of APP with the related protein APLP2 is lethal, indicating that there is some redundancy of function between the two *(68).* Recent reports have hinted at another function, possibly involving signal transduction. γ-Secretase cleavage of the APP CTFs C99 and C83 (also referred to as CTFβ and CTFα) eventually leads to the secretion of Aβ or p3, respectively, but also liberates a cytoplasmic fragment, called CTFγ or the APP intracellular domain (AICD, Fig. 1 *[69,70]).* The AICD, much like the Notch intracellular domain (NICD; *see* below), translocates to the nucleus after γ-secretase cleavage, where it may regulate gene transcription, possibly through binding with Fe65 *(71–74).* Although the precise events mediated by AICD in the nucleus are not clear at present, recent work suggests that a downstream consequence may be alterations in cellular calcium homeostasis *(75).*

Notch

Notch is a ~300 kDa type 1 transmembrane domain (TMD) protein with a large extracellular domain containing 36 tandemly repeated epidermal growth factor (EGF)-like domains that mediate ligand binding *(76).* Notch is cleaved in the Golgi by furin or a furin-like convertase and the resulting two fragments remain associated at the cell surface as a heterodimer *(77).* After binding of one of the DSL family of ligands *(D*elta, *S*errate/Jagged or *L*ag-2), Notch is cleaved by TACE to release the extracellular domain and produce a membrane bound CTF, also known as NEXT (for *N*otch *ex*tracellular *t*runcation) *(78,79).*

Notch signaling is vital for proper development of both vertebrates and invertebrates *(80),* and it is deficient Notch processing that is thought to be responsible for the lethal effects of PS1 knockout in mice *(45).* Central to Notch signaling is γ-secretase processing of the NEXT fragment to produce the NICD *(79,81),* which then translocates to the nucleus and forms an active transcription factor in association with members of the CSL family of proteins *(C*BF-1, *S*u(H) and *L*ag-1) *(82).*

ErbB4

ErbB4 is a ~200 kDa receptor tyrosine kinase that regulates mammary gland differentiation, cardiovascular and neural development *(83).* It is, like APP and Notch, a type 1 membrane protein. ErbB4 interacts with the EGF family ligand heregulin (HRG), after which the ~120 kDa ectodomain is shed by the activity of an as yet undefined metalloprotease *(84,85).* This cleavage also leaves behind a membrane anchored ~80 kDa fragment (m80).

Recently, it was discovered that the m80 fragment is further processed to a soluble fragment (s80) by a γ-secretase-like activity *(85,86).* This cleavage is inhibited by the expression of dominant-negative forms of PS1 and by treatment with a variety of γ-secretase inhibitors. What ErbB4-s80 is doing in the nucleus is, at present, a mystery, because it has only weak transcriptional activity and the kinase domain appears to prefer a cytosolic localization *(85).* However, it is worth noting that in the case of the AICD co-expression of the known binding partner Fe65 is required to obtain a robust stimulation of transcription *(72).*

A Common Theme?

The three confirmed γ-secretase substrates exhibit striking parallels (Fig. 2). All three are type 1 membrane proteins, and all require shedding of the ectodomain as a prerequisite for γ-secretase cleavage of the membrane-associated fragment. Two of the three confirmed substrates undergo cleavage of the ectodomain by TACE. In the case of ErbB4, the cleavage is by an uncharacterized metalloprotease activity. Although undetermined at this time, it is possible that TACE will be involved in ErbB4 cleavage as well. The cytoplasmic fragment also translocates to the nucleus in all three cases following γ-secretase cleavage, where it likely

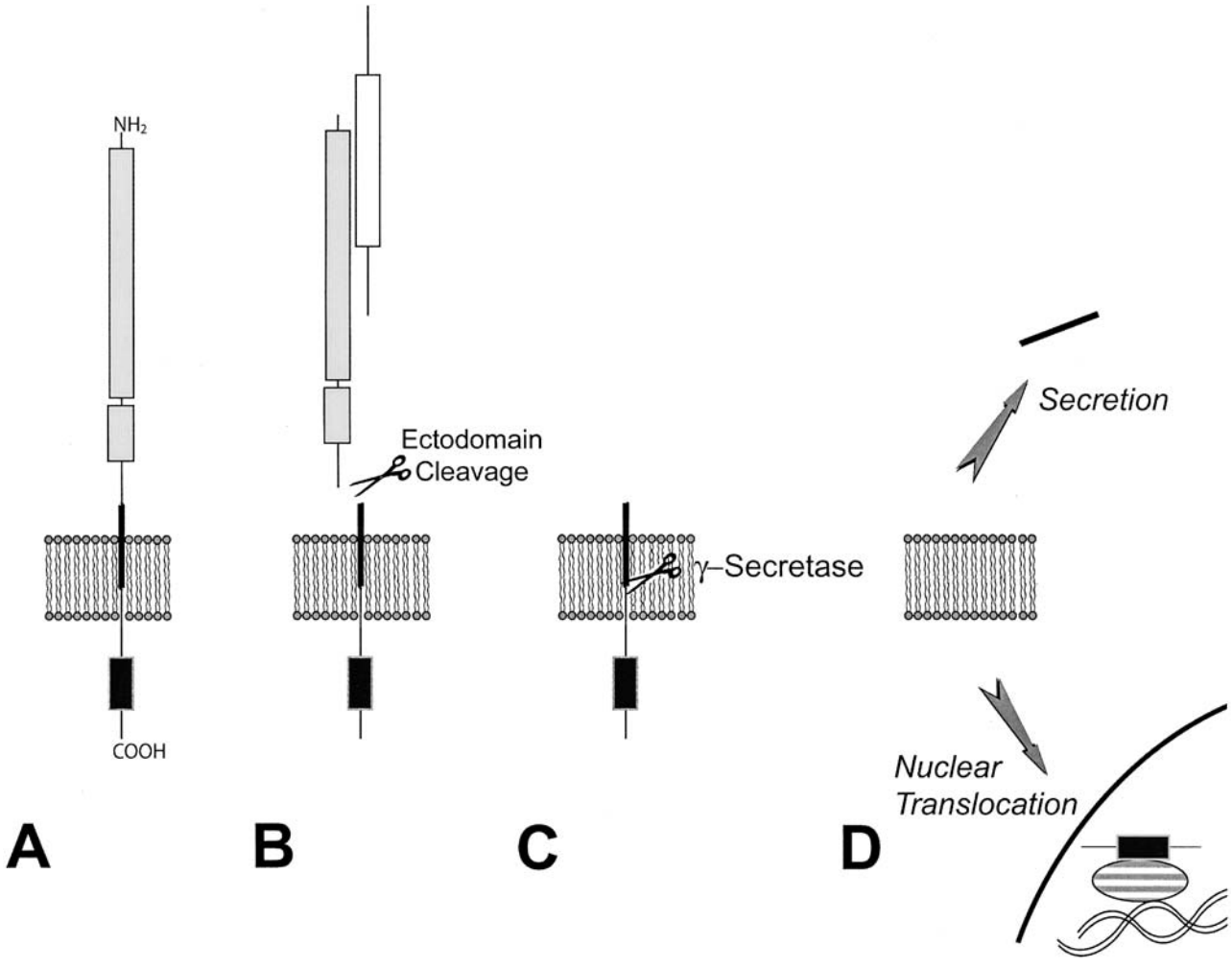

Fig. 2. Common Processing Events for γ-Secretase Substrates. **(A)** APP, Notch, and ErbB4 are transmembrane domain proteins, with a large extracellular domain. **(B)** The full-length protein undergoes enzymatic cleavage, by TACE or a related metalloprotease, and the ectodomain is shed. For both ErbB4 and Notch, this is dependent on ligand binding; the ligand for APP, if any, is unknown. **(C)** The remaining membrane-bound carboxyl terminal fragment is cleaved by γ-secretase; this may happen within the membrane. **(D)** γ-Secretase cleavage eventually liberates a small secreted peptide (Aβ or p3 in the case of APP; peptides secreted from Notch and ErbB4 have not been characterized, but likely exist), and a soluble, cytosolic fragment (AICD for APP, NICD for Notch, and s80 for ErbB4) which is then translocated to the nucleus. AICD and NICD each have nuclear binding partners (Fe65 and CSL family proteins, respectively); the binding partner for ErbB4, if any, is unknown.

plays a role in gene transcription. Although the cleavage site for ErbB4 has not been specifically determined, the γ-cleavage site for both APP and Notch may be very similar *(87–89).*

It is interesting to speculate as to how far these parallels extend. For example, it has recently been shown that γ-secretase-mediated cleavage of the Notch transmembrane domain can produce secreted, Aβ-like peptides *(90).* Although theoretically reasonable, this study utilized APP/Notch chimeras, and therefore will require confirmation with

respect to naturally occurring Notch. Taken to its logical endpoint, it is also reasonable to postulate that an as yet unknown ligand may exist for APP. However, for this to be true, the ligand would have to occur ubiquitously and at sufficient levels to account for the high rate of constitutive APP processing. Similarly, we do not yet know if ErbB4-s80 possesses a nuclear binding partner such as the CSL proteins for NICD or Fe65 for AICD, although the low rate of transcriptional activation for the expression of ErbB4 alone implies that one may exist *(72,85).* Finally, the analogy is informative as to what γ-secretase substrates are *not*. For instance, it seems from the available evidence regarding nuclear translocation that γ-secretase is not merely responsible for the cellular housekeeping chore of general transmembrane domain degradation. In any case, the resemblances between APP, Notch, and ErbB4 will likely help guide the future identification of novel γ-secretase substrates.

Inhibitors of γ-Secretase

In addition to being a major therapeutic target for the treatment of AD, γ-secretase is also an unusual enzymatic activity. Perhaps its most atypical feature is that the cleavage sites for all of the known γ-secretase substrates lie buried within the membrane, presumably where no water is present to participate in hydrolysis. In addition to this characteristic, γ-secretase activity first requires cleavage of a larger substrate by a different enzyme, such as TACE or BACE. These properties have become the defining feature of what has been called regulated intramembranous proteolysis (RIP) *(91),* one of the best-studied examples of which is the processing of the sterol regulatory element binding protein (SREBP) *(30).* Interestingly, in the case of SREBP, it has been postulated that cleavage does not actually take place within the membrane, but may occur near the cytosolic face after the protein undergoes a partial unwinding of the α-helical transmembrane domain following a cleavage event on the lumenal side *(92).* For APP, a similar "cut-expose-cut" model has been proposed *(93).* Alternatively, the γ-secretase complex may form a pore in the membrane to allow water to enter into the active site *(94).*

Studies conducted using γ-secretase inhibitors have proven useful for characterizing substrates and cleavage sites and are, to some extent, prototypical models of what may one day be therapeutic agents to treat the disease. Inhibitors of γ-secretase can be grouped into three very broad categories: high-potency transition state analogs, small peptide-based inhibitors, and nonpeptidic inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs).

Transition State Analogs

In the late 1990s, evidence was accumulating that γ-secretase was an aspartyl protease. This conclusion hinged on findings that γ-secretase activity could be inhibited by several compounds that were known to inhibit aspartyl proteases, such as the prototypical aspartyl protease inhibitor pepstatin *(93)* and peptidomimetic compounds designed to model the transition state of the substrate during cleavage *(95).* Further development of transition state analogs have generated the most potent inhibitors of γ-secretase activity to date, with effective half maximal inhibitor concentrations (IC_{50}) in the low nanomolar range. The most notable of these are: L685,458, which was the first of these compounds shown to covalently bind to presenilin when synthesized as a photoactivatable form *(96)*; compound E, which is arguably the most potent, cell-permeable inhibitor, with an IC_{50} of ~0.3 nM *(97)*; and DAPT, which was the first reported high-potency compound to be tested in vivo *(98).* In general, all of these compounds are potent inhibitors of both major forms of Aβ production (Aβ40 and Aβ42), hence earning the name "pan-Aβ" inhibitors. However, in nearly every case they are also highly effective inhibitors of γ-secretase-mediated Notch and ErbB4 cleavage as well.

Small Peptide Based (Peptidomimetic) Inhibitors

Modified small peptides were actually the first reported inhibitors of γ-secretase activity, although they are widely considered to have low therapeutic potential owing to relatively low potencies (IC_{50} typically in the low micromolar range) in comparison to the newer transition-state analogs. Nevertheless, they have proven to be useful tools for studying γ-secretase activity in vitro. Peptide aldehydes such as carbobenzoxyl-valinyl-phenylalanal (z-VF-cho), z-IL-cho, z-YIL-cho, z-C(tBu)IL-cho, and Boc-GVV-cho are also selective (to varying degrees) for the Aβ40 component of γ-secretase activity *(40,85,99–103).* Similar selectivity is also observed for some difluoroketone compounds *(104).* Although these effects were originally attributed to variable cell penetrance, this selectivity has recently been shown to occur in a broken cell assay of γ-secretase activity, supporting the existence of pharmacologically dissociable activities *(103).* Some preliminary studies have also been conducted on small peptide epoxide inhibitors such as z-IL-epoxide *(105)* and boc-K(DNP)IL-epoxide *(103).* Epoxides are similar to peptide aldehydes with regards to having some selectivity for the Aβ40 generating component of γ-secretase, but have significantly longer wash-out profiles, suggesting that they might be functionally irreversible. However, similar to the more potent transition state analogs, Aβ40 selective compounds are also effective inhibitors of γ-secretase cleavage of Notch *(79,81)* and ErbB4 *(85).*

Other Nonpeptidic Inhibitors

These compounds share the high IC_{50}s characteristic of many of the small peptide inhibitors. However, at least two classes of these compounds also exhibit some form of selectivity. Petit et al. *(106)* recently reported that compounds based on 4-chloro-isocoumarin inhibitors of α-chymotrypsin were capable of inhibiting both Aβ40 and Aβ42 production without affecting the cleavage of NICD from a constitutively active Notch construct. It was also recently reported that the NSAID ibuprofen was able to reduce the amount of amyloid deposited in the Tg2576 transgenic mouse model of AD *(107).* Weggen et al. *(108)* were able to show that the likely mechanism for this effect was the selective inhibition of Aβ42 production, and that a limited set of other NSAIDs also possessed this property. The effect was not mediated through the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2), nor were any effects detected on the γ-secretase-mediated cleavage of Notch. Interestingly, the decrease in Aβ42 secretion was mirrored by a corresponding increase in the production of a normally minor Aβ species, Aβ38. These results suggest that rather than acting as an inhibitor *per se,* some NSAIDs can act to modify γ-secretase activity so that cleavage is shifted away from the longer, more amyloidogenic form of the peptide.

Multiple γ-Secretase Activities?

Observations that a variety of different compounds are capable of differentially affecting the γ-secretase activities responsible for the production of Aβ40, Aβ42, and the Notch intracellular domain hint at the existence of multiple, pharmacologically dissociable γ-secretase activities *(40,93,99,100,102–104,106);* whether or not this will also turn out to be true for the γ-secretase-mediated cleavage of ErbB4 has yet to be determined. Furthermore, a recent study of APP transmembrane domain insertion and deletion mutants indicated that the Aβ40 and Aβ42 activities may be spatially distinct as well *(109).* PS1 deficiency reduces all types of γ-secretase cleavage *(38,40,41,81)* and the knockout of PS2 alone has minimal effect *(48).* Therefore, it seems unlikely that PS1 comprises the active site of an isoform of the enzyme responsible for some types of γ-secretase cleavages and PS2 forms another.

It is possible that the existence of multiple conformers of γ-secretase might account for these results. One conformation might prefer to cleave closer to the lumenal side of the mem-

brane and generate shorter Aβ peptides. Because the majority of Aβ peptides produced are 40 or fewer amino acids in length, this would be the predominant form of the enzyme. A less common conformer might cleave further away, closer to the cytoplasmic face of the membrane, and produce longer secreted peptides. This pathological conformer would be favored by FAD-linked PS1 mutations, which might destabilize the enzyme and prevent it from assuming its preferred conformation, thus making them "loss of function" mutants *(109).* In this context, exactly how mutations in PS2 can also lead to an increased production of Aβ42 remains a mystery. One intriguing possibility is that the mutations in PS2 are true pathological "gain of function" mutants. Although little supporting data exist at present, this hypothesis may explain why there are so few FAD-linked PS2 mutations relative to PS1 (6 vs 72; *c.f.* www.alzforum.org for a complete listing). The multiple conformation hypothesis could similarly explain the existence of compounds and APP mutations that can shift cleavage towards the production of shorter or longer Aβ peptides *(40,93,108).* An obvious problem with this hypothesis is the possibility that one might have to eventually postulate the existence of greater than 2 γ-secretase conformers in order to account for inhibitors that reduce both Aβ40 and Aβ42 activity without affecting NICD production *(106).*

An additional possibility is suggested by several groups independently reporting the likely amino terminal sequence of the AICD *(87–89).* Surprisingly, instead of the 59 or 57 amino acid length predicted by the secretion of Aβ40 and Aβ42, respectively, the AICD is primarily 50 amino acids long. This putative cleavage site is highly similar in APP, Notch and APLP1 and APLP2; valine is the preferred residue at the P1′ site, and is located 3-4 residues N-terminal to a highly charged trio of amino acids (presumably the stop-transfer sequence). As yet, no convincing evidence has been presented to account for the missing 7–9 amino acids. This raises the intriguing possibility that the PS1-dependent γ-secretase activity is not the cleavage event that generates Aβ, but might merely generate the substrate for an array of "Δ-secretase" carboxypeptidases *(89).* This could explain the varying effects of the current inhibitor compounds. Highly potent transition state analogs that target PS1 block all types of Aβ producing γ-secretase activity because they prevent generation of substrate for the Δ-secretases, whereas the differential effects of other compounds are attributable to their interactions with these enzymes. Although this hypothesis appears attractive, it suffers from the shortcoming that it cannot obviously account for the effects of the FAD-linked presenilin mutations, the pathogenic mechanism of which appears to be through selectively increasing Aβ42 production. Clearly, substantial work remains to be done to elucidate the specifics of this pathway.

Conclusions

γ-Secretase inhibitors have recently been shown to adversely affect thymocyte development *(57,58),* a warning sign for adverse interactions with the immune system. In the first of what will likely be several new substrates, the discovery that ErbB4 is also processed by γ-secretase further increases the opportunity for mechanism-based toxic side effects *(85).* In this case, γ-secretase inhibition blocks the growth inhibition/differentiation effects of the ErbB4 ligand heregulin. Although it is difficult to predict how this might manifest in vivo, the involvement of ErbB4 in several types of cancer should be cause for intense scrutiny *(83).* The recent observation that γ-secretase may also be involved in E-cadherin cleavage and the subsequent disassembly of adherens junctions raises similar concerns *(56).* Finally, γ-secretase cleavage of APP, the prime target of therapy, may produce side effects of its own, either through the dysregulation of calcium homeostasis *(75)* or toxicity due to CTFβ accumulation *(110,111).*

Significant progress has been made towards the design and development of γ-secretase

inhibitors in the past few years, with the eventual goal of developing an effective AD therapy *(112).* Unfortunately, given their lack of selectivity for the different components of γ-secretase activity, the most potent of these compounds will likely have deleterious consequences in vivo. Nevertheless, the findings by several independent labs that the distinct components of γ-secretase activity can be pharmacologically separated suggests that this avenue for the development of AD therapeutics is not yet closed.

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