

## Minireview

Regulation of APP cleavage by  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases

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**Abstract** Proteolytic cleavage of the amyloid protein from the amyloid protein precursor (APP) by APP secretases is a key event in Alzheimer's disease (AD) pathogenesis.  $\alpha$ -Secretases cleave APP within the amyloid sequences, whereas  $\beta$ - and  $\gamma$ -secretases cleave on the N- and C-terminal ends respectively. The transmembrane aspartyl protease BACE has been identified as  $\beta$ -secretase and several proteases (ADAM-10, TACE, PC7) may be  $\alpha$ -secretases. A number of studies have suggested that presenilins could be  $\gamma$ -secretases, although this remains to be demonstrated conclusively. Inhibition of  $\beta$ - and  $\gamma$ -secretase, or stimulation of  $\alpha$ -secretase, is a rational strategy for therapeutic intervention in AD. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:** Alzheimer's disease; Amyloid protein precursor; Secretase; Dementia

## 1. Introduction

Alzheimer's disease (AD) is characterised by the presence of proteinaceous deposits in the brain, comprising extracellular amyloid plaques and congophilic amyloid angiopathy and intracellular neurofibrillary tangles [1]. The main component of the extracellular amyloid deposits is the amyloid protein (A $\beta$ ), which is derived by proteolytic cleavage from the amyloid protein precursor (APP), a type I transmembrane glycoprotein [2].

The A $\beta$  region of APP comprises a sequence of 42–43 amino acid residues located partly within the ectodomain and partly within the transmembrane domain of APP (Figs. 1 and 2). APP is cleaved by three types of proteases, which are designated  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases (Fig. 1). Processing by  $\beta$ - and  $\gamma$ -secretase cleaves on the N- and C-terminal ends of the A $\beta$  region respectively, releasing A $\beta$ , whereas  $\alpha$ -secretase cleaves within the A $\beta$  sequence [3].  $\gamma$ -Secretase cleaves at several adjacent sites to yield A $\beta$  species containing 39–43 amino acid residues.

A substantial body of evidence indicates that accumulation of A $\beta$  in the brain, particularly longer species containing 42 or 43 residues (long A $\beta$ ), is an important step in the pathogenesis of AD [4]. Genetic and biochemical studies on familial forms of AD have shown that a variety of pathogenic mutations in the APP gene and in genes encoding proteins known as presenilins increase the production of long A $\beta$  [5]. Long A $\beta$  is also more abundant in sporadic (non-familial) forms of AD

[6], suggesting that the accumulation of long A $\beta$  is a common mechanism underlying all forms of AD. Furthermore, transgenic mice with increased A $\beta$  levels have some neurodegenerative changes (e.g. dystrophic neurites, gliosis) and cognitive defects similar to AD (reviewed in [7,8]), which supports this view. Long A $\beta$  aggregates more readily than the more commonly produced A $\beta$ 40 species, and may seed the formation of A $\beta$ 40 fibrils, which ultimately act as a template for plaque formation [9]. It is generally believed that aggregated A $\beta$  is neurotoxic, although the mechanism of neurotoxicity is not well understood [4].

Because of their role in the production of A $\beta$ , the APP secretases have been under intense investigation. Recent studies have identified several candidate APP secretases. This review describes the current status of research on APP secretases and on secretase inhibitors which may prove useful for AD therapy.

## 2. $\alpha$ -Secretase

A major route of APP processing is via the  $\alpha$ -secretase pathway, which cleaves on the C-terminal side of residue 16 of the A $\beta$  sequence, generating an 83-residue C-terminal fragment (C83) (Figs. 1 and 2) [10]. Subsequent cleavage by  $\gamma$ -secretase releases a short peptide (p3) containing the C-terminal region of the A $\beta$  peptide (Fig. 1). The biological significance of p3 and its role, if any, in amyloidogenesis remains obscure. As cleavage of APP by  $\alpha$ -secretase destroys the A $\beta$  sequence, it is generally thought that the  $\alpha$ -secretase pathway mitigates amyloid formation, although this has not been demonstrated unequivocally. In addition, the C-terminally truncated form of APP released by  $\alpha$ -secretase may have trophic actions [11] which could antagonise the neurotoxic effects of aggregated A $\beta$  [12].

Studies by Nitsch et al. [13] have shown that stimulation of muscarinic receptors can increase cleavage of APP via the  $\alpha$ -secretase pathway, and inhibit A $\beta$  production [14]. However, at the time of writing, muscarinic receptor agonists have been largely unsuccessful in the treatment of AD in clinical trials [15], although not all promising muscarinic agonists have been tested clinically.

The exact subcellular localisation of the  $\alpha$ -secretase is unclear, although the *trans*-Golgi [16] has been proposed as one of the sites of  $\alpha$ -cleavage. More recently, a membrane-bound endoprotease at the cell surface has been found to have  $\alpha$ -secretase-like activity [17]. However, the localisation of  $\alpha$ -secretase solely to regions of the late secretory pathway is difficult to reconcile with the suggestion that stimulation of  $\alpha$ -secretase inhibits  $\beta$ -secretase processing [18] and that inhibition of  $\alpha$ -secretase cleavage promotes A $\beta$  production through

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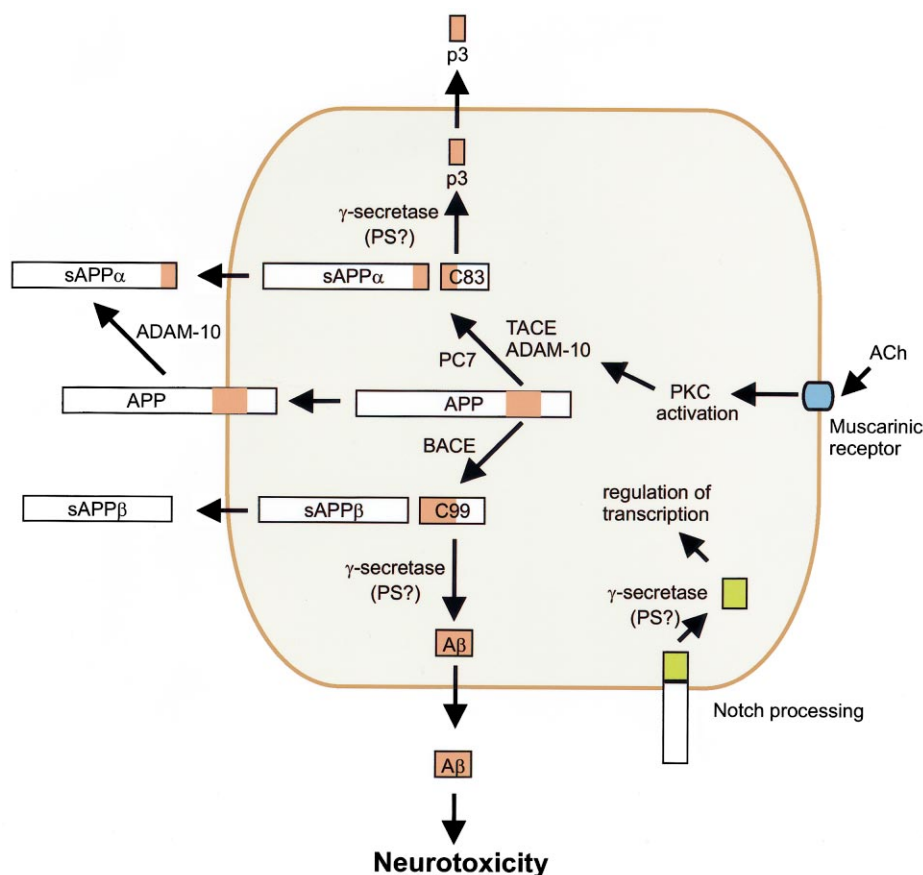


Fig. 1. Pathways of APP processing by  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases. Cleavage by  $\alpha$ -secretase (PC7, TACE or ADAM-10) produces sAPP $\alpha$  and a C-terminal fragment C83. Both TACE and ADAM-10 can be activated by protein kinase C (PKC) which is regulated by the muscarinic acetylcholine (ACh) receptor. C83 is cleaved by  $\gamma$ -secretase to produce p3. Cleavage of APP by  $\beta$ -secretase (BACE) produces sAPP $\beta$  and C99.  $\gamma$ -Secretase, also believed to play a role in the proteolysis of Notch, cleaves C99 to release A $\beta$  which has neurotoxic properties. Presenilins (PS) 1 and 2 have been proposed as  $\gamma$ -secretases.

the  $\beta$ -pathway [19], as the alternative  $\beta$ -secretase cleavage may occur much earlier in the secretory pathway [20].

One possible explanation for the uncertainty about the localisation of  $\alpha$ -secretase is that there may be more than one enzyme.  $\alpha$ -Secretase activity has both constitutive and regulated components. Although the constitutive activity has not yet been identified, regulated  $\alpha$ -secretase cleavage appears to be under the control of protein kinase C (PKC) [21]. Phorbol esters increase  $\alpha$ -secretion of APP above basal levels, while PKC inhibitors leave a residual cleavage action [18].

Two members of the ADAM (a disintegrin and metalloprotease) family, tumour necrosis factor- $\alpha$  (TNF $\alpha$ )-converting enzyme (TACE or ADAM-17) and ADAM-10, are candidate  $\alpha$ -secretases (Fig. 2). TACE cleaves pro-TNF $\alpha$ , releasing the extracellular domain (TNF $\alpha$ ) in a similar manner to APP. The inhibition or knockout of TACE decreases the release of the  $\alpha$ -cleaved product sAPP $\alpha$  [22]. However, cells deficient in TACE still have a residual  $\alpha$ -secretase activity that cannot be increased by phorbol esters [22]. Thus, TACE may play a role in regulated PKC-dependent  $\alpha$ -secretion.

Overexpression of ADAM-10 increased  $\alpha$ -secretase cleavage of APP in a phorbol ester-inducible manner [17]. A dominant-negative form of ADAM-10 with a point mutation in the zinc-binding site was found to inhibit basal and stimulated  $\alpha$ -secretase activity, but did not totally abolish sAPP $\alpha$  production [17]. ADAM-10 exists only in a proenzyme (inactive)

form in the Golgi, but becomes activated at the plasma membrane [17]. Thus, TACE and ADAM-10 may both be  $\alpha$ -secretases.

Although the identity of the constitutive  $\alpha$ -secretase activity is not known, one possibility is the prohormone convertase PC7 (Fig. 2). Overexpression of PC7 in HEK293 cells has been found to increase sAPP $\alpha$  secretion, while overexpression of  $\alpha_1$ -antitrypsin Portland (an inhibitor of precursor convertases) was found to inhibit endogenous sAPP $\alpha$  production [23]. However, as both TACE and ADAM-10 as initially synthesised as proproteins, the possibility that PC7 acts upstream as a pro- $\alpha$ -secretase cleaving enzyme cannot be excluded [23].

As it is likely that several proteases contribute to  $\alpha$ -secretase activity, it may be difficult to regulate APP processing pharmacologically through this pathway. However, most studies aimed at developing inhibitors of A $\beta$  production have focussed on the two enzymes directly responsible for cleaving A $\beta$  from APP,  $\beta$ - and  $\gamma$ -secretase.

### 3. $\beta$ -Secretase

A  $\beta$ -site APP cleaving enzyme (BACE or Asp2) has been identified by several groups both by genetic screening and by direct enzyme purification and sequencing [24–28]. BACE is an unusual member of the pepsin family of aspartyl proteases, which has an N-terminal catalytic domain, containing two

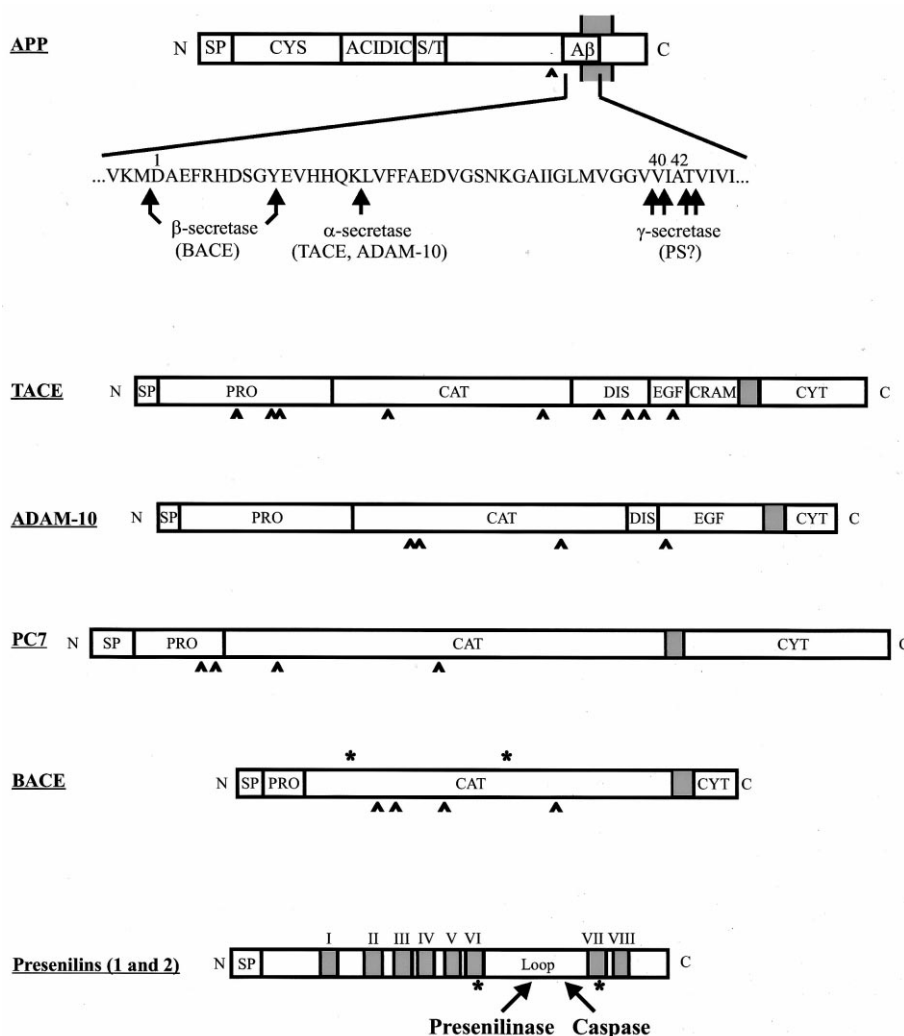


Fig. 2. Functional domains of APP and putative APP secretases. The figure also shows the amino acid sequence of APP around the amyloidogenic region with the secretase cleavage sites indicated by arrows. Abbreviations: SP = signal peptide, CAT = catalytic domain, CYS = cysteine-rich domain, S/T = serine/threonine domain, PRO = proprotein sequence, DIS = disintegrin domain, CYT = cytosolic domain, CRAM = crambin-like domain, – denotes potential *N*-linked glycosylation sites, \* denotes putative catalytic aspartate residues. Shaded regions show putative transmembrane domains.

important aspartate residues, linked to a 17-residue transmembrane domain and a short C-terminal cytoplasmic tail [24]. BACE contains four potential *N*-linked glycosylation sites and a propeptide sequence at the N-terminus (Fig. 2). Within the cell, BACE is expressed initially as a preproprotein, and then efficiently processed to its mature form in the Golgi [29]. A related transmembrane aspartyl protease (BACE2 or Asp1) [25] shows similar substrate specificity [30] but is not highly expressed in the brain [31].

However, BACE possesses many of the features of  $\beta$ -secretase. BACE can cleave full-length APP at Asp1 of the A $\beta$  sequence and also at Glu11, which is an alternative cleavage site [24]. The Swedish NL mutation, which is known to enhance  $\beta$ -secretase cleavage [32], also promotes cleavage of APP at Asp1 by BACE [24]. BACE is expressed coordinately with APP in many regions of the brain, particularly in neurons, and has a subcellular distribution similar to that of  $\beta$ -secretase [24]. Interestingly, the Flemish familial AD mutation in APP increases production of A $\beta$  from APP by BACE2 but not BACE [30], suggesting that BACE2 contributes

to A $\beta$  production in individuals bearing the Flemish mutation.

BACE has structural homology to a protease required for the HIV life cycle and many of the inhibitors of this protease can now be tested for their ability to inhibit BACE [33]. However, an important question is whether inhibition of BACE is an appropriate strategy for therapeutic intervention in AD. It is likely that BACE has several substrates (other than APP) and that it has an important physiological function. Inhibition of this function could have toxic consequences. Perhaps, the development of BACE knockout mice may help to answer some of the concerns about the physiological importance of BACE and the consequences of inhibiting its activity.

#### 4. $\gamma$ -Secretase

Cleavage of the APP C99 fragment (Fig. 1) by  $\gamma$ -secretase is the final step in the production of A $\beta$ . The exact position of cleavage by  $\gamma$ -secretase is critical for the development of AD. Production of the more amyloidogenic long A $\beta$  species by

cleavage by  $\gamma$ -secretase adjacent to residues 42 or 43 is closely associated with disease pathogenesis [4,5].

Although the  $\gamma$ -secretase has not been identified, presenilin (PS) 1 and 2 are two candidates. PSs are integral membrane proteins with eight putative transmembrane domains, encoded by genes on chromosomes 14 and 1 [34] (Fig. 2). A large number of mutations have now been identified in PS, mostly in PS1, which cause familial AD and many of these mutations have been shown to cause an increase in the proportion of long A $\beta$  species [5,35].

There are several arguments supporting the view that PSs are  $\gamma$ -secretase. As knockout of both PS1 and PS2 completely inhibits all  $\gamma$ -secretase activity [36], this demonstrates that PSs are required for  $\gamma$ -secretase activity. Furthermore, PSs are localised to subcellular compartments (i.e. ER–Golgi) known to be the site of  $\gamma$ -secretase processing [37]. Subcellular and biochemical fractionation experiments have shown that PS and  $\gamma$ -secretase copurify as a high molecular weight complex [38]. In addition,  $\gamma$ -secretase inhibitors can affinity-label PS subunits [39,40] and both PSs reportedly bind to APP [41,42]. Thus, if  $\gamma$ -secretase is not identical to the PS, then the protease activity is at least intimately associated with PS in vivo. Inhibitor studies show that  $\gamma$ -secretase is likely to be an aspartyl protease [43]. Wolfe et al. [44] found that mutation of two aspartate residues (Asp257 and Asp385) in the transmembrane domain of PS1 inhibits  $\gamma$ -secretase activity. Similar results have been obtained by mutating a corresponding aspartate residue (Asp366) in PS2 [45], indicating that the one or both aspartate residues may be important for cleavage by  $\gamma$ -secretase (Fig. 2). Wolfe et al. [46] have suggested that the two aspartate residues in PS1 form part of an active catalytic site. However, in contrast to this idea, Capell et al. [47] found that PS1 mutants lacking Asp257 secrete significant amounts of A $\beta$ .

However, the identity of  $\gamma$ -secretase as PS has not yet been established. For example, PS has never been directly shown to have protease activity. PS may simply be a regulatory subunit of  $\gamma$ -secretase, or a protein that is somehow involved in the trafficking of proteins targeted to  $\gamma$ -secretase. Furthermore, it is unclear how aspartates situated in a transmembrane domain could participate in a hydrolytic cleavage. Not only would transmembrane  $\alpha$ -helices lack the ability to form active site pockets typical of known enzymes, but also the lipid environment is unlikely to have enough water to facilitate hydrolytic cleavage. Wolfe and coworkers [46] have proposed a model in which the PS transmembrane domains form a pore, which could allow water to enter the membrane. However, there is as yet no experimental verification of this model.

Regulated intramembraneous proteolysis (RIP), similar to  $\gamma$ -secretase cleavage of APP, has now been postulated to occur in several membrane proteins [48]. However, the term RIP may be misleading, as proteolytic cleavage within a membrane has never been demonstrated directly. For example, it is possible that  $\alpha$ - or  $\beta$ -secretase cleavage of APP results in movement of the C-terminal polypeptide and exposure of the  $\gamma$ -secretase sites to the aqueous environment.

A number of  $\gamma$ -secretase inhibitors have now been synthesised [39]. However, as discussed for  $\beta$ -secretase,  $\gamma$ -secretase may be an important enzyme for the processing of other proteins (e.g. Notch) [47]. Therefore, due regard needs to be taken of the possibility that  $\gamma$ -secretase inhibitors may have unwanted side effects. Notch is involved in the regulation of neuronal

differentiation, spermatogenesis, oogenesis and myogenesis, and cleavage of the C-terminus by a  $\gamma$ -secretase-like activity (regulated by PS) is an important step in its biological function [49]. It is possible that  $\gamma$ -secretase can be inhibited sufficiently to lower A $\beta$  production in the brain without altering the Notch signalling pathway. However, this remains to be demonstrated in vivo.

## 5. Will inhibiting A $\beta$ production be an effective treatment for AD?

Almost all of the work on APP secretases has been based on the belief that inhibition of A $\beta$  (especially A $\beta$ 42) production will block or even reverse the cognitive decline in AD. While there is strong evidence in favour of the amyloid hypothesis of Small and McLean [4], it is not yet proven. It is possible that the use of  $\beta$ - or  $\gamma$ -secretase inhibitors for AD therapy will provide the ultimate test of this hypothesis. If the cognitive decline in AD patients can be arrested using secretase inhibitors, this will provide compelling evidence for a direct role of A $\beta$  in AD pathogenesis. Indeed, recent studies involving a novel strategy for treatment of AD suggest that removal of amyloid or A $\beta$  from the brain may be of great therapeutic benefit. APP transgenic mice immunised with A $\beta$  have been shown to have much lower A $\beta$  amyloid in the brain [50]. In addition, the pathologic change normally seen in these mice can be inhibited by immunisation [50,51], supporting the view that diminishing the amyloid burden will be of benefit. Ultimately, the proof of this concept must rest with human trials using secretase inhibitors.

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