The Role of Presenilin and its Interacting Proteins in the Biogenesis of Alzheimer’s Beta Amyloid

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Abstract The biogenesis and accumulation of the beta amyloid protein (Aβ) is a key event in the cascade of oxidative and inflammatory processes that characterises Alzheimer’s disease. The presenilins and its interacting proteins play a pivotal role in the generation of Aβ from the amyloid precursor protein (APP). In particular, three proteins (nicastrin, aph-1 and pen-2) interact with presenilins to form a large multi-subunit enzymatic complex (γ-secretase) that cleaves APP to generate Aβ. Reconstitution studies in yeast and insect cells have provided strong evidence that these four proteins are the major components of the γ-secretase enzyme. Current research is directed at elucidating the roles that each of these protein play in the function of this enzyme. In addition, a number of presenilin interacting proteins that are not components of γ-secretase play important roles in modulating Aβ production. This review will discuss the components of the γ-secretase complex and the role of presenilin interacting proteins on γ-secretase activity.

Keywords Alzheimer’s disease · Amyloid precursor protein · Beta amyloid · Presenilin · Interacting proteins · Gamma secretase

Abbreviations APP amyloid precursor protein Aβ beta amyloid AICD APP intracellular domain aph-1 anterior pharynx defective 1 pen-2 presenilin enhancer 2 PS1 presenilin 1 PS2 presenilin 2 TMP21 transmembrane protein 21

Introduction: the generation of beta amyloid by presenilin and its binding proteins

Alzheimer’s disease (AD) is a complex, progressive neurodegenerative disorder that is neuropathologically characterised by extensive neuronal loss and the presence of neurofibrillary tangles and senile plaques. While the majority of AD cases are sporadic, ~5% of AD cases are familial (FAD) with mutations in three genes, amyloid precursor protein, presenilin 1 (PS1) and presenilin 2 (PS2) accounting for the majority of cases. A major feature of both sporadic and familial forms of AD, is the accumulation and deposition of a small peptide referred to as beta amyloid (Aβ) within...
brain tissue of AD sufferers. The mechanisms that underlie the disease processes are poorly understood. However, the accumulation of Aβ is thought to play a pivotal role in neuronal loss or dysfunction through a cascade of events that include the generation of free radicals, mitochondrial oxidative damage and inflammatory processes (reviewed in Refs. [1, 2]). The primary event that results in the abnormal accumulation of Aβ is thought to be the dysregulated proteolytic processing of its parent molecule, the amyloid precursor protein (APP).

The APP molecule is a transmembrane glycoprotein that is proteolytically processed by two competing pathways, the non-amyloidogenic and amyloidogenic (Aβ forming) pathways. How these pathways are regulated remains unclear. However, there are many factors including diet, hormonal status, and genetic mutations that influence the processing of APP to generate Aβ (reviewed in Refs. [1, 3]). Three major secretases are postulated to be involved in the proteolytic cleavage of APP. These include z-secretase (of which the metalloproteases ADAM17/TACE and ADAM 10 are likely candidates), beta APP cleaving enzyme (BACE, formally known as β-secretase) and the γ-secretase. The z-secretase cleaves within the Aβ domain of APP thus precluding the formation of Aβ and generating non-amyloidogenic fragments and a secreted form of APP (z-APPS). In the amyloidogenic pathway (Fig. 1), BACE cleaves near the N-terminus of the Aβ domain on the APP molecule, liberating another soluble form of APP, β-APPS and a C-terminal fragment (C99) containing the whole Aβ domain. The final step in the amyloidogenic pathway is the intramembranous cleavage of the C99 fragment by γ-secretase, to liberate the Aβ peptide (reviewed in Ref. [1]).

Identification of multiple cleavage sites within the APP molecule has suggested the presence of other secretases, namely ε- and ζ-secretase; that generate the APP intracellular domain (AICD) and a longer fragment of Aβ (Aβ46), respectively [4–7]. Although different enzymes may exist, the same γ-secretase complex that generates Aβ may also generate AICD and Aβ46 as these products are inhibited by γ-secretase inhibitors [4–7] indicating that this enzyme has multiple cleavage sites. However, over-expression and mutagenesis studies have shown that the production of Aβ and AICD may be independent catalytic events [8, 9].

The γ-secretase enzyme is thought to be an aspartyl protease that has the unusual ability to regulate intramembrane proteolysis (RIP) for a growing list of type 1 integral membrane proteins which include, APP, APP like proteins (APLPs), E-Cadherin, CD44, lipoprotein receptor related protein (LRP), Notch, sterol regulatory element-binding protein (SREBP), interferon response element (IRE1) and activated transcription factor 6 (ATF-6) (for recent review see [10]). The mechanism of RIP and γ-secretase activity is unknown. However a transient hydrophilic environment for catalysis within the lipid membrane must be created. Furthermore, the enzyme (or an enzyme domain) must have the ability to bend and unwind the α-helical substrates, exposing their amide bonds to hydrolysis. It is therefore conceivable that the γ-secretase enzyme is an integral protein of the lipid bi-layer and contains a number of proteins that may have different functions within an enzyme complex. The exact conformation or molecular architecture of the γ-secretase enzyme remains unknown. However, large molecular mass complexes of ~250 kDa [11, 12], ~500 kDa [13–17] and ~2,000 kDa [13, 18] have been identified.

Over the last few years biochemical and genetic approaches have identified four components of the γ-secretase complex, presenilins, nicastrin, anterior pharynx defective (aph-1) and presenilin enhancer 2 (pen-2). Over-expression and expression knockdown studies have provided strong evidence that these proteins are essential for γ-secretase catalytic activity [15, 19–24]. Subsequent reconstitution studies in non-mammalian cells have provided evidence that these are the only components responsible for γ-secretase catalytic activity [25–27]. However the active component and the active catalytic site of this enzymatic complex are yet to be identified.

The roles of presenilin and its binding partners within the γ-secretase complex in Aβ generation

Presenilin 1: the catalytic component of the γ-secretase enzyme

Presenilin 1 (PS1) is thought to be the most critical component of the γ-secretase complex as evidence indicates that it is the catalytic component of this proteolytic enzyme. The finding that mutations in PS1 account for the majority of inherited early onset forms of AD and result in the overproduction of the highly amyloidogenic Aβ42 [28–30] provided the initial evidence that PS1 facilitates γ-secretase activity. Subsequent studies showed that PS1 ablation or mutagenesis of two highly conserved aspartate residues within transmembrane domains 6 and 7 of PS1 results in a reduction in Aβ levels [31–34]. Furthermore, aspartyl protease inhibitors and transition state analogue inhibitors which are designed to target the active site of the protease, all reduce Aβ40 and Aβ42 levels and have
been shown to affinity label and bind to PS1 [35–37]. In addition, physical interactions between PS1 and the APP-C99 fragment have been identified [38–40]. The studies outlined above provide substantial evidence to indicate that PS1 is the catalytic subunit of γ-secretase. However, this transmembrane protein is not typical of a conventional aspartyl protease, and lacks the typical D(T/S)G motif for an aspartyl protease active site (recently reviewed in [41]). Aspartyl proteases require the presence of two aspartate residues for the hydrolysis of the peptide bond. Although PS1 lacks the typical D(T/S)G aspartyl protease active site it does contain the two aspartyl residues (D257 and D385) which are either critical for the active site on the γ-secretase complex or constitute the active site [42]. Other proteases that contain atypical aspartyl protease motifs and have weak homology with PS1 include the bacterial membrane embedded aspartyl protease, type 4 prepilin protease and the signal peptidases, which are all transmembrane proteins. These proteins share the unusual aspartyl motif GxGD and perform intramembranous cleavage of type I membrane proteins (reviewed in [43]). The topology and atypical characteristics of these proteins make them an unprecedented class of protease and as is the case for PS1, would require interaction with other proteins within the complex.

The interaction between the components of the γ-secretase complex is not sufficient to create a macromolecular enzyme complex such as γ-secretase (i.e. total mass of all the components is ~200 kDa). Thus,
dimer or trimer formation must occur within the complex. To date the only member of the complex that is known to undergo this process is PS1. The full length PS1 protein is rapidly endoproteolytically cleaved within its characteristic large hydrophilic loop into amino- and carboxy-terminal fragments (NTF/CTF) of ~27 and ~17 kDa, respectively [44, 45]. These fragments are thought to interact with each other to form the catalytic component of the γ-secretase enzyme since in the absence of full length PS1, co-expressed NTF and CTF were able to generate γ-secretase activity in presenilin deficient mammalian cells [46, 47]. The stoichiometry and the nature of the interaction between these fragments remain unclear. It has been shown by many studies that the NTF:CTF form a heterodimer in mammalian cells [12, 48–51] leading to suggestions that this heterodimer is the active γ-secretase [52]. However, Cervantes and colleagues [53] provided evidence that the presenilin fragments can form a tetramer by identifying heterodimers as well as NTF and CTF homodimers in yeast. Evidence for heterodimer and NTF homodimer (but not CTF homodimer) formation has been provided by photo-affinity labelled cross-linking studies [54]. This formation provides a core of aspartyl residues required for aspartyl protease activity. However, it has yet to be established whether the hypothetical “core” is formed between fragments from one PS1 molecule or multiple molecules within the complex. Further, if PS1 is the “catalytic core” of the γ-secretase complex the roles of the other interacting proteins need to be determined.

Nicastrin: the substrate docking site and scaffolding for building the γ-secretase complex

Nicastrin was discovered to be part of the γ-secretase complex when it was found to co-immunoprecipitate with PS1 [55]. Nicastrin is a type I transmembrane protein with a 670 amino acid long hydrophilic N-terminal domain, a transmembrane domain, and a relatively short cytoplasmic C-terminus of twenty amino acid residues [55]. The protein undergoes glycosylation and sialylation within the secretory pathway to yield a mature ~150 kDa protein, the largest component of the γ-secretase complex [14], [56–58]. Nicastrin has been shown to be a critical component of the γ-secretase complex as inhibition of nicastrin function in vitro and in vivo results in a complete loss of APP and Notch cleavage [14, 55, 59–61]. Within this complex, nicastrin appears to be involved in the early formation of the complex, particularly in the formation of the first subcomplex between itself and aph-1 (Fig. 1). Recent evidence suggests that nicastrin is critical for the correct assembly of the γ-secretase complex within the endoplasmic reticulum and the intracellular trafficking of the complex to the cell surface [62, 63]. Furthermore, nicastrin is essential for the interaction between the complex and APP-C99 and thus may act as a receptor for γ-secretase substrates [63, 64]. Therefore, although nicastrin is a critical component, evidence to date suggests that it is unlikely to be a catalytic component of the γ-secretase complex. Instead, nicastrin acts as a scaffold for the building of the active complex, and a possible substrate docking site.

Anterior pharynx defective 1 (aph-1): scaffolding partner for nicastrin and may have possible catalytic activity

The aph-1 protein is a ~30 kDa, 308 amino acid long 7-transmembrane protein [65] existing in two homologous forms, located on chromosome 1 and chromosome 15 (aph-1a & aph-1b respectively; [19]). In addition, as the result of alternate splicing, a long and short isoform of aph-1a have also been reported, with the short isoform more abundantly expressed in most tissues [21]. Although the exact function of these isoforms remains unknown, multiple γ-secretase complexes containing aph-1 isoforms are thought to exist [66, 67]. The aph-1a isoform appears to be incorporated into active γ-secretase complexes. Deletion of aph-1a causes a reduction in Aβ generation [66] and is the major isoform present in γ-secretase complexes during embryonic development [67]. The γ-secretase complexes incorporating aph-1b are thought to have a redundant function as aph-1b has been found not to be involved in the regulation of other γ-secretase components or γ-secretase activity [66].

The aph-1 protein forms a stable and intermediate ~140 kDa complex with Nicastrin, before binding PS1. Aph-1 has been shown to interact with immature and mature forms of presenilins and nicastrin [21]. The GXXXG motif located within transmembrane 4 of aph-1 has been shown to be important in this interaction [68], [69]. The aph-1: nicastrin sub-complex appears to play a role in the stabilisation and assembly of PS1 in the γ-secretase complex (Fig 1; [24, 70, 68]). However, the exact role of aph-1 in the γ-secretase complex remains unknown. Expression studies have shown that removal of aph-1 destabilises the presenilin holoprotein and the addition of aph-1 leads to the accumulation of the holoprotein [11, 19–24, 71]. Thus together with nicastrin, aph-1 is thought to contribute the initial formation and trafficking of the γ-secretase complex and thereby provide a scaffold for the proteolytic processing of PS1 and the formation of the active site.
Aph-1 is critical for the activity of the fully constructed \( \gamma \)-secretase complex. Evidence has been provided that as well as binding to immature components of \( \gamma \)-secretase in early stages of complex formation, aph-1 also interacts with the mature forms of PS1, nicastrin and pen-2 [68, 72]. Furthermore, recent evidence has been provided to suggest that this interaction occurs on the cell surface where it also binds the \( \gamma \)-secretase substrate, Notch and facilitates its cleavage [72]. These studies suggest that apart from the scaffolding function it shares with nicastrin, aph-1 has another function within the fully constructed, active \( \gamma \)-secretase complex. Structural and functional similarities between aph-1 and other proteases that possess the ability for intramembranous cleavage (such as rhomboid, [68, 73]) suggests that this transmembrane protein may have an enzymatic function within the complex. This postulated novel function for aph-1 needs to be validated by future studies.

Presenilin Enhancer 2 (pen-2): the elusive presenilinase or “molecular clamp” that holds the complex together?

The pen-2 protein is a \(~12\) kDa, 101 amino acid long protein containing two transmembrane domains and its N- and C-terminals facing the lumen of the endoplasmic reticulum [74, 75]. Pen-2 has been shown to interact with Nicastrin, aph-1, and also PS1. Without this interaction pen-2 has been shown to be degraded, possibly by the proteasome [76, 77]. Ablation of pen-2 results in significantly reduced PS1 endoproteolysis and A\( \beta \) production, suggesting that pen-2 is essential for both presenilinase and \( \gamma \)-secretase activity [15, 19, 22–24]. Likewise, combinational expression studies have shown that when pen-2 is co-expressed with the other components of the \( \gamma \)-secretase complex in a step-wise manner, endoproteolysis of the PS1 holoprotein was enhanced [11, 22, 24–27, 71]. A trimeric sub-complex between presenilin, nicastrin and aph-1 has been identified in cells lacking pen-2 [24, 70] suggesting that pen-2 may be the final protein incorporated into the \( \gamma \)-secretase complex (Fig. 1). Recent evidence has suggested that pen-2 is incorporated into the \( \gamma \)-secretase complex through an interaction with presenilins. The “DYLSF” domain of pen-2 and a “NF” motif on transmembrane 4 of PS1 have been shown to be critical for the interaction between these proteins [78–80]. The cleavage of PS1 occurs within the hydrophilic loop and is distal from the interaction site on transmembrane 4, suggesting that another domain of pen-2 is responsible for the cleavage of PS1 or pen-2 may present PS1 to the elusive “presenilinase” which may be another member of the \( \gamma \)-secretase complex. The C-terminal end of pen-2 has been shown to be important for \( \gamma \)-secretase activity since altering the length of this region by addition or deletion of residues has been shown to reduce A\( \beta \)40 and 42 generation without altering the binding of pen-2 to the complex [78] suggesting that pen-2 may have an alternative function within the complex. One suggestion put forward by Hasegawa and colleagues [78] is that the C-terminus of pen-2 may be a linker/space molecule that maintains spatial interactions between proteins within the complex. However, recent evidence using pen-2 C-terminal loss of function mutations suggest that the C-terminus acts as a “molecular clamp” holding together the presenilin fragments and the whole \( \gamma \)-secretase complex [81]. If this is the case then PEN-2 is an integral part of the catalytic process holding the complex together whilst the \( \gamma \)-secretase products are generated.

The newest member of the complex: TMP21, a modulator of \( \gamma \)-secretase activity

Recently another member of the \( \gamma \)-secretase complex was immuno-purified from wild-type PS1 and PS2 blastocye derived cells [82]. The type 1 transmembrane protein termed TMP21 is a member of the p24 cargo-family which may have a signalling role in the sorting and transport of proteins from the endoplasmic reticulum to the Golgi [83, 84]. It was shown by Chen and colleagues [82] that TMP21 is a member of the complex as it was isolated in a high molecular weight presenilin complex, interact with all of the known components of the \( \gamma \)-secretase complex, co-localised with the complex components in the ER, Golgi and cell surface and destabilised from the complex in the absence of the presenilins and pen-2. Although the over-expression of TMP21 did not alter \( \gamma \)-secretase activity, its suppression resulted in and increase in A\( \beta \)40 and 42 generation without altering the production of A\( \beta \)c. However, suppression of TMP21 did not alter the production of A\( \beta \)c, notch cleavage to generate NICD or cleavage of E-cadherin to generate CICD. These results are consistent with the notion that \( \gamma \) and \( \epsilon \)-secretase cleavage activities are independently regulated and indicate a role for TMP21 in modulating \( \gamma \)-secretase activity to generate A\( \beta \). This role for TMP21 appears to be independent of its role in protein transport since the suppression of both TMP21 and p24a (a member of the p24 cargo family that interacts with TMP21) does not result in additional increases in A\( \beta \) production to that observed following the suppression of TMP21 only [82]. This finding led the authors to postulate that there are two pools of TMP21, a major pool that is stabilised by, p24a and has
no role in Aβ production and a minor pool that modulates Aβ production, independent of p24a. Although, further investigation is required to determine the precise mechanism of action, it appears that TMP21 may function to regulate intramembrane proteolysis controlling γ-secretase activity and thus preventing the over-production of Aβ. This modulator role for TMP21 is important to consider when elucidating mechanisms on how γ-secretase activity is altered (for example by presenilin mutations) resulting in enhanced Aβ40 and Aβ42 production.

Presenilin 2 (PS2): capable of forming a fully functional γ-secretase complex

The presenilin proteins share an overall homology of 67% with the highest similarities in the predicted transmembrane domains. Fewer similarities are found at the N-terminus and in the central region of the hydrophilic loop [85, 86]. Although there are some structural similarities between PS1 and PS2 several lines of evidence suggest that these proteins may have distinct functions. Mice lacking PS1 die before birth and have severe skeletal and brain deformities, whilst those lacking PS2 only develop a mild pulmonary fibrosis and haemorrhage with age [33, 87–90]. Compared to neuronal cultures isolated from PS1 ablated mice, those isolated from PS2 knockout mice exhibit higher Aβ production and are less sensitive to γ-secretase inhibitors [89, 91]. Lai and colleagues [91] also provided evidence to suggest that distinct PS1 containing and PS2 containing complexes exist [91]. If this is the case, evidence to date suggests that PS2 containing complexes have different functions and have less γ-secretase processing power than PS1 containing complexes. However, recent in vitro evidence has suggested that chimeras of PS1 and PS2 fragments retain γ-secretase activity [92] suggesting that active complexes containing both PS1 and PS2 could occur. However, this is unlikely to occur in vivo as PS2 and PS1 transgenic mice have differential effects on γ-secretase activity [93]. In this study mice lacking PS1 were crossbred with transgenic mice containing wild type or mutant PS2/PS1. Compared to mice harbouring wild-type PS1, those harbouring wild-type PS2 had a ~4-fold reduction in Aβ40 and ~2-fold reduction Aβ42 brain levels. In addition, the authors reported that although mutations in PS1 lead to higher brain levels of Aβ40 and Aβ42 than PS2 mutations, those mice harbouring PS2 mutations had higher brain levels of Aβ42, whilst Aβ40 brain levels remain unchanged [93]. Taken together these studies suggest that PS1 containing complexes predominate in generating Aβ40 and Aβ42. However, the finding that PS2 is involved in Aβ42 (but not Aβ40) generation suggests that two Aβ generating γ-secretase complexes (γ-40 secretase and γ-42 secretase) exist, with γ-42 secretase complexes containing both PS1 and PS2.

The roles of presenilin binding proteins not incorporated into the active γ-secretase complex

In addition to Aβ generation, the presenilins have been implicated in a variety of intracellular processes including membrane trafficking, Notch signalling, neuronal plasticity, cell adhesion, regulation of calcium homeostasis, the unfolded protein response, and apoptosis. Therefore it is not surprising that the presenilins have a growing list of binding partners (see Table 1). Though the presenilin binding proteins that are incorporated into the γ-secretase complex are critical for the activity of this enzyme, those that are not integral components have equally important roles in modulating Aβ production. A number of non-complex presenilin binding proteins have been shown to alter γ-secretase activity (see Table 1). However, only a few have had supporting evidence that their direct interaction with the presenilins modulate γ-secretase activity. These are described below and shown in Fig. 2.

Calsenilin

Calsenilin (also called DREAM or kChIP3) is a calcium binding protein, that was first identified through yeast two hybrid screening using the PS2 C-terminus as the bait protein [94]. The interaction between calsenilin and the presenilins appear to have a role in APP processing. It was initially demonstrated that calsenilin can form a complex with PS1 and PS2 and regulate presenilin fragment formation in mammalian cells [95]. This study also showed that calsenilin may facilitate presenilin mediated apoptosis, since calsenilin was shown to preferentially interact with the C-terminal fragment of PS2 that results from caspase cleavage of the presenilin holoprotein [96]. Expression studies in vivo (Xenopus) and in vitro (mammalian cells) have shown that the interaction between presenilin and calsenilin may be involved in ER calcium release and apoptosis [97, 98]. Over-expressing calsenilin in neuronal cells resulted in cell death which was enhanced by the co-expression of wild-type or PS1 mutations [99]. These studies have suggested that the interaction between calsenilin and presenilins may function in apoptotic pathways. However, several lines of evidence...
| Interacting protein | Proposed function | Interaction with presenilins shown to modulate Aβ production | Reference |
|---------------------|-------------------|------------------------------------------------------------|-----------|
| **Complex components** | | | |
| APH-1 | Component of the γ-secretase complex; role for initial formation of the complex, or a proteolytic role | Yes | [19, 65, 67, 123]. |
| Nicastrin | Component of γ-secretase complex; possible role as a receptor for APP-C99 | Yes | [14, 55–58]. |
| PEN-2 | Component of the γ-secretase complex, role in proteolytic cleavage of the presenilins | Yes | [19, 24, 70, 76, 77]. |
| TMP21 | Recently identified component of the γ-secretase complex, role in modulating Aβ production | Yes | [82]. |
| **γ-secretase substrates** | | | |
| APP-C99 | Substrate for the γ-secretase complex: precursor to Aβ generation | Yes | [38–40]. |
| E/N cadherin | γ-secretase substrate; role in Cell-Cell Adhesion | No | [124]. |
| IRE1 | γ-secretase substrate; role in unfolded protein response | No | [125, 126]. |
| LRP | γ-secretase substrate; Lipid metabolism | Yes | [119]. |
| Notch1 | γ-secretase substrate; precursor to NICD generation | Yes | [112]. |
| **Interacting binding proteins** | | | |
| ABP-280, Filamin homolog1 | Actin binding protein | No | [127]. |
| Bcl-2 | Anti-apoptotic molecule | No | [128]. |
| Bcl-X<sub>L</sub> | Anti-apoptotic molecule | No | [129]. |
| Calmyrin | Possible calcium-myrstol switch | No | [130]. |
| CALP- calsenilin like protein | Novel member of the calsenilin/KChIp protein family | No | [131]. |
| Calsenilin | Calcium binding protein | Yes | [94, 96–99, 101, 102]. |
| CLIP-170 | Linking membrane organelles to microtubules | No | [132]. |
| DRAL | LIM-domain containing protein | No | [133]. |
| FHL2 | Role in PI3K/Akt activation | No | [134]. |
| FKBP38 | Role in mitochondria mediated apoptosis | No | [135]. |
| Go | Signalling molecule | No | [136]. |
| GSK3β | Wnt signalling, serine threonine protein kinase | No | [137]. |
| HCS/ZETA | Subunits of the catalytic 20S proteasome | Yes | [138]. |
| Met1 | Putative methyltransferase | No | [139]. |
| **Modifier of cell adhesion protein (MOCA)** | | | |
| Omi/HtrA2 | Serine protease, proapoptotic | No | [141]. |
| PAG | Neuronal proliferation protein | No | [142]. |
| PAMP and PARL | Metalloproteases | No | [143]. |
| PKA | Serine/Threonine protein kinase; β-catenin phosphorylation | No | [144]. |
| Plakoglobin | Role in b-catenin/Tcf-4 activity | No | [145]. |
| PLD1 | Phospholipid-modifying enzyme | Yes | [146]. |
| PSAP | PDZ like protein | No | [147]. |
| QM/Jif1 | Negative regulator of c-Jun | No | [148]. |
| Rab proteins | Vesicle mediated protein trafficking | Yes | [149, 150]. |
| RyR2 | Cardiac ryanodine receptor | No | [151]. |
| SEL-10 | Ubiquitination of proteins | Yes | [152]. |
| Sorcin | Calcium binding regulator of ryanodine receptor | No | [153]. |
| Syntaxin 1A | Synaptic plasma-membrane protein | No | [154]. |
| Syntaxin 5 | ER-Golgi vesicular transport | Yes | [155, 156]. |
| Tau | Microtubule binding protein | No | [137]. |
| Telencephalin | Neuron specific adhesion molecule | No | [40]. |
| TPP1 | Tetratricopeptide repeat-containing protein | No | [157]. |
| Ubiquilin | Ubiquitin domain-containing protein | No | [158–160]. |
| X11 family of proteins | Cytoplasmic adaptor proteins | Yes | [110, 111]. |
| β-catenin/δ-catenin (NPARP) | Wnt signalling. Cell adhesion | No | [161–163]. |
| μ-Calpain | Calcium-dependent thiol protease | No | [164]. |
suggest that calsenilin may also be implicated in APP processing and Aβ generation. Initial in vitro studies showed that over-expression of calsenilin in mouse neuroblastoma cells resulted in a gene dose dependent accumulation of Aβ42 [100]. Using a γ-secretase luciferase based assay and a cell free system established by Li and colleagues [18] to characterise the γ-secretase complex in purified cell membranes, Jo and colleagues [101] recently reported that the over-expression of calsenilin resulted in an increase in γ-secretase activity. This calsenilin/presenilin, mediated activity was markedly reduced using a well-characterised γ-secretase inhibitor (L-685, 458). Furthermore, compared to wild-type mice, brain Aβ40 and Aβ42 levels were reduced in calsenilin ablated mice [102]. Together these studies would suggest that calsenilin has a second function, to facilitate γ-secretase activity.

X11/Mint proteins

A family of cytoplasmic adaptor proteins called X11 (also known as munc-18-interacting proteins—mints) have also shown to have a direct role in APP processing. In particular, several studies have reported that X11α and X11β stabilise APP and inhibit the secretion of Aβ and APP from neuronal cells [103–106]. The molecular mechanisms by which the X11s influence APP processing and Aβ production are not clear. However, this effect appears to be modulated by a direct interaction between X11 and APP. The interaction between the phosphotyrosine binding (PTB) domain within X-11 and the YENPTY motif within APP appears to modulate Aβ generation, since introduction of point mutations within these domains enhanced Aβ generation [103, 106–109]. The binding
of X11 appears to stabilise APP, leading to its intracellular accumulation [103, 106–109]. It is conceivable that the X11–APP interaction may inhibit secretase accessibility to APP resulting in impaired Aβ secretion. The X11 proteins have multiple binding domains and have been shown to interact with the PS1 C-terminal fragment through their PDZ domain [110]. The authors also showed an interaction between PS1 and APP in the presence of X11 that was otherwise ablated in the absence of X11. This suggests that an interaction between APP and PS1 is mediated through X11. A subsequent study has reported that X11β and alcadein (and X11β-binding protein) expression prevents the binding of PS1 to APP [111]. The significance of the results from these interaction studies remains unclear. However, they do suggest that X11 proteins may act as inhibitory molecules preventing PS1 mediated proteolytic processing of APP and thus attenuating Aβ production.

Competing substrates

Some presenilin binding proteins reduce the likelihood of Aβ generation by competing for the catalytic activity offered by γ-secretase. One example is Notch1 which undergoes cleavage by γ-secretase to liberate an intracellular domain (NICD) that translocates to the nucleus where it co-activates transcription of genes involved in several developmental pathways [112–115]. The first evidence of substrate competition came from one study that treated neurons with the Noch1 ligand, delta, and found a dose dependent reduction in Aβ levels [116]. In contrast, the over-expression of APP in these neurons resulted in a reduction in Notch signaling [116]. Further evidence for substrate competition was provided by Kimberley and colleagues [117] where APP and Notch based substrates directly competed for γ-secretase activity in a cell free assay. Similar results were achieved in a subsequent study where cells expressing Notch showed a reduction in Aβ production [118]. Additional evidence for substrate competition has been provided recently for another substrate of γ-secretase, the cytoplasmic tail of the low-density lipo-protein receptor—related protein (LRP), which has also been shown to interact with PS1 [119]. The co-expression of the LRP C-terminal fragment and APP in CHO cells has been shown to result in a reduction in secreted levels of Aβ40 and Aβ42 [119].

Taken together, these studies not only suggest substrate competition but also the presence of a single γ-secretase. However, the presence of multiple γ-secretase complexes cannot be ruled out. As discussed above there is evidence in the literature for the formation of γ-secretase complexes with distinct functions. All of the studies described above are undertaken in conditions where there is a large amount of substrate available which could down-regulate the activity of one γ-secretase for another. Whether substrate competition occurs under physiological conditions and if there are certain conditions that promote a higher expression of one substrate over another remains to be determined.

Concluding comments

It is well established that together with presenilin, its binding proteins, nicastrin, aph-1 and pen-2 are the only proteins that constitute the γ-secretase complex responsible for generating Aβ from APP. The function(s) of each γ-secretase component within the complex is gradually becoming apparent. However, there are many facets of γ-secretase activity that are yet to be identified such as the domain responsible for actual catalytic activity; the mechanisms of enzymatic activity within the lipid bi-layer; and the existence of single or multiple γ-secretase complexes. Further investigation using appropriate protein expression models and reconstitution studies such as those used to validate the protein components of γ-secretase complex is required to provide some insight into the processes that underlie the catalytic activity of this enzyme. Indeed, reconstitution of the γ-secretase complex in insect cells and purification of this complex from the cells have provided researchers with the first 3D images of the structure of this intricate enzyme [120, 121]. Identification of the specific catalytic domain(s) within the complex would provide a site that could be a potential target to develop agents that specifically modulate Aβ levels without altering the other known (and unknown) activities of the γ-secretase enzyme. Recent insight into this notion was provided by the finding that certain compounds selectively block the cleavage of APP but not Notch [122]. However, it is important to note that other presenilin interacting proteins not incorporated into the complex and the pathways they are involved in play important roles in APP processing and Aβ generation. These interactions assist in many facets of γ-secretase activity, including modulating substrate/enzyme availability, and thus are important to consider when developing appropriate therapeutic strategies.

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