Cell. Mol. Life Sci. 66 (2009) 1534 – 1555
1420-682X/09/091534-22
DOI 10.1007/s00018-009-8435-9

Cellular and Molecular Life Sciences

Review

Presenilin-dependent regulated intramembrane proteolysis and γ-secretase activity

J. V. McCarthy*, C. Twomey and P. Wujek

Signal Transduction Laboratory, Biochemistry Department, University College Cork, Cork (Ireland),
Fax: +353-(0) 21-490-1382, e-mail: jv.mccarthy@ucc.ie

Received 25 July 2008; received after revision 24 November 2008; accepted 10 December 2008
Online First 4 February 2009

Abstract. Inhibiting the production of amyloid-β by antagonising γ-secretase activity is currently being pursued as a therapeutic strategy for Alzheimer's disease (AD). However, early pre-clinical studies have demonstrated that disruption of presenilin-dependent γ-secretase alters many presenilin-dependent processes, leading to early lethality in several AD model organisms. Subsequently, transgenic animal studies have highlighted several gross developmental side effects arising from presenilin deficiency. Partial knockdown or tissue-specific knockout of presenilins has identified the skin, vascular and immune systems as very sensitive to loss of presenilin functions. A more appreciative understanding of presenilin biology is therefore demanded if γ-secretase is to be pursued as a therapeutic target. Herein we review the current understanding of γ-secretase complexes; their regulation, abundance of interacting partners and diversity of substrates. We also discuss regulation of the γ-secretase complexes, with an emphasis on the functional role of presenilins in cell biology.

Keywords. Presenilin, γ-secretase complexes, regulated intramembrane proteolysis, signal transduction, Alzheimer's disease.

Introduction

The presenilins were initially identified through genetic linkage of families with autosomal dominant forms of familial Alzheimer's disease (FAD), but have now emerged as key components of γ-secretase protease associated with all forms of Alzheimer's disease (AD) (reviewed in [1–4]). Genetic studies of families with FAD have shown that nearly 50% of FAD patients bear mutations in either of the two presenilin-encoding genes, *PSEN1* or *PSEN2* [5]. The *PSEN1* and *PSEN2* genes encode the ~50kDa presenilin-1 (PS1) and presenilin-2 (PS2) proteins respectively, which are rapidly endoproteolytically cleaved into active amino- and carboxyl-terminal fragments (NTF/CTF), which subsequently interact with each other to form stable heterodimers. Subsequent biochemical, molecular and genetic studies have shown that the presenilins are the catalytic component of the multiprotein γ-secretase enzyme complexes [6–8]. Presenilin-dependent cleavage of amyloid-β (Aβ) precursor protein (APP) contributes to the generation of Aβ peptides and the formation of pathological Aβ senile plaques. Mutations in *PSEN1* and *PSEN2*, which contribute to the onset of FAD, lead to an increase in the generation of the more amylodiogenic 42 amino acid Aβ peptide ($A\beta_{42}$). This shift in $A\beta_{42}$ abundance, coupled with the fact that $A\beta_{42}$ is more amylodiogenic than the predominant

* Corresponding author.

$A\beta_{40}$ species, is believed to accelerate the pathogenesis of AD. Therefore, inhibition of presenilin-dependent γ-secretase is considered a potential therapy for Aβ lowering and AD intervention [140, 71].

From biochemical and genetic analyses, insights into the role of presenilins in normal and pathological processes are emerging, and it has become evident that presenilins have biological roles beyond γ-secretase cleavage of APP, some of which are independent of γ-secretase activity (reviewed in [1–3]). Using yeast-two-hybrid or co-immunoprecipitation approaches, several groups have reported that the presenilins interact with a diversity of proteins, thereby linking presenilins to a wide range of cellular processes, ranging from the modulation of cell viability to cell adhesion, protein trafficking, calcium homeostasis and regulation of gene transcription [3, 4, 9, 10]. It was subsequently shown that in addition to cleaving APP, the presenilins are involved in the intramembranous cleavage of numerous type-I integral membrane proteins [1–3]. Following receptor-ectodomain shedding, the presenilins are involved in the cleavage of the membrane-tethered carboxyl-terminus of more that sixty-six membrane proteins, liberating their intracellular domains (ICDs) into the cytosol. In some cases these ICDs translocate to the nucleus and regulate the transcription of target genes [1, 3, 4], but other cleavage events are proposed to function as a means of clearing redundant protein fragments from the membrane following ectodomain shedding [11]. Thus, the presenilins are involved in many signalling events independent of previously reported AD-associated activities. This has lead to the proposal that disruption of one or many of the presenilins' activities within signalling complexes may also contribute to the pathogenesis of AD, or other disorders [1, 3, 9, 12].

Consistent with these hypotheses, studies that inactivated the PSEN1 gene in mice produced severe developmental abnormalities and perinatal lethality [13–16], indicating an essential role for PS1 in development. To overcome the perinatal lethality following germ line targeting of PS1 and embryonic lethality in germ line presenilin-double knockouts [17, 18], several groups have made use of $PS1^{+/-}PS2^{-/-}$ mice which carry the most severe reduction of presenilin alleles compatible with survival [19]. Others have utilised PS1 transgene rescued-PS1 null mice [20, 21] or previously developed brain sub-region-specific gene knockout techniques based on the Cre/LoxP system [22–24] to study the long-term role of PS1 in age-associated development, brain function and cognitive behaviours. The phenotype of $PS1^{+/-}PS2^{-/-}$ 'partial deficient' mice is normal up to approximately six months, when the majority of the mice develop skin lesions similar to seborrheic keratosis, the common benign wart seen frequently in elderly humans, and an autoimmune disease characterized by dermatitis, glomerulonephritis, keratitis and vasculitis, as seen in human systemic lupus erythematosus [19]. Subsequent studies have also employed the Cre/LoxP system to more closely study the long-term role of PS1 in lymphoid T cells [25] and B cells [26]. Deficiency in both PS1 and PS2 functions antagonise T cell homeostasis and signalling [25], while B cells deficient in PS1 and PS2 present a deficit in both lipopolysaccharide (LPS) and B-cell antigen receptor-induced proliferation and signal transduction events [26]. From these studies it has been concluded that in addition to the central nervous system, the skin [20, 27] and the immune system appear to be especially sensitive to a partial loss of presenilin functions [19, 28]. Furthermore, these studies emphasise the necessity for a better, more rigorous understanding of presenilin-associated processes and functions, which should better steer the search for candidate signalling pathways and genes contributing to familial and sporadic AD, and possibly reveal novel presenilin- and/or γ-secretase-dependent biological processes.

Assembly of the γ-secretase complexes

To date, over 160 FAD mutations have been identified in PS1 and 10 FAD mutations in PS2 (http://www.molgene.ua.ac.be/ADMutations). The presenilins are synthesized as inactive holoproteins, which undergo endoproteolysis to generate an active NTF/CTF heterodimer. Both PS1 and PS2 can function independently within the γ-secretase complexes and FAD mutations alter γ-secretase processing of APP, thereby contributing to the pathogenesis and progression of AD [2–4].

The presenilins are widely and differentially expressed in mammalian tissues, with a broad cellular distribution suggesting multiple presenilin-dependent functions. The presenilins are located at the nuclear envelope, endoplasmic reticulum, Golgi apparatus, early secretory pathway and the plasma membrane. PS1 and PS2 share a high degree of homology (67%) with conservation between some transmembrane domains reaching 95% [29]. Accordingly, there is significant similarity in their translated structures. Each of the presenilin genes encodes a multi-transmembrane protein and, although it is well over a decade since these proteins were discovered, a consensus has yet to be reached as to their precise topology [30–32]. While all are agreed on the presence of a large cytosolic hydrophilic loop domain, the arrangement of their ten hydrophobic domains has

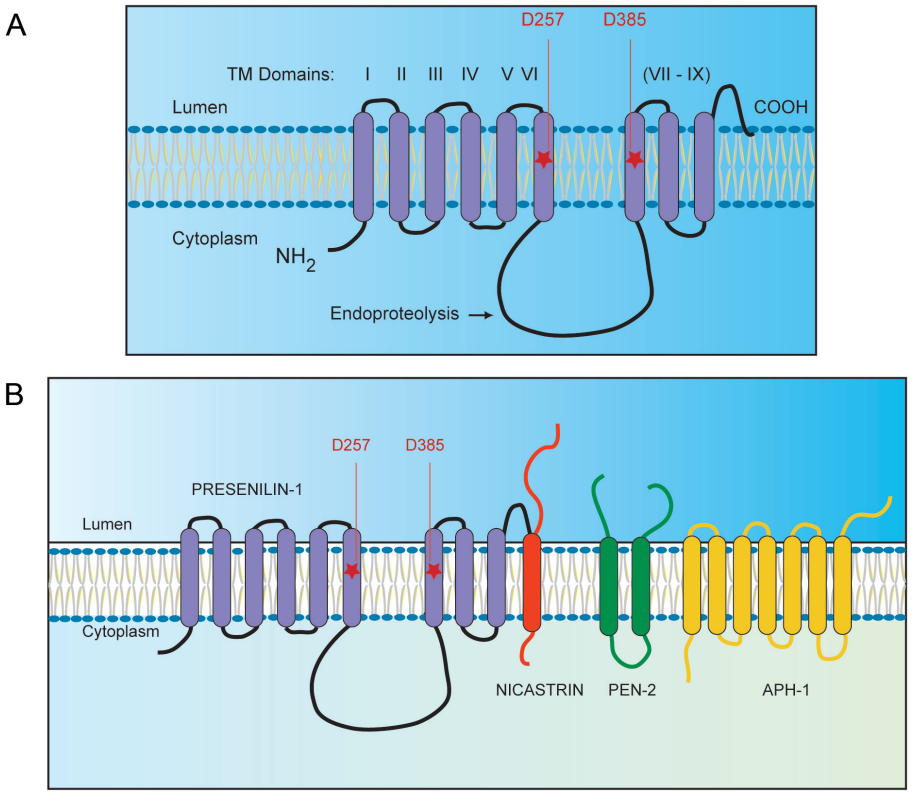

Figure 1. *(A)* Schematical representation of the nine-transmembrane topology of presenilin. The two conserved aspartate residues in transmembrane (TM) domain VI and VII, required for catalytic activity, indicated. An approximate location of site of endoproteolysis is also indicated. *(B)* Topological representation of the γ-secretase complexes. The γ-secretase protease complexes are composed of four primary proteins presenilin, nicastrin, Aph-1 and Pen-2 in a 1:1:1:1 stoichiometry. The catalytically active γ-secretase complex consists of four proteins with a total 19 transmembrane domains; this has significantly hampered attempts to obtain structural information of the γ-secretase complexes.

lead to much debate. The most recent data, suggesting a nine transmembrane model for the presenilins, is based on sequence analysis, computational predictions, compilation of published data and experimental approaches such as N-linked glycosylation scanning [32, 33]. This model places the N-terminus of the presenilins in the cytosol, with the hydrophilic loop cytosolically orientated between transmembrane domain 6 and 7 and the C-terminus either in the lumen or inserted into the membrane (Fig. 1A).Given such a high degree of sequence and topographical homology, it is no surprise that there is functional redundancy between PS1 and PS2, as evident from the ability of either homologue to rescue the egg-laying defect of *Caenorhabditis elegans* hermaphrodites lacking sel-12 [34]. Despite these similarities, the presenilins are differentially expressed during development [35] and the dramatically different phenotypes of PS1-deficient and PS2-deficient mice suggest incomplete functional redundancy and a complex functional repertoire for presenilins.

Several independent research reports have given evidence supporting the concept that the presenilins are the catalytic centre of the γ-secretase complexes [8]. The first evidence of presenilin requirement for γ-secretase was elucidated by De Strooper and colleagues [16], who demonstrated that PS1-deficient mice had greatly reduced Aβ production and consequential accumulation of APP carboxyl terminal γ-secretase substrates, C83 and C99 [16]. Secondly, the description of presenilins as aspartyl proteases found credibility when introduction of mutations into the two conserved aspartyl residues located in transmembrane domains 6 and 7 of either PS1 or PS2 resulted in loss of γ-secretase activity [36–38]. This suggested that presenilins are aspartyl proteases and the catalytic core of the γ-secretase complexes. This hypothesis was further supported by the use of transition-state analogue inhibitors of aspartyl proteases. These inhibitors were found to covalently bind to the presenilins and inhibit γ-secretase cleavage of APP and other γ-secretase substrates [39, 40].

Despite this strong evidence that the presenilins are the catalytic core of γ-secretase complexes, overexpression of the presenilins alone does not increase γ-secretase activity and presenilins alone are not sufficient for γ-secretase protease activity. As outlined below, the identification of three presenilin-associated cofactors, nicastrin, anterior pharynx-1 (Aph-1) and presenilin enhancer-2 protein (Pen-2) [32], and subsequent purification of the multi-protein γ-secretase complexes proved that presenilin and these three cofactors collectively form active γ-secretase protease complexes (Fig. 2). Reconstitution studies in yeast and insect cells have subsequently provided conclusive

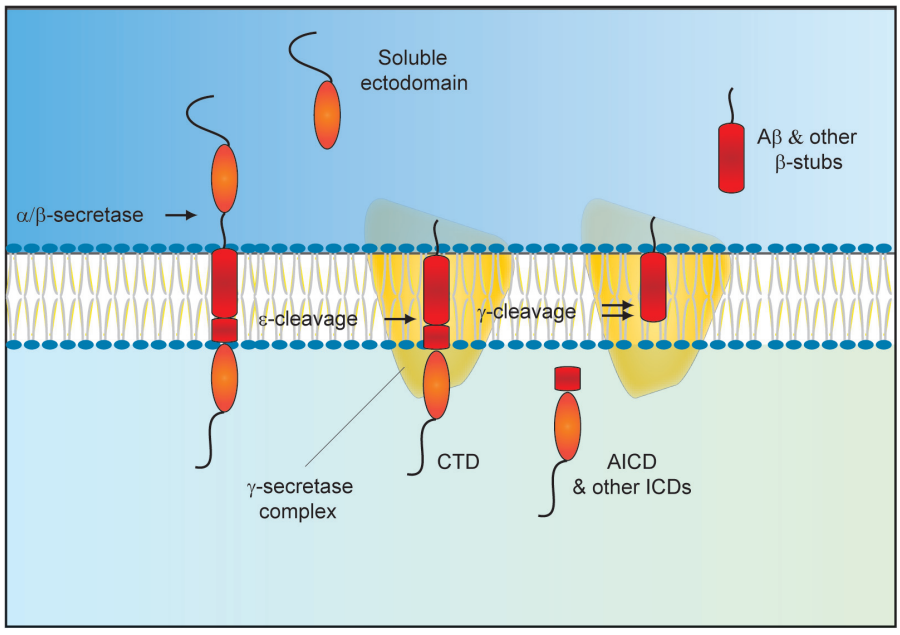

Figure 2. Schematic representation of the γ-secretase complexes and proteolytic cleavage of a substrate. In this model, the progressive cleavage of a γ-secretase substrate is illustrated. Firstly, cleavage in the substrate ectodomain by α/β–secretase produces soluble ectodomain and membrane-anchored carboxyl-terminal domain (CTD). Next, γ-secretase is recruited to the substrate (as discussed in text) and cleaves the CTD at the ε-cleavage-site to liberate the substrate intracellular domain (ICD). Finally, γ-secretase cleaves the remaining membrane-associated fragment at the γ-cleavage site to liberate Aβ or related peptide fragment.

evidence that γ-secretase is a multi-protein complex, consisting primarily of these four proteins [41–44].

Nicastrin was the first of the γ-secretase cofactors to be independently identified via its interaction with presenilin [45]. Nicastrin is a type I membrane protein with a large and glycosylated ectodomain. In formation and assembly of the γ-secretase complexes, nicastrin is involved in the proper assembly of the γ-secretase complexes within the endoplasmic reticulum and their intracellular trafficking to the plasma membrane [46, 47]. It has also been referred to as 'the γ-secretase substrate receptor' because of its role in the initial recognition of γ-secretase substrates [47]. Subsequent to ectodomain shedding, nicastrin recognises the short amino-terminal stubs of γ-secretase substrates [47], and thereby provides an important mechanism by which ectodomain shedding can act as a rate-limiting step in the recognition and further proteolysis of γ-secretase substrates [32, 47]. Once bound to nicastrin, the substrates are locked into the lipid bilayer, introduced to the presenilin component of the complex where the γ-secretase cleavage event occurs. The remaining cofactors, Aph-1 and Pen-2 were discovered via genetic screens for mutations that alter presenilin activity in *C. elegans* [48]. Aph-1 encodes a unique protein whose topology consists of a seven transmembrane-spanning structure with a predicted molecular weight of ~29kDa, while Pen-2 is a two transmembrane domain, 101 amino acid protein (~10kDa) [49] (Fig. 1B). In humans, there are two Aph-1 genes, but three variants of the Aph-1 protein, Aph-1a (S or L splice variants) and its homologue Aph-1b, which differentially incorporate into distinct γ-secretase complexes with potentially different functions [50, 51]. In contrast, in rodents there are three Aph-1 genes, resulting from a rodent-specific duplication of the Aph-1b gene, creating Aph-1c [52]. Aph-1 has been reported to form a complex with nicastrin that precedes association with presenilin. This Aph-1/nicastrin complex is proposed to enable the stabilisation and integration of presenilin into the assembling γ-secretase complexes [53–55]. Pen-2 has an essential role in the endoproteolytic generation of the presenilin NTF/CTF heterodimers [56, 57], and consistent with a role in the γ-secretase complex, Pen-2 knockouts have an identical phenotype to presenilin and Aph-1 knockouts in *C. elegans* [49]. Analyses in mammalian cells demonstrate that presenilin, nicastrin, Aph-1 and Pen-2 regulate each other's maturation and γ-secretase activity. For example, reduced expression of nicastrin results in a loss of Pen-2 stability and reciprocally, reduction of cellular Pen-2 interferes with maturation of nicastrin and presenilin stability [58]. Interestingly, along with the presenilin genes, Pen-2 is the only other identified member of the γ-secretase complexes in which mutations have been linked to FAD; a recent study revealed a missense mutation (D90N) in three family members diagnosed with AD, though clearly more studies are necessary to reveal the full effects of such mutations [59].

Though the critical constituents of the γ-secretase complexes have been identified, the size at which the protease complexes migrate suggests either the existence of oligomers of the constituent proteins or the complexes themselves, or alternatively, the presence of additional non-essential regulatory proteins that

influence γ-secretase activity. In accordance with the latter as well as a recent report that the stoichiometry of γ-secretase components (presenilin : Pen-2 : nicastrin : Aph-1) is 1:1:1:1 [60], a number of proteins have been shown to influence the function of the protease complexes, including TMP21, CD147, Rer1p, calsenilin, pigment epithelial-derived factor (PEDF) and β-adrenergic receptor. The cargo protein TMP21 co-immunoprecipitates with γ-secretase complex proteins [61] and has been reported to differentially modulate γ-cleavage of APP and generation of $A\beta_{40}$ and $A\beta_{42}$, without altering generation of APP-derived APP intracellular domain (AICD) [61]. TMP21 has therefore dual functionality; protein transport and quality control within pathways of the endoplasmic reticulum and Golgi as well as being a highly specific modulator of presenilin-dependant cleavage at the γ-cleavage site [61,62]. The type I membrane glycoprotein CD147 also co-purifies with the γ-secretase complexes and like TMP21, reduced expression of CD147 increased $A\beta_{42}$ production, suggesting that CD147 may function as a negative regulator of γ-secretase [63]. In addition, the retrieval receptor Rer1p which has been shown to mediate endoplasmic reticulum localisation of binding proteins interacts with Pen-2 and precludes formation of a functioning γ-secretase complex [64]. An additional independent study has shown that Rer1p competes with Aph-1 for binding to immature nicastrin, and thereby again negatively impacts on the formation of a functional γ-secretase complex [65]. It was also recently demonstrated that PEDF and the β-adrenergic receptor are both potent up-regulators of γ-secretase activity and that they promote its translocation to the plasma membrane [66, 67]. Ongoing biochemical and genetic studies will no doubt lead to the identification and characterisation of other putative γ-secretase-interacting proteins that are perhaps needed for either proper formation of the complexes, selective interaction with substrates, or function of the complexes during enzymatic cleavage of substrates. These and similar studies may eventually contribute to our understanding of the broader cellular functions of both γ-secretase and its substrate proteins.

It is important to emphasis that given that there are two presenilins and three Aph-1 proteins, and that both PS1 and PS2 have functional activity within the γ-secretase complexes, it has been proposed that between four and six functionally distinct γ-secretase complexes could exist [50, 52, 68]. Consistent with this proposal, differential contribution of the Aph-1 genes to γ-secretase activity have been reported [68, 69]. Independent studies have reported that the Aph-1a-containing γ-secretase complexes are critical for Notch signalling, while deficiency of Aph-1b/c-containing γ-secretase complexes antagonises cleavage of the γ-secretase substrate neuregulin-1 (Nrg1), but not other substrates (ErbB4, Syndecan and Notch) [69]. Furthermore, while Aph-1a is widely expressed, Aph-1b/c γ-secretase complexes are prominently expressed in brain areas relevant to schizophrenia pathogenesis [69]. Additionally, though a comprehensive assessment of the overlapping functionality of distinct PS1 and PS2-mediated γ-secretase activities is lacking, it is know that the efficacy with which they can cleave their substrates can vary considerably. Using purified cell membranes from blastocyst derived from wild type, $PS1^{-/-}PS2^{+/+}$ and $PS1^{+/-}PS2^{-/-}$ deficient animals, it was demonstrated that PS1-associated γ-secretase complexes display considerably higher specific activity (>150-fold) than PS2-associated γ-secretase complexes [34]. Likewise, in an *in vivo* transgenic system, PS2 was shown to be far less efficient than PS1 at processing APP, even though PS2 FAD mutations robustly increased $A\beta_{42}$ production [70]. It is equally important to emphasise that the γ-secretase complexes are responsible for two distinct proteolytic cleavage events within the transmembrane domain of APP and other less well-characterised substrates (Fig. 2). Subsequent to ectodomain release, proteolytic cleavage at the ε-cleavage site produces the amino terminus of the AICD and other substrate-derived ICDs, while subsequent proteolysis at the γ-cleavage site is responsible for the production of Aβ and other β-stubs [71–73]. Proteolysis at the ε-cleavage site is nearly always exclusive, producing ICDs of homogeneous length. In contrast, proteolysis at the γ-cleavage site is heterogeneous, producing Aβ peptides and β-stubs of varying lengths [74, 75]. However, FAD mutations in either APP or the presenilins affect proteolysis at both cleavage-sites [76].

Therapeutic strategies: γ-secretase modulators and inhibitors

Currently, five drugs are approved for the symptomatic treatment of AD [77]. Arising from its central role in the proteolysis of APP and subsequent AD pathologies, the γ-secretase aspartyl protease is a prime target for pharmacological intervention in the progression of AD. Prior to the discovery of a γ-secretase target molecule (presenilins) or resolution of any structural information on the γ-secretase complex, drug discovery efforts had produced a large variety of very potent and selective γ-secretase inhibitors [6, 71]. While many γ-secretase inhibitors failed to differentiate between the cleavage site in substrates (ε-site and γ-sites) and non-selectively

target γ-secretase cleavage of an assortment of substrates, many have subsequently been shown to directly target the presenilins or APP [78].

Many of the peptidic γ-secretase inhibitors contain a hydroxyethylene dipeptide isostere [79, 80] or a difluoroketone moiety [81] which can mimic the tetrahedral *gem*-diol transition state that is formed during the catalytic reaction of aspartyl proteases. These transition state analogue (TSA) inhibitors (e. g. *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester (DAPT), L685458, compound E) target the active site of the enzyme and have been shown to bind presenilins [39,40] and directly interact with the two-presenilin catalytic aspartates, forming the proposed γ-secretase active site [82]. In this manner, the TSA inhibitors have been shown to block substrate access to the active site, and inhibit γ-secretase-mediated cleavage of substrates in both cell-free and cell-based assays. Significantly, in an *in vivo* model of AD, the TSA inhibitor DAPT at a dose of 100 mg/kg lowered brain cortex Aβ levels by 40 % within 3 hours, and 20 % after 18 hours of administration [83].

Another distinct panel of very potent γ-secretase inhibitors encompasses benzodiasepines, benzolactams, sulfones, sulfonamides, and caprolactames which belong to structural classes that do not contain any functional groups known to mimic the transition states of aspartyl proteases and are therefore collectively known as the non-TSA inhibitors [84]. Enzyme kinetics and radio-ligand binding studies indicate that these non-TSA inhibitors bind to an allosteric site distinct from the enzyme catalytic active site. The non-TSA inhibitors include semagacestat (LY-450139; Eli Lilly) which has progressed to the clinic [85], and a phase III study due to complete in 2012 will further determine whether this compound (LY-450139) has therapeutic potential in AD.

With an increased appreciation for the complexity of γ-secretase complexes and functionality comes the realisation that an ideal therapeutic γ-secretase inhibitor should either exclusively or selectively reduce APP processing and subsequent generation of Aβ peptides with minimal impact on other γ-secretase substrates. A subset of non-steroidal anti-inflammatory drugs (NSAIDs; ibuprofen and sulindac) and their derivatives have been shown to diminish the production of $A\beta_{42}$ and associated Aβ pathology without affecting cleavage of alternative substrates. These gamma secretase modulators (GSMs) reduce $A\beta_{42}$ levels and in parallel enhance production of the APP-derived carboxyl-terminal truncated peptides such as $A\beta_{38}$, $A\beta_{37}$ and $A\beta_{34}$ in both cultured cells and APP transgenic animals [78, 86, 87]. Importantly and in contrast to γ-secretase inhibitors, independent reports have indicated that the effects of GSM are not accompanied by inhibition of γ-secretase-mediated generation of the intracellular cytoplasmic domains of APP or other γ-secretase substrates, including Notch or ErbB-4 receptors [88–90]. Neither the inhibition of cyclooxygenase (COX) nor peroxisome proliferator-activated receptor-γ (PPAR-γ) are involved in the Aβ-lowering effects of GSM, but rather it has been shown that GSMs bind to an allosteric site discrete from the γ-secretase active site/transition state analogue inhibitor-binding site [91], and some GSMs have been shown to bind to APP in the Aβ region, on or near the GXXXG motif [78, 87]. Significantly, further *in vivo* studies have demonstrated that tarenflurbil (*R*-flurbiprofen), a non-NSAID $A\beta_{42}$-lowering GSM, has serious potential as a new AD therapeutic [87, 88, 90].

An alternative therapeutic approach that has emerged from kinetic- and structure-based studies is to develop compounds that disrupt the interaction between the substrate and the γ-secretase enzyme complex. Nicastrin is involved in the recognition of γ-secretase substrates and presenilin binds to its substrates on a 'docking site' that is distinct form the catalytic enzyme active site. A subsequent conformational transition is then required to translocate the substrate to the active site. Consistent with this approach, TSA-inhibitor-bound and inactivated γ-secretase complexes can still interact with APP [92]. Based on the assumption that distinct γ-secretase substrates have discrete 'docking sites', substrate specificity could be achieved by selectively interfering with discrete substrate-enzyme-complex binding. The fact that it has been shown that Notch and APP do not compete for presenilin binding [93] and specifically designed helical peptides selectively inhibit γ-secretase cleavage of APP over Notch [94] adds credibility to this therapeutic approach. These and other therapeutic approaches (Dimebon (Medivation) [95, 96], PBT2 (Prana Biotechnology) [97, 98], TRx-0014 (TauRX), Gammagard (Baxter International)) highlight several promising therapeutic strategies and drugs with novel mechanisms of action that are being pursued with the aim of favourably modifying disease progression.

Presenilin-Interacting Proteins

Despite this strong evidence that presenilins are the catalytic core of γ-secretase complexes, it has also been shown that only a small proportion (<14 %) of total PS1 appears to be engaged in an active γ-secretase complex [99]. These findings corroborate the suggestions that beyond γ-secretase activity and generating Aβ, the presenilins mediate other bio-

logical functions and that FAD mutations have multiple pathological effects.

In addition to the presenilin-binding partners that form the γ-secretase complexes, PS1 and PS2 can differentially interact with numerous intracellular proteins independent of their ability to function within the γ-secretase complexes and so have been reported to function in the regulation of a diversity of cellular processes including development and tissue homeostasis (Table 1) [3, 4, 9, 10, 12]. Importantly, though there is a high degree of homology between both presenilins, the sequence divergence of the hydrophilic cytosolic domain in PS1 and PS2 enables them to interact with overlapping and different intracellular binding partners. Consistent with this notion, PS1 and PS2 appear to engage in independent high molecular weight protein complexes and biological functions including cell viability, calcium homeostasis, the unfolded protein response (UPE) and ER stress, protein trafficking and gene transcription [2–4, 9, 10]. Though the precise biological relevance of presenilin interaction with some proteins remains obscure, it is evident that certain proteins that bind to the presenilins are associated with the γ-secretase complexes (nicastin, Aph-1, Pen-2, TMP21 and CD147) or are known γ-secretase substrates (APP, Notch, Jagged, E-cadherin, N-cadherin and Ire1), indicating important functions in cell proliferation, development and tissue homeostasis, while other presenilin-associated proteins can likewise be grouped on functionality; for example presenilins bind to glycogen synthase kinase 3β (GSK3β) [100, 101], its upstream regulator, the p85 subunit of phosphatidylinositol 3-kinase (PI3K) [102] and two substrates of GSK3β, tau [101] and β-catenin [103]. These events strongly implicated and have subsequently lead to the identification and characterisation of an important role for presenilins in regulating signalling events involving GSK3β and its substrates [102]. Likewise, several presenilin interacting proteins are important for modulating calcium homeostasis [2–4]. Presenilins interact with the ryanodine receptors (RyR and RYR2) [104, 105], sorcin, a modulator of calcium release by ryanodine receptors [106] and calsenilin [107], a calcium-binding protein associated with calcium-dependent apoptosis. Through the characterisation of these interactions, an important role for presenilins in modulating calcium homeostasis has been identified. Importantly, certain presenilin-interacting proteins can also occasionally influence the activity of γ-secretase and production of Aβ peptides [108]. For example, independent reports have demonstrated that the presenilins interact with and are regulated post-translationally by SEL-10-mediated ubiquitination, which affects turnover of presenilin proteins and increases the production of Aβ peptides [108]. Likewise, the reported association between calsenilin and presenilins is proposed to increase γ-secretase activity in both culture cells and cell free systems [109]. However, if the interaction between PS1 and β-catenin is disrupted, it selectively antagonises γ-secretase cleavage of N-cadherin but not APP. It remains to be seen whether other presenilin-binding proteins which interact with the presenilins can influence substrate selective γ-secretase cleavage, or lead to the identification of novel presenilin-associated functions.

In addition to identifying presenilin-interacting proteins, it is equally important to explore and understand the events that regulate or induce the association between presenilins and their binding partners. Independent reports have demonstrated that the presenilins are regulated post-translationally by endoproteolysis [110], caspase-cleavage [111–113], phosphorylation [114–116] and ubiquitination [108, 117]. In addition to regulating presenilin protein half-life, detailed studies have demonstrated that in certain cases posttranslational modification of the presenilins can also influence presenilin's ability to interact with other proteins. For example, GSK3β and CDK5-mediated phosphorylation of PS1 has been shown to regulate PS1 stability [115, 118] and association with β-catenin [116].

Regulated Intramembrane Proteolysis and γ-secretase

In recent years a highly conserved system of membrane-to-nucleus signalling has emerged [119, 120]. This novel mechanism, termed regulated intramembrane proteolysis, is quite different from the classic model, which proposes the mediation of receptor-initiated signals through a cascade of intracellular signals and intermediate messengers. In contrast, regulated intramembrane proteolysis involves the selective enzymatic cleavage of type I or II membrane proteins, involving sequential cleavage events [121]. The first cleavage is referred to as ectodomain shedding and occurs within the ectodomain at a peptide bond close to the transmembrane domain. This results in the release of a soluble ectodomain into the extracellular milieu and generation of a truncated membrane-associated carboxyl terminal domain (CTD). Subsequently, a second intramembrane cleavage event occurs within the transmembrane domain of the CTD and generates a CTD-derived ICD which acts as a biological effector in alternative intracellular sites. Ectodomain shedding is constitutive or induced in response to ligand stimulation. Members of the disintegrin and metalloprotease (ADAM) family,

Table 1. Presenilin-Binding Proteins. Abbreviations: **ABP-280**, actin-binding protein-280; **Aph-1**, anterior pharynx-defective 1; **APP**, amyloid precursor protein; **BACE**, β-site of APP cleaving enzyme; **BCl**, B-cell lymphoma; **CALP**, calsenilin-like protein; **DNER**, Delta/Notch-like EGF-related receptor; **DSG2**, desmoglein-2; **E-cadherin**, endothelial-cadherin; **FHL-2**, four and a half LIM domains protein 2; **FKBP38**, FK506 binding protein 8 (38kDa); **GRB2**, growth factor receptor bound protein 2; **GSK3β**, glycogen synthase kinase3β; **GFAPε**, glial fibrillary acidic protein-epsilon; **HtrA2**, HtrA serine peptidase 2; **InP_3R**, Inositol trisphosphate receptor; **ZETA**, proteasome Z subunit; **IRAK2**, insulin receptor-associated kinase 2; **LDLR**, low-density lipoprotein receptor; **LRP**, LDL receptor-related protein; **MET1**, methionine requiring-1; **MOCA**, modifier of cell adhesion; **NMDA**, N-methyl-D-aspartate; **NPR-C**, natriuretic peptide receptor-C; **PAG**, proliferation-associated gene; **PARL**, presenilin-associated rhomboid-like protein; **PKA**, protein kinase A; **PSAP**, PS-1-associated protein; **RIP1**, receptor interacting protein-1; **Rab GDI**, Rab GDP-dissociation inhibitor; **RyR**, ryanodine receptor; **sel-10**, suppressor/enhancer of Lin-12; **TRAF6**, tumour necrosis factor receptor associated factor 6; **TMP21**, transmembrane trafficking protein; **VE-cadherin**, vascular endothelial-cadherin.

Binding Partner	Binding domain	Proposed Function	Reference
Acetylcholinesterase	N-terminus	Hydrolyzes the neurotransmitter acetylcholine	[166]
ABP-280	Loop domain	Link actin filaments and membrane glycoproteins	[167]
Aph-1	C-terminus	Assembly and activation of γ-secretase	[48]
APP	C-terminus	Alzheimer's disease; γ-secretase substrate	[168, 169]
BACE	N-terminus	Aspartyl protease	[170]
Bcl-xL	Loop domain	Negative regulator of apoptosis	[171]
Bcl-2	Full length	Negative regulator of apoptosis	[172]
β-catenin	Loop domain	Wnt signalling; cell adhesion	[103]
Calmyrin	Loop domain	Calcium signalling; myristoyl switch	[173]
Calsenilin	C-terminus	Calcium-mediated signalling	[107]
CD147		Regulatory subunit of γ-secretase	[63]
CALP/KChIP4	C-terminus	Member of calsenilin/KChIP protein family	[174]
μ-Calpain	C-terminus	Calcium-dependent thiol protease	[175]
CLIP-170/Restin	N-terminus	Link membrane organelles to microtubules	[176]
DRAL/FHL2/SLIM3	Loop domain	LIM-domain containing protein	[177]
δ-catenin	Loop domain	Wnt signalling; cell adhesion	[103]
E/N cadherin		Cell-cell adhesion	[178]
Filamin	Loop domain	Actin binding protein	[167]
FKBP38	C-terminus	Anti-apoptosis protein	[179]
G-protein G_0	C-terminus	Brain G-protein	[180]
GRB2		Growth factor receptor signalling	[181]
GSK3β	N-terminus	Serine-threonine protein kinase; Wnt signalling,	[101]
GFAPε	N-terminus	Intermediate filament protein	[182]
HC5/ZETA	Loop domain	Subunits catalytic 20S proteasome	[183]
$InsP_3R$		Calcium release channel	[184]
IRAK2		Il-1R and NGFR signalling	[133]
IRE1	Full length	Unfolded protein response; γ-secretase substrate	[185, 186]
LRP	N-terminus	Lipid Metabolism; γ-secretase substrate	[187]
Met1	Loop domain	Putative methyltransferase	[188]
MOCA/PBP		DOCK-1-like protein	[189, 190]
NMDA Receptor		Synaptic plasticity & LTP	[191]
Nicastrin	C-terminus	Assembly and activation of γ-secretase	[45, 157]
Notch 1	Full length	Developmental regulator of cell fate and neurogenesis; γ-secretase substrate	[192]
Omi/HtrA2	C-terminus	Serine protease; pro-apoptotic	[193]
P85	C-terminus	PI3K regulatory subunit	[102]
PAG	Loop domain	Thioredoxin peroxidase	[194]
PAMP/PARL	C-terminus	Putative metalloproteases	[195]
Phospholipase D1	Loop domain	Phospholipase	[196, 197]
Plakoglobin		Cell Adhesion	[198]
PEN2	Full length	Assembly and activation of γ-secretase	[49]

Table 1 *(Continued)*

Binding Partner	Binding domain	Proposed Function	Reference
PKA		Serine-threonine protein kinase; β-catenin phosphorylation	[21]
PSAP	C-terminus	PDZ-like protein; Apoptosis	[199]
RIP1		TNF-α Receptor signalling	[200]
RAB11	Loop domain	GTPase; regulation of vesicular transport	[201]
RabGDI	N-terminus	Rab GDP dissociation inhibitor	[202]
RYR2	C-terminus	Cardiac ryanodine receptor; calcium signalling	[104]
RyR	N-terminus	Mouse brain ryanodine receptor; calcium signalling	[105]
Sorcin	Loop domain	Regulator of ryanodine receptor	[106]
Sel-10		E3 ubiquitin ligase	[108]
Syntaxin 1A	Loop domain	Synaptic plasma membrane protein	[203]
Syntaxin 5	Full length	ER-Golgi vesicular transport	[204]
tau	N-Terminus	Microtubule-binding protein	[101]
Telencephalin	C-terminus	Neuron-specific adhesion molecule	[205]
TRAF6		IL-1R1 and NGFR signalling	[133, 206]
TMP21		Protein transport	[61]
TPIP	N-terminus	Tetratricopeptide repeat-containing protein	[207]
Ubiquilin	C-terminus	Ubiquitin domain-containing protein	[208]
X11α & X11β	C-terminus	Neuronal adapter proteins	[209]

matrix metalloprotease and the aspartyl proteases β-site APP-cleaving enzymes 1 and 2 (BACE1 and BACE2) are responsible for ectodomain shedding of most reported substrates. The proteases associated with intramembrane proteolysis are multi-transmembrane intramembrane-cleaving proteases (I-CLiPs) which are highly selective, and substrate recognition involves key conserved amino acid residues within the enzyme active site that determine the substrate to be cleaved as well as the rate and location of this cleavage event [2]. The γ-secretase protease is the founding member of a class of I-CliPs that consists of signal peptide peptidase (SPP), site 2 protease (S2P) and rhomboids [122]. Uniquely, all I-CliPs hydrolyse their particular substrates within their transmembrane domains, and the protease active site is located either within the intramembranous hydrophobic region or located at the membrane-cytosol interfaces of the proteases.

Diversification of γ-secretase substrates

Since the discovery of APP and its processing by the γ-secretase complexes, γ-secretase has been shown to be responsible for the cleavage of approximately sixty-six type I integral membrane proteins [1–3] (Table 2). However, we are still far from a complete understanding of how γ-secretase activity is regulated, how the protease-complexes recognise substrates and the physiological functions of all of these proteolytic events. From the diverse list of reported γ-secretase substrates (Table 2), it is apparent that γ-secretase substrates do not have one uniting function; for example, endothelial (E)-, neuronal (N)- and vascular endothelial (VE)-cadherins are involved in cell adhesion [123–126]; the insulin receptor and insulin-like growth factor receptor (IGF-R1) are receptor tyrosine kinases [127, 128]; the Notch receptor is involved in developmental biology and cell differentiation [129–131], Notch, VEGFR-1, ErbB4 and IGF-1R are implicated in a range of cellular responses including regulation of cell proliferation, differentiation and angiogenesis [132]; while the interleukin-1 receptors (IL-1R1 and IL-1R2) and major histocompatibility complex (MHC) class I molecule HLA-A2 participate in regulation of the immune response [133–135]. Similarly, the ICDs that are generated following γ-secretase cleavage of the transmembrane proteins do not have a unifying function, though many have been reported to influence cellular signalling processes in some way [2, 3]. Predominantly through the use of pharmacological manipulation and genetic inactivation/deletion of presenilins, independent studies continue to report the existence of novel γ-secretase substrates. For a subset of these purported substrates, including Notch [129, 136], ErbB4 [137] and N-cadherin [138], an emerging and unifying function of regulated intramembrane proteolysis is the regulation of membrane-to-nuclear signalling by generation of ICDs which can function as transcriptional activators or repressors. Others, such as the ICDs derived from

the $p75^{NTR}$ and syndecan-3, suggest that presenilin-dependent regulated intramembrane proteolysis may also function in non-nuclear signalling events such as regulating formation/disassembly of cell-surface heteromeric receptor complexes [139], while γ-secretase cleavage of E-cadherin contributes to adherens junction disassembly, whereas others are inherently labile species, produced as a 'by-product' of proteolysis and receptor degradation [11]. Despite the preliminary and inconclusive nature of some studies and the apparent diversity of γ-secretase substrates, from comparative biochemical and genetic studies it is becoming apparent that regulated intramembrane proteolysis is critically involved in the regulation of several pathways of signal transduction and essential for embryonic development, tissue homeostasis, defects which contribute to the onset of disease [1, 2, 132, 140]. Developmental abnormalities have been described in PS1-deficient mice; these include impaired neurogenesis, altered somitogenesis, impaired vasculogenesis, defective angiogenesis, malformation of the axial skeleton and manifestation of autoimmune disease and oncogenesis [14–28]. A strong association between presenilins and the immune system has been reported from phenotypic characterization of several *in vivo* models of presenilin biology and identification of γ-secretase substrates. The phenotype of $PS1^{+/-}$ $PS2^{-/-}$ 'partial deficient' mice revealed a novel *in vivo* function for presenilins in the immune system [19], which has been substantiated in T cell and B cell-specific presenilin-deficient animals [25, 26]. The $PS1^{+/-}PS2^{-/-}$ animals are relatively normal up to about 6 months of age, after which the majority of the mice develop a systemic lupus erythematosus (SLE)-like autoimmune disease [19]. Ablation of presenilin in T cells results in inefficient generation of CD4+ T cells, a phenotype that correlates with evidence of impaired T cell receptor (TCR) signalling [25], while selective loss of presenilins in B cells compromises responsiveness to LPS and B cell antigen receptor-induced proliferation and signal transduction events [26]. Likewise, abnormal vasculogenesis and angiogenesis have been reported in PS1 and Aph-1a deficient mice [68, 141]. These phenotypic characteristics are further emphasised at the genetic and biochemical level where we and others have shown that γ-secretase is able to regulate immune function through cleavage and nuclear translocation of the C-terminal domain of HLA-A2, a MHC class I protein [135], IL-1R type 1 (IL-1R1) [133] and IL-1R type 2 (IL-1R2) [134]. Furthermore, a role for presenilins in vasculogenesis is also supported by the observation that Notch [142], vascular endothelial growth factor receptor-1 (VEGFR-1) [66], vascular endothelial (VE)-cadherin [126], and ErbB4 [143] are γ-secretase substrates and functionally modulated by regulated intramembrane proteolysis [132]. We have recently reported that the insulin-like growth factor-1 receptor (IGF-1R) is modulated by regulated intramembrane proteolysis [128], significant because IGF-1 signals through IGF-1R, is pro-angiogenic and associated with diabetic retinopathy [132].

Determining how γ-secretase complexes recognise and differentiate between their many substrates is essential for understanding the role of presenilins in biological processes. There is accumulating evidence that suggests γ-secretase cannot efficiently cleave full-length type I proteins, but that proteolysis of γ-secretase substrates is dependent upon a previous ectodomain shedding cleavage event which liberates a soluble ectodomain and generates a membrane-bound CTD which is subsequently cleaved by γ-secretase [2, 144]. Ectodomain shedding may remove inhibition to γ-cleavage, but more likely allows access to nicastrin which has been shown to function as a γ-secretase substrate docking site by selectively interacting with the N-terminus of truncated substrates, post-ectodomain shedding. Alternatively, ectodomain shedding may allow for subtle conformational changes in the transmembrane α-helix and thereby allow the γ-secretase complexes access to the substrate cleavage site. This is consistent with sequence analysis studies of several different γ-secretase substrates, which indicate that a conserved consensus sequence for γ-secretase cleavage is very relaxed or does not exist at all, suggesting that γ-secretase cleavage may be dictated to some extent by the conformational topography of the substrate's transmembrane domain [145]. Whatever the specifics on regulation and consequence of ectodomain shedding, it has been shown that the size of the ectodomain also determines whether a type I membrane protein is a γ-secretase substrate or not [144]. Recently, compelling preliminary studies have shown that the spatial segregation of γ-secretase and its substrates serves as a regulatory step controlling γ-secretase. It is proposed that γ-secretase localised in non-raft membranes facilitates proteolysis of diverse substrates during development, but that the translocation of γ-secretase to lipid rafts in adults ensures more selective substrate cleavage [146]. The occurrence of constitutive and ligand-induced cleavage of APP, Notch [129, 147], $p75^{NTR}$ [148] and IL-1R1 [133] has been demonstrated suggesting that some degree of regulation surrounds ligand-induced proteolysis. A well-documented mediator of regulated ectodomain shedding and γ-secretase cleavage is protein kinase C (PKC), whether stimulated directly by phorbol esters, or as a consequence of the activation of receptors coupled to phosphoinositide turnover [149]. Recently, posttrans-

Table 2. List of known γ-secretase substrates. Abbreviations: **APLP**, amyloid precursor-like protein; **APMA**, 4-aminophenylmercuric acetate; **ApoER2**, apolipoprotein E receptor-2; **BDNF**, brain-derived neurotrophic factor; **CASK**, calmodulin-associated serine/threonine kinase; **CSF-1**, colony-stimulating factor 1; **DBP**, vitamin D-binding protein; **DCC**, deleted in colorectal cancer; **EGF**, epidermal growth factor; **EIPA**, 5-(*N*-ethyl-*N*-isopropyl)amiloride; **GHR**, growth hormone receptor; **GluR**, glutamate receptor; **GSK-3β**, glycogen synthase kinase 3β; **HA**, hyaluronic acid; **HLA**, human leukocyte antigen; **IFNaR2**, subunit of the type I IFN receptor; **IGFI-R**, insulin-like growth factor I receptor; **IL-1R**, interleukin-1 receptor; **ICD**, intracellular domain; **JNK**, c-Jun N-terminal kinase; **LAR**, leukocyte-common antigen related; **LPS**, lipopolysaccharide; **LRP**, low density lipoprotein receptor-related protein; **MAG**, myelin associated glycoprotein; **MCD**, methyl-β-cyclodextrin; **NCAM-L1**, neural cell adhesion molecule L1; **ND**, not determined; **NMDA**, N-methyl-D-aspartate; **NRADD**, neurotrophin receptor alike death domain protein; **NRG-1**, neuregulin-1; **NTR**, neurotrophin receptor; **PDGF**, platelet-derived growth factor; **PMA**, phorbol 12-myristate 13-acetate; **RA**, retinoic acid; **RPTP**, receptor protein tyrosine phosphatase; STS, staurosporine; **TCF**, T cell factor; **TFP**, trifluoperazine; **TM**, transmembrane domain; **TPA**, 12-O-tetradecanoylphorbol 13-acetate; **UPR**, unfolded protein response; **VEGF**, vascular endothelial growth factor; **VGSC**, voltage-gated sodium channel; **VLDL**, very low density lipoprotein.

Substrate	Function	Mechanism regulating ectodomain shedding/γ-secretase cleavage	Function of cleavage/ ICD	References
Alcadeins (α1, γ)	Cadherin-related membrane protein – unknown role	Constitutive?	Gene transactivation activity	[210]
APLP1/2	Synaptogenesis/neuritic outgrowth?	Constitutive? RA and BDNF-induced	Gene transactivation activity	[211–215]
ApoER2	Endocytic and signalling receptor	Ligand & PMA-induced	Unknown	[216–218]
APP	Unresolved physiological role Alzheimer's disease pathology	Constitutive? PMA- APMA-, RA- & BDNF-induced	Gene transactivation activity	[213, 214, 219–225]
Bri2 (Itm2b)	type II-oriented transmembrane protein	Constitutive? ADAM10-induced		[226]
CD43	Cell adhesion molecule	PMA-induced	Unknown	[227, 228]
CD44	Cell adhesion molecule	TPA-, Ionomycin-, Anti-CD44- & HA-fragment-induced	Gene transactivation activity	[74, 229–233]
CSF-1 Receptor	Protein-tyrosine kinase	LPS- and PMA-induced	Unknown	[234]
CXCL16 & CX3CL1	Transmembrane chemokine ligands	ADAM-10	Unknown	[235–238]
DCC	Netrin-1 receptor	Unknown	Gene transactivation activity	[239, 240]
Delta	Ligand of Notch receptor	Notch-binding and APMA-induced	Gene transactivation activity	[99, 241–243]
DNER	Neuronal Notch receptor ligand	Constitutive	Unknown	[244]
DSG2	Structural component of desmosomes	Constitutive	Unknown	[244]
Dystroglycan	Member of multiprotein dystrophin-glycoprotein complex	Constitutive	Unknown	[244]
E-Cadherin	Cell adhesion molecule	Ionomycin- and STS-induced	Disassembly of cadherin-based adherens junctions Gene transactivation activity	[123]
EphB2	Receptor Tyrosine Kinase	Ligand, ADAM10 and ionomycin	Unknown	[245]
Ephrin-B1	Ligand of Eph receptor tyrosine kinase	Constitutive PMA-induced	Formation of cellular protrusions enriched in polymerized actin	[246]
Ephrin-B2	Receptor tyrosine kinase	EphB-induced	Src activation	[247]
Erb4	Receptor tyrosine kinase	Constitutive Ligand-and TPA-induced	Gene transactivation activity Apoptosis	[143, 248–250]
γ-Protocadherins	Cell adhesion molecules	Constitutive PMA-, ionomycin and STS-induced	Gene transactivation activity	[251–253]
GHR	Growth hormone receptor	PMA- and PDGF-induced Calf serum treatment of serum-starved cells	ICD accumulates in nucleus Gene transactivation activity?	[254, 255]
GluR subunit 3	Subunit of glutamate receptor	Constitutive? Unknown	ICD associates with GluR subunits	[256]
HLA-A2	MHC Class I protein	Constitutive?	Unknown	[257]
IFNaR2	Subunit of the type I IFN-α receptor	Constitutive Ligand-, PMA- & EGF-induced	Gene transactivation activity	[258, 259]

Table 2 *(Continued)*

Substrate	Function	Mechanism regulating ectodomain shedding/γ-secretase cleavage	Function of cleavage/ ICD	References
Insulin receptor	Receptor tyrosine kinase	Constitutive and PMA	ICD accumulates in nucleus	[127]
IGFI-R	Receptor tyrosine kinase	Constitutive and PMA	Unknown	[128]
Ire1 α/β	ER transmembrane protein with kinase and endoribonuclease activities	Constitutive, Stimulated by UPR induction	ICD accumulates in nucleus Gene transactivation activity?	[186]
IL-1R II	Cytokine receptor	Constitutive PMA-induced	Unknown	[134]
IL-1RI	Cytokine receptor	Constitutive Ligand-and PMA-induced	Unknown	[133]
Jagged	Ligand of Notch receptor	Constitutive? PMA-induced	Gene transactivation activity	[241, 242]
LAR	Receptor tyrosine phosphatase	Constitutive? PMA-induced	ICD accumulates in nucleus	[260]
LDLR	Lipoprotein receptor	Constitutive	Unknown	[244]
LRP	Endocytic and signalling receptor	PMA-induced	Gene transactivation activity	[261]
Megalin	Endocytic and signalling receptor	Constitutive Ligand (DBP) -,PMA- and EIPA-induced	Unknown	[262]
N-Cadherin	Cell adhesion molecule	Constitutive Ionomycin-, PMA-, MCD- and STS-induced stimulation of NMDA receptor	Gene transactivation activity Promotes degradation of the transcription factor CBP	[138]
NCAM-L1	Cell adhesion molecule	Constitutive PMA- and MCD- induced	Unknown	[263, 264]
Nectin-1α	Adherens junction protein	TPA-induced	Rearrangement of cell-cell junctions? Promotion of cell migration?	[265]
Notch 1–4	Signalling receptor	Ligand-induced	Gene transactivation activity	Reviewed in [130]
NPR-C	Natriuretic peptide receptor	Metalloprotease-induced	Unknown	[244]
NRADD	Death-receptor-like protein	PMA- and APMA-induced	ICD translocates to the nucleus	[266]
NRG-1	Growth factor	Binding to ErbB4-ErbB2 receptors Cell depolarization	Gene transactivation activity	[267]
$P75^{NTR}$	Neutrophin receptor	Constitutive Ligand-, PMA- and TPA-induced	Gene transactivation activity Receptor-signal termination Apoptosis	[268, 269]
PLXDC2	Nervous system protein	PMA-induced	Unknown	[244]
Polyductin/ fibrocystin	Cell Receptor	Constitutive and PMA	ICD accumulates in nucleus	[270]
RPTP (κ, μ)	Cell adhesion receptors	TFP-, ionomycin-, cell contact formation and anti-RPTPκ-induced	Gene transactivation activity	[271]
SorLA	Sorting receptor	Constitutive Ligand- and PMA-induced	Gene transactivation activity	[272]
SorCS1–3	Sorting receptors	Constitutive PMA-induced	Unknown	[273, 274]
Sortilin	Sorting receptor	Constitutive PMA-induced	Unknown	[273, 274]
Syndecan 1–3	Cell surface heparan sulfate proteoglycan co-receptor	Ligand-, Forskolin- and PMA-induced	Release of ICD regulates subcellular distribution of CASK	[244, 275]

Table 2 *(Continued)*

Substrate	Function	Mechanism regulating ectodomain shedding/γ-secretase cleavage	Function of cleavage/ ICD	References
Tie1	Receptor-tyrosine kinase	Constitutive VEGF- and PMA-induced	controlling signalling by Tie2	[276]
Tyrosinase and tyrosinase-related proteins 1–2	Type I membrane proteins specialized in pigment synthesis	Unknown	Localization of Tyr and related proteins	[277]
VGSC β1–4 subunits	Subunits of sodium channel	Constitutive TPA-induced	DAPT inhibits β2-mediated cell-cell adhesion and cell migration	[278, 279]
Vasorin	TGF-β inhibitor	Metalloprotease-induced	Unknown	[244]
VE-Cadherin	Cell adhesion molecule	ADAM-10	Endothelial permeability	[126]
VLDL Receptor	Endocytic and signalling receptor	Ligand-induced	Unknown	[217]
VEGFR-1	Growth factor receptor	VEGF and PEDF-induced	Negative regulation of angiogenesis	[66, 132]

lational modification of γ-secretase substrates has also been shown to regulate substrate cleavage, where ubiquitination of human Notch-CTD [150] and palmytolation of the $p75^{NTR}$ [151] are a prerequisite for γ-secretase cleavage. An important factor to consider is that ubiquitination of target proteins is also a regulatory event in receptor endocytosis and endosomal trafficking. PKC-mediated effects on vesicular trafficking increase the formation of APP-containing secretory vesicles [152], and inhibition of receptor-mediated endocytosis has been shown to increase APP and Notch receptor shedding and reduce γ-secretase mediated generation of Aβ and Notch intracellular domain (NICD) [131, 153–155]. This suggests that accelerated trafficking of substrate to the cell surface might underlie the increase in α-secretase cleavage induced by PKC, or that inhibition of endocytosis could increase substrate cleavage by prolonging the interaction of substrates with cell surface α-secretase and prevent access to γ-secretase on the sorting endosome. In this regard, it has been shown that catalytically inactive presenilin or γ-secretase inhibitors affect cell surface accumulation of βAPP [156, 157]. Similarly, PS1 has been shown to regulate epidermal growth factor receptor (EGFR) turnover and signalling in the endosomal-lysosomal pathway [158]. Subsequent studies have shown that γ-secretase cleavage of $p75^{NTR}$ and Notch occurs in endosomes [153, 159, 160] and that γ-secretase cleavage and signalling of endogenous Notch is reduced in *Drosophila* mutants that impair entry into sorting endosomes [131]. Whether specific groups or all γ-secretase substrates are regulated in similar ways remains to be seen.

Future Perspectives

Despite significant advances in the identification and functional characterisation of each γ-secretase complex component, the assembly, regulation and function of the γ-secretase complexes have emerged to be considerably more complex than initially predicted, with the existence of several γ-secretase complex subtypes and a diversity of interacting partners and substrates [2, 4]. Coupled with the highly hydrophobic nature of the complexes and difficulties experienced in obtaining a structure of the γ-secretase complexes [161–165], it is apparent that a more comprehensive understanding of the regulation and mechanism of the γ-secretase complexes will be of significant importance for cell biology and for deciding anti-γ-secretase therapeutic strategies. Pharmacological inhibitors that directly target the catalytic site of γ-secretase and do not discriminate between potential subtypes of γ-secretase complexes nor γ-secretase substrates will most-probably cause major deleterious or undesirable side effects because of their interference with other γ-secretase-mediated signalling events. The next generation of γ-secretase inhibitors and modulators will most likely focus on compounds that specifically target APP processing, but which can also discriminate between distinct γ-secretase substrates. Recently, significant progress has been made towards achieving this goal [78, 86, 87, 140]. These and related therapies will function through modulation of γ-secretase and enzyme interactions, by inhibiting select substrate binding to the enzyme complexes, or by preventing the substrate from accessing the catalytic core. Coupled with a better structural and mechanistic understanding of γ-secretase, this therapeutic approach should ultimately allow for the development of more potent and substrate-selective γ-secretase modulators.

Acknowledgements. This work was supported by a grant from Science Foundation Ireland (02/IN1/B218). We apologise to authors whose relevant work we did not refer to directly due to space limitations.

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