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Review

Understanding BACE1: essential protease for amyloid-β production in Alzheimer's disease

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Abstract. The identification of the aspartic protease BACE1 (β-secretase) was a defining event in research aimed at understanding the molecular mechanisms that underlie Alzheimer's disease (AD) pathogenesis. This is because BACE1 catalyses the rate limiting step in the production of amyloid-β (Aβ) the principal component of plaque pathology in AD, the excessive production of which is believed to be a primary cause of neurodegeneration, and cognitive dysfunction in AD. Subsequent discoveries showed that genetic deletion of BACE1 completely abolishes Aβ production and deposition *in vivo,* and that BACE1 activity is significantly increased in AD brain. In this review we present current knowledge on BACE1, discussing its structure, function and complex regulation with a view to understanding BACE1 function in the brain, and BACE1 as a target in blocking aberrant Aβ production in AD.

Keywords. Alzheimer's disease, BACE1, aspartic protease, amyloid-beta, neurodegeneration.

Introduction

Alzheimer's disease (AD) is a slowly progressive neurodegenerative disease that is the most common cause of dementia in the elderly population. One of the major challenges in AD research is identifying the primary mechanisms that mediate the progressive and insidious neurodegeneration that characterises the disease, in order to develop better therapeutic strategies. The clinical features of AD are accompanied by progressive neuronal and synaptic loss in brain regions associated with memory and higher mental function, and two defining pathological hallmarks, amyloid plaques and neurofibrillary tangles (NFTs). NFTs are insoluble aggregates of the microtubule-binding protein tau, and amyloid plaques are primarily composed of aggregated Aβ (amyloid β-peptide) 40 – 42 amino acids in length. A large body of evidence now indicates that amyloid−β (Aβ) production is a critical feature in the deterioration of the brain during AD [1]. Thus, processes that control Aβ production, aggregation and clearance are centre stage in efforts to understand and develop effective treatments for the disease. Critical to the understanding of Aβ production is the control of its cleavage from the type I transmembrane protein APP (amyloid precursor protein), which occurs by sequential proteolysis by β and γ secretase to generate the N and C termini of Aβ respectively. The enzyme β-secretase, now more commonly known as BACE1, is especially significant in this regard as its cleavage of APP at the β site initiates and is the rate limiting step in the production of Aβ. Thus, detailed knowledge of the identity, integrity, activity and regulation of β−secretase in

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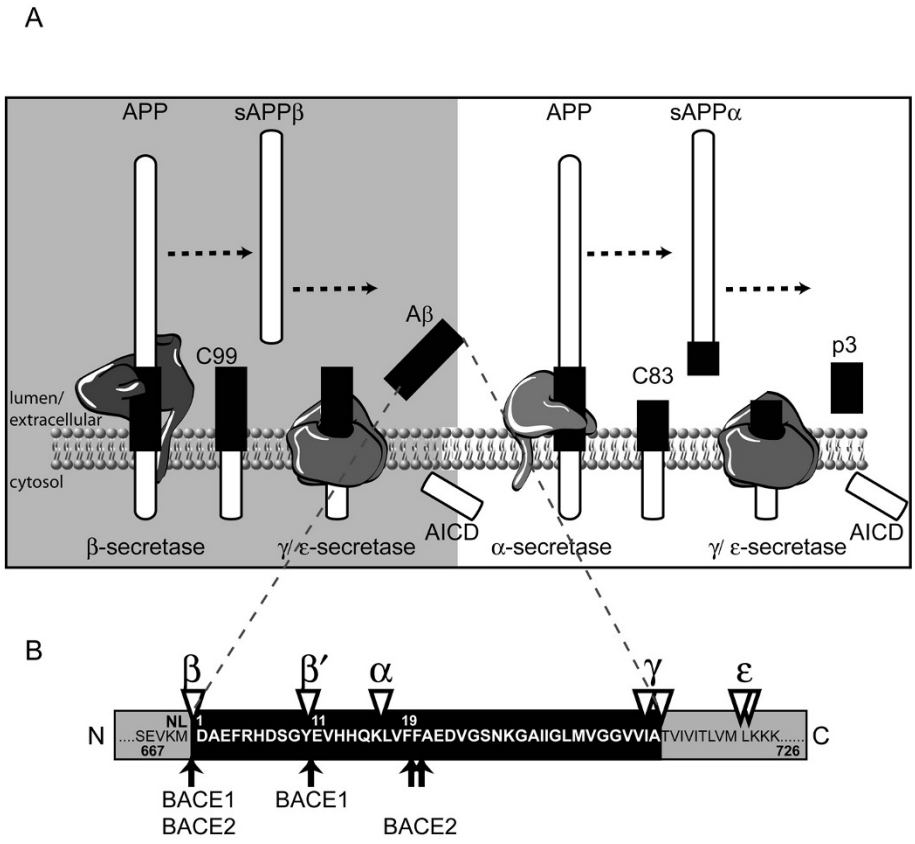

Figure 1. Amyloidogenic and non-amyloidogenic processing of APP. (*A*) During amyloidogenic processing (left hand side), β-secretase cleaves APP, to generate a 99 amino acid membrane bound stub (C99) and soluble sAPPβ. C99 is further cleaved by γ-secretase within the membrane, thereby liberating Aβ. In non-amyloidogenic processing (right hand side) α-secretase cleaves APP generating soluble sAPPα and an 83 amino acid (C83) membrane stub which is subsequently cleaved by γ-secretase to generate non amyloidogenic p3. The carboxyl terminal fragment AICD can be generated from both processing pathways through γ / ε-secretase. (*B*) Schematic representation of the primary amino acid sequence of APP from amino acids 667 – 726 (APP-770 numbering) with Aβ (1 – 42) shown as bold text within the shaded box. The APP_{SWE} mutation is shown next to β-secretase processing site. The β, β', α, γ and ε-secretase processing sites are highlighted as arrowheads, and the known cleavage sites of BACE1 and BACE2 are shown as arrows.

the human brain and, in particular, in AD is essential in the understanding and treatment of AD pathogenesis.

Proteolytic cleavage of APP and Aβ production

Aβ derives from the sequential cleavage of the single transmembrane amyloid precursor protein by the enzymes β and γ secretase (Fig. 1). In this amyloidogenic pathway, APP is cleaved by β−secretase, between Met^{671} and Asp^{672} (APP 770 numbering) to generate a secreted amino terminal APP derivative (sAPPβ), and a membrane inserted C-terminal fragment (β-CTF), 99 amino acids long (C99). C99 is further cleaved by the intramembranous γ-secretase cleavage mainly around APP residues 711-713 to produce the Aβ peptide (Fig. 1), mostly Aβ 1-40 amino acids long (Aβ40), with a small amount of Aβ 1-42. Aβ 1-42 is increased in most cases of autosomally dominant inherited forms of familial AD (FAD), is more amyloidogenic, and is believed to be a major neurotoxic species in AD [1]. β-secretase processing of APP can also occur within the Aβ sequence, at the β′ site between Tyr^{681}-Glu^{682} (Fig. 1). The alternative non-amyloidogenic competing pathway cleaves APP within the Aβ sequence between amino acid 16 and 17, generating a secreted APP derivative sAPPα, and a shorter CTF of 83 amino acids (C83), which is subsequently cleaved by γ-secretase to form a non amyloidogenic 3 kDa peptide (p3). A carboxy terminal fragment (AICD) can be generated from both processing pathways through γ/ε secretase processing (Fig. 1). Although Aβ is produced normally in the CNS, the α and β pathways may compete for APP substrate and APP is believed to be cleaved preferentially through the non-amyloidogenic pathway in normal brain, with the balance shifting to the amyloidogenic pathway in AD.

History, discovery and identification of β-secretase

From the initial discovery that Aβ was a normal proteolytic product of APP, it was surmised that two enzymes must exist to initiate cleavage of this small 1-40/42 peptide from its larger single transmembrane precursor protein. The unknown enzyme cleaving APP at the beginning of Aβ, prior to amino acid 1, was called β-secretase. In 1992, the discovery of the Swedish APP (APP_{SWE}) double mutation, located at -2 and -1 (Lys^{670}-> Asn/Met^{671}-> Leu) of the β-secretase site [2], and a cause of rare autosomal dominant forms of AD, drew attention to the possi-

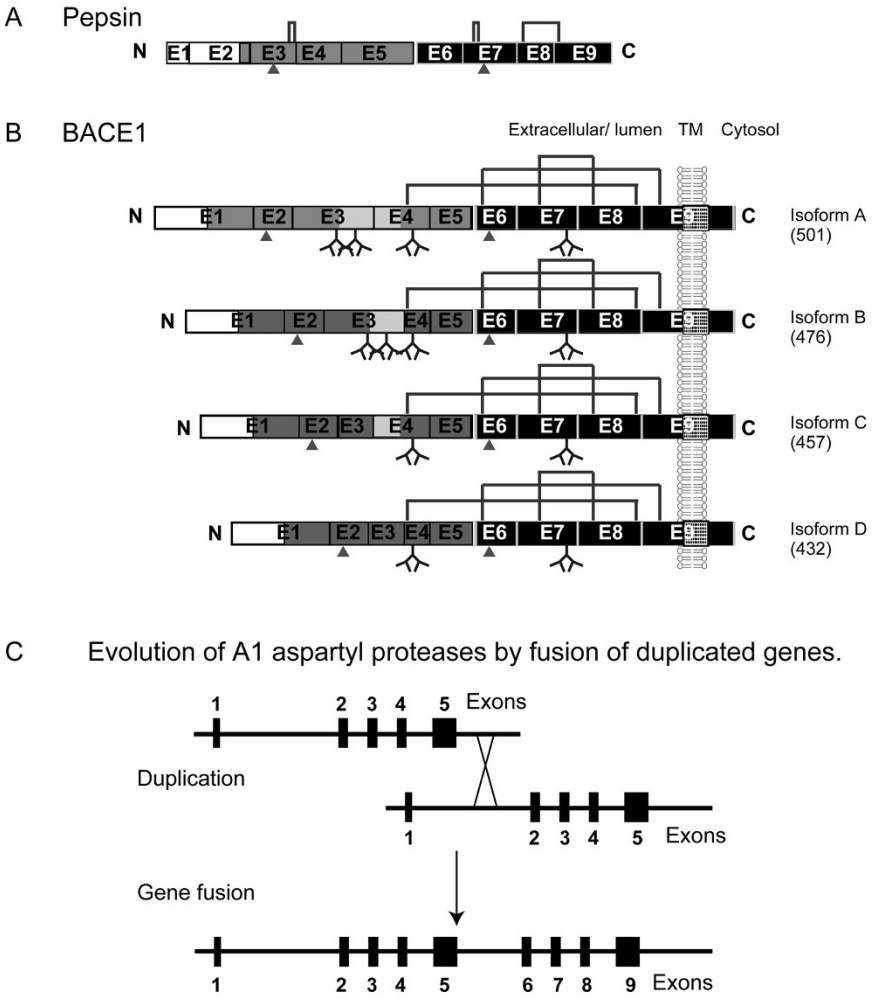

Figure 2. Exon distribution and general structure of BACE1. Schematic representations of A1 aspartic protease family members, pepsin (*A*) and BACE1 (*B*) drawn to scale. E1 - E9 represent exon numbering. Pro-domains for each protein are shown as white boxes. N-terminal catalytic lobes are coloured dark grey and the C-terminal catalytic lobes are coloured as black. The light grey coloured boxes in BACE1 are regions excised in the differing splice variants. Disulphide bridge distributions are shown as straight connecting lines and the active sites aspartates are indicated with triangles. Note the differences in location of the disulphide bridge Cys residues between pepsin and BACE1. Branches indicate locations of potential N-glycosylation sites (Asn^{153}, Asn^{172}, Asn^{223} and Asn^{354}) for BACE1. Isoform name and number of amino acids are shown to the right of the BACE1 illustrations. (*C*) Proposed mechanism for the evolution of the 9 exon structure of mammalian A1-APs. As a result of a gene duplication event and fusion by unequal homologous recombination the ancestral AP gene was created. Exons have a general organisation of 1:4:4 in the new gene when compared to the original genes (adapted from [36]).

bility that this mutation could cause AD by being more favourable to β-secretase cleavage. This was substantiated in studies showing that APP_{SWE} significantly enhanced the proteolytic activity of β-secretase causing a 10 fold increase in Aβ production [3].

Following an intensive search to identify β-secretase, an aspartic protease fitting all the requirements for β-secretase was identified in 1999 and 2000 by several groups using different methodologies, including affinity purification [4] expression cloning strategies [5], and BLAST searching genomic libraries [6 – 8]. This protease was named memapsin-2 (E.C. 3.4.23.46), and Asp-2 and is now more commonly referred to as BACE1 (β-site APP cleaving enzyme 1). Soon after the discovery of BACE1, a homologue was described and identified by sequence homology searches using genomic clone libraries of the Down's critical region and human EST databases [9–11]. The gene identified was named memapsin-1 (E.C. 3.4.23.45) and is also referred to as Asp-1, ALP-56, CDA-13, DRAP (Down's region aspartic protease), but is now more commonly called BACE2.

Both BACE1 and BACE2 can process APP at the β-site, but BACE2 has a preference to cleave between amino acids 19 and 20 of the Aβ sequence, thus precluding Aβ formation. A number of studies provide strong evidence that BACE1 is the major β-secretase responsible for Aβ generation in the brain. Thus BACE1 cleaves at the β and also the β′ site (between amino acid 10 and 11 of Aβ) of APP and has a higher preference to cleave APP_{SWE} [4 – 7]. *BACE1* mRNA has highest expression levels in the mammalian brain [12], and is found in organelles of the secretory pathway [5] displaying optimal activity at pH 4.5 [13], which is consistent with its detection in acidic organelles of the endosomes and trans-Golgi network where Aβ is predominantly generated [3, 14, 15]. Most importantly, targeted deletion of BACE1 in APP transgenic mice completely abolishes the production and deposition of Aβ [16 – 18].

BACE genes and BACE1 splice variants

The *BACE1* gene is on chromosome-11 at 11q23.2-q23.3 and the *BACE2* gene is located on chromosome-21 at locus 21q22.3 in the Down's obligate region [9–11]. *BACE1* and *BACE2* genes contain nine exons coding for proteins of 501 and 518 amino acids respectively. BACE genes have TATA-less promoters, and bear a strong resemblance to housekeeping genes [19–21]. The nucleotide sequences of *BACE1* and *BACE2* promoter regions differ considerably [21]. *BACE1* promoter activities are highest in neurons compared to non-neuronal cells [22–24], whereas *BACE2* promoter activities are very low in neuronal cells compared to those of *BACE1* [20, 22]. This is in agreement with *BACE1* mRNA levels which are highest in the CNS [5, 8, 25–27], where *BACE2* mRNA is detected at low levels [8, 10–12, 25–28].

A total of 4 splice variants can be synthesised from the *BACE1* gene, encoding BACE1 of 501, 476, 457 and 432 amino acids in size respectively [29] (Fig. 2). The most common normally spliced variant is the largest I-501 amino acid form. Alternative splicing in exon 4 deletes 75 base pairs, to form the I-476 variant, while the last 44 residues encoded by exon-3 are absent in I-457. The combination of residues excised in isoform B and C comprises the smallest I-432 variant. The significance of the BACE1 and BACE2 splice variants is discussed later in this review in the context of activity, post-translational modification and subcellular locations. Unless otherwise stated, further references to BACE1 proteins are to their largest transcript I-501 variants. *BACE1* mRNA translation is also regulated at both the 5′ and 3′ untranslated regions (discussed below).

Molecular evolution of BACE1 and BACE2

BACE1 and BACE2 are suggested to represent recently evolved proteins that may have arisen by ancestral gene duplication [6]. To investigate this we performed BLAST analysis of human BACE1 (P56817 – SwissProt accession number) and BACE2 (Q9Y5Z0 – SwissProt accession number), and have identified novel BACE family sequences in *C. savignyi* (sea squirt), *M. domestica* (opossum), *S. purpuratus* (purple sea urchin), *T. rubripes* (Japanese fugu fish), *T. nigroviridis* (green spotted puffer fish) and *X. tropicalis* (pipid frog) (Fig. 3). We were able to generate a maximum likelihood (ML) tree of BACE1 protein orthologues by first aligning sequences using ClustalW, and refining the alignment by eliminating sequences that failed a Chi-square analysis. This was followed by ML tree generation (using the Multiphyl webserver [30]) shown in Fig. 3. As can be seen from Figure 3, the BACE family of proteins display high conservation across species, and interestingly both BACE1 and BACE2 are vertebrate specific. BACE1 is more tightly conserved than BACE2 showing the presence of shorter branch lengths (Fig. 3). Most strikingly though, the tree shows that BACE1 and BACE2 may have arisen as a result of a possible gene duplication event (hatched circle) from an early ancestor which is present in the sea squirts (*C. savignyi*) and purple sea urchin (*S. purpuratus*). This duplication event may thus have occurred after the diversification of *Craniates* (fish) from *Tunicates* (sea squirts) over 400 million years ago. This would be consistent with current knowledge of genome duplication events, where it is hypothesised that two rounds of whole genome duplication occurred recently in chordate evolution (for review [31]).

In line with this, BACE1 and BACE2 have been shown previously to be associated with regions of the human genome that may have arisen by genome duplication (paralogons) [32]. We also discovered that the region of chromosome-11 where BACE1 resides is paralogous to a region of chromosome-21 where BACE2 resides. Paralogous genes within these regions can be viewed at: http://wolfe.gen.tcd.ie/dup/. Interestingly, both APP on chromosome-21 and the APP homolog APLP-2 on chromosome-11 are present in the same described paralogous regions, suggesting a possible synteny between BACE2 and APP and BACE1 and APLP-2. Examination of the evolution of the secretase processing sites of APP was also performed and shows clearly that the Aβ sequence is highly conserved across vertebrates, as are the secretase cleavage sites of APP. The most conserved order of secretase sites are $\gamma > \varepsilon >$ BACE2 $> \alpha >$ β-site and the β′-site. While this may not reflect the direct order of evolution of enzyme recognition sites and their processing, it does point toward the notion that β-secretase processing of APP may be the most recently evolved.

BACE enzymes compared to other A1 aspartic protease family members.

BACE1 and BACE2 are the newest described members of the A1 aspartic protease family, commonly known as the pepsin family (for a list of enzyme family classification http://merops.sanger.ac.uk/). Human aspartic proteases of this family include pepsin, cathepsin-E, cathepsin-D, renin, pepsinogen-C and napsin. The BACE proteins represent a novel subgroup of this family, being the first reported aspartic proteases to contain a transmembrane domain and carboxyl terminal extension [12], and also possessing unique

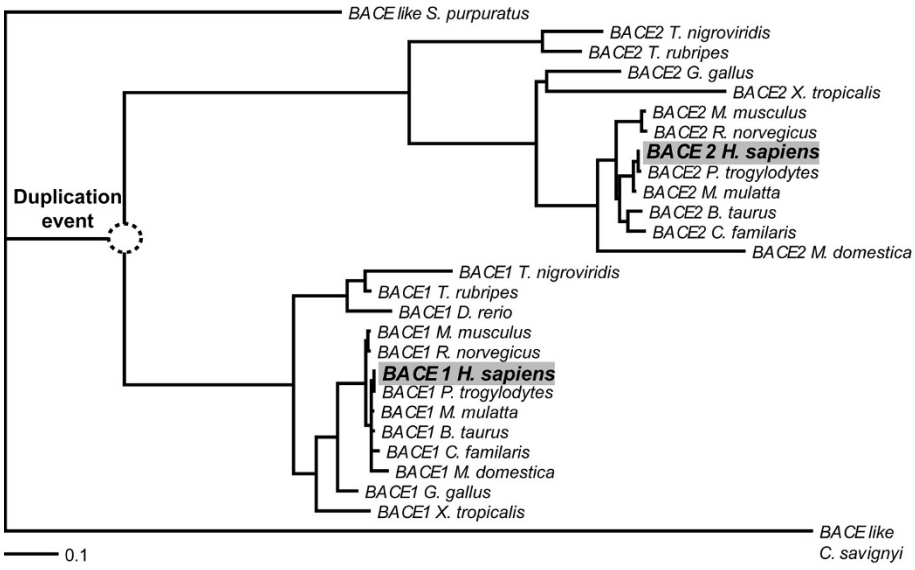

Figure 3. Evolutionary origins of BACE1 and BACE2. Protein Maximum-likelihood (ML) tree generated from aligned BACE sequences identified in human (*H. sapiens*), chimpanzee (*P. trogylodytes*), rhesus monkey (*M. mulatta*), cow (*B. taurus*), dog (*C. familiaris*), mouse (*M. musculus*), rat (*R. novegicus*), opossum (*M. domestica*), pipid frog (*X. tropicalis*), chicken (*G. gallus*), purple sea urchin (*S. purpuratus*), sea squirt (*C. savignyi*) Japanese fugu fish (*T. rubripes*), green spotted puffer fish (*T. nigroviridis*) and zebra fish (*D. rerio*). BACE1 and BACE2 clades show they are vertebrate specific genes and have evolved from a common ancestor present in tunicates (purple sea urchin and sea squirts) by gene duplication after the divergence of craniates from tunicates (hatched circle).

disulphide bridge distribution (Fig. 2) [33, 34]. The eight known functional human A1 aspartic proteases vary in genomic structure, with BACE2 being the largest and napsin the smallest. The nine exons are distributed as 1:4:4, whereby exon-1 codes for a signal peptide and pro-domain, exons 2 – 5 code for the N-terminal catalytic lobe, and exons 6 – 9 code for the C-terminal catalytic lobe (Fig. 2). The 9 exon homologous biolobar structure is believed to have evolved through duplication and unequal fusion of the related ancestral 5 exon encoded A2 family of aspartic proteases (Fig. 3C), including enzymes such as HIV-1 retro-pepsin, which only become active upon homodimerisation [35–37]

Key features of A1 aspartic proteases are their bilobar structure, with an essential catalytic Asp dyad located at the interface of the homologous N- and C-terminal lobes, with maximal enzyme activity occurring in an acidic environment. These Asp residues activate water molecules to mediate nucleophilic attack on the substrate peptide bond [38], and mutation of the catalytic active site aspartic residues abolishes enzyme activity. For BACE1 (I-501) the Asp dyad locates at amino acids, 93 – 96 (D*TGS) and 289 – 292 (D*SGT). A1 aspartic proteases are usually synthesised as inactive pre-pro-enzymes (zymogens), where pro-domain removal is necessary for enzymatic activity. Pro-BACE2 is catalytically inactive, reflecting typical A1 aspartic protease zymogen properties [39]. However, this is not the case for BACE1, which possesses enzymatic activity [40], prior to its cleavage at a pro-protein convertase recognition sequence (Arg^{42}-X-X-Arg^{45}) [41] by furin or a furin like convertase [12, 42–44]. All A1-aspartic proteases have six conserved Cys residues which form three disulphide bridges. However, as with BACE2, only two of the six Cys residues (Cys^{278} and Cys^{330}) for BACE1 occur at similar positions to the other A1 aspartic proteases, with the other 4 Cys, and all three disulphide bridges (Cys^{216}/Cys^{420}, Cys^{380}/Cys^{330} and Cys^{278}/Cys^{44}) occurring at considerably different positions to other A1 aspartic proteases [12] (Fig. 2). BACE1 disulphide bridges maintain correct folding and orientation of BACE, but are not vital to its enzymatic activity [33, 34]. In addition, the unique transmembrane regions of BACE1 and BACE2 confer an evolutionary specialisation, allowing their sequestration to membranes of specific organelles and the plasma membrane. This serves to expose their catalytic lobes to the lumenal regions of vesicles such as endosomes or Golgi where the low pH environment sustains their optimal protease activity, while their C-termini are exposed to the cytoplasm, enabling post-translational modification and protein-protein interaction.

BACE1 in 3D: components involved in catalysis

Insight into the three-dimensional structure of BACE1 is vital to understanding how the enzyme works catalytically, and in developing inhibitors which block BACE1 activity as a therapy for AD. X-ray-crystallography of BACE1 has determined numerous structures of BACE1 complexes, and residues and regions that are important for substrate specificity and proteolysis [45–50]. To date, there are over 70 known

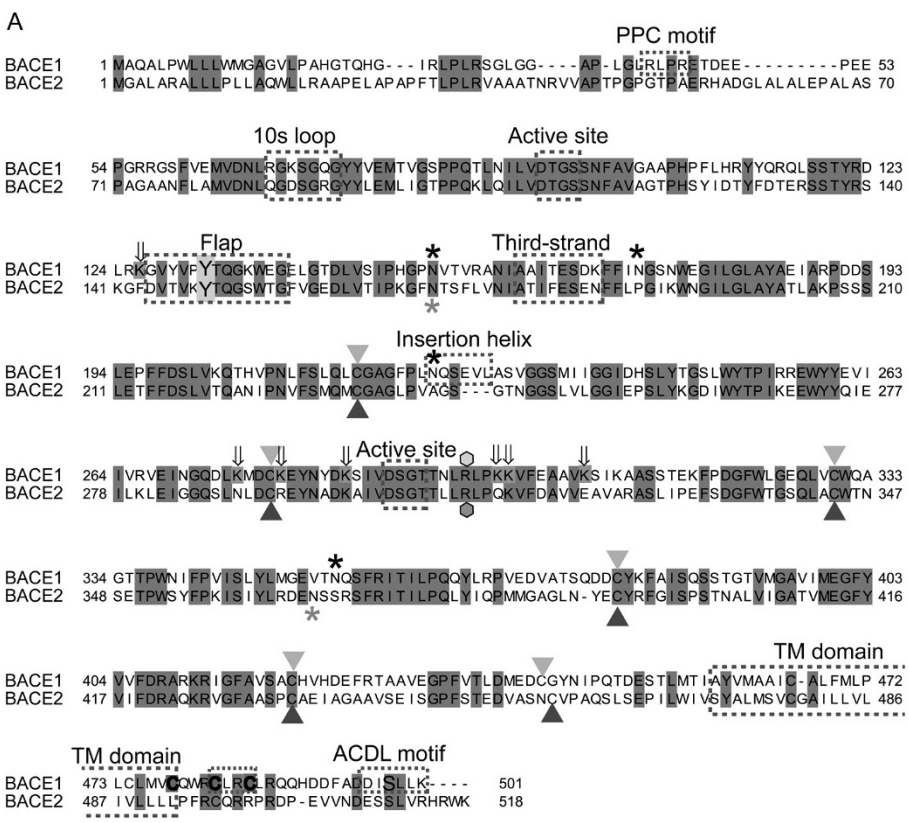

Figure 4. Comparison of BACE1 and BACE2 amino acid sequence. (*A*) Pairwise alignment of human BACE1 and human BACE2 primary amino acid sequences. Shaded boxes highlight similar amino acids shared between the proteins. 10s loops, active sites, flaps, third-strands, insertion helices, transmembrane domains (TM), acid cluster dileucine (ACDL) and pro-protein convertase (PPC) motifs are outlined with dashed boxes. Cys residues involved in disulphide bridge formation, asparagine residues potentially involved in N-glycosylation and Lys residues that have been shown to be acetylated are indicated with triangles, * and ⇓ respectively. Cys residues that have been shown to undergo palmitoylation and a serine that can be phosphorylated in the BACE1 C-terminus are shown in bold text. The arginine residues which comprise part of the S2 binding site and which are unique to BACE1 and BACE2 are indicated by polygons.

structures of BACE1 in complex with inhibitor, seven without inhibitor and one of BACE2 in the protein data bank http://www.rcsb.org/. The number of crystal structures of BACE1 is testimony to the variety of compounds being tested as β-secretase drug candidates [for review [51]]. The X-ray structure of BACE1 protease domain was first determined to 1.9Å resolution, with BACE1 bound to an eight residue transition state analogue inhibitor (OM99-2) [49]. OM99-2 is a $P_4 - P_4'$ peptide (P_4-P_3-P_2-P_1*-P_1'-P_2'-P_3'-P_4') based on the amino acid composition of APP_{SWE} (Glu-Val-Asn-Leu*Ala-Ala-Glu-Phe) but incorporating a non-cleavable hydroxyethylene isostere (*) at P_1 and P_1', blocking normal proteolytic BACE1 cleavage between the P_1 and P_1' scissile bond. Further enzyme subsites were identified for BACE1 with enzyme bound to other eight residue [48] and longer transition state inhibitors [50], and the crystal structure of free BACE1 has been studied [45, 47].

These crystal structure studies show BACE1 has strong conservation with A1 aspartic proteases, for which pepsin is prototypic, and also more recently with BACE2 [39]. Ribbon diagrams of the 3-D structure described for BACE1, BACE2 and pepsin, describing their major structural features, are shown in Figure 5, and can be viewed with the linear amino acid sequence of BACE1 and BACE2 in Figure 4. Regions of commonality include: the conformation and location of the catalytic Asp dyad in the middle of the active site cleft at the interface between N- and C-terminal lobes, and the shielding of the active site by a flexible antiparallel hairpin-loop, known as a flap [39, 49]. Overall accommodation of the eight peptide substrate ($P_1 - P_4$) residues occurs at enzyme subsites (S_1-S_4) and $P_1' - P_4'$ at enzyme subsites S_1'-S_4' in a similar way to other aspartic proteases. Thus, hydrogen bonds between the active site aspartates and 10 hydrogen bonds from different parts of the active site and flap bond to the substrate /inhibitor backbone in the active site cleft, with a high degree of conservation. There are key differences between the BACE1 crystal structure and other aspartic proteases that may be exploited in BACE inhibitor design. The most obvious difference is the larger molecular surface of BACE1, due to the presence of five insertions (four loops and one helix) all in the C-terminal lobe, in addition to the presence of a 35 residue C-terminal extension, the latter being highly ordered in structure and possibly forming a stem with the transmembrane domain [49]. In addition, although the general organisation of the active site subsites is similar to other aspartic proteases, their specificity and conformation display key differences [46–49]. Moreover, the active site of BACE1 is larger, having additional subsites (S_5-S_7),

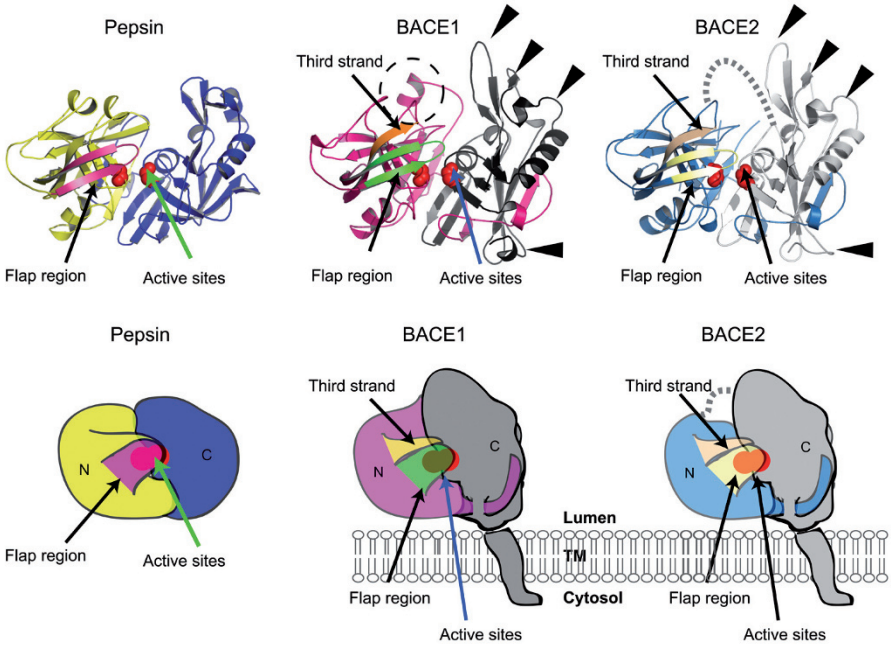

Figure 5. Structural features of BACE1 compared to pepsin and BACE2. Structures for pepsin (1PSN), BACE1 (1SGZ) and BACE2 (2EWY) were obtained from the protein data bank (http://www.rcsb.org/pdb/home/home.do). Ribbon diagrams were visualised using the Pymol software. The shown cartoon illustrations underneath the ribbon structures represent the surface structures of each protein and a possible orientation of BACE1 and BACE2 to the membrane. The N-terminal lobes of pepsin, BACE1 and BACE2 are coloured gold, magenta and blue respectively, and the C-terminal catalytic lobes are coloured dark blue, dark grey and silver respectively. The flap regions of pepsin, BACE1 and BACE2 are shown in their respective colours of purple, green and wheat. The third-strands in BACE1 and BACE2 are shown adjacent to the flaps and coloured orange and beige respectively. The active site aspartates of each enzyme are coloured in red space fill. The BACE1 and BACE2 insertion loops are indicated with arrow heads and the BACE1 insertion helix is highlighted with a hatched circle. The dashed line in BACE2 represents a disordered region in the BACE2 crystal structure.

and although it works well with the eight substrate residues as is normal for other aspartic proteases, it can also accommodate a greater number of substrate residues (11) [50]. The larger opening of the active site occurs due to structural differences near subsites, and the absence of a constricting pepsin helix loop across from the active site [46–49]. The S_1 and S_3 subsites consist mostly of hydrophobic residues and their conformations are very different to pepsin. S_4 and S_2 are much more hydrophilic than these subsites in other aspartic proteases, where S_2 in BACE1 and BACE2 contains Arg (Arg^{296}, Arg^{310} respectively), absent in other aspartic proteases and linked to more effective cleavage of APP_{SWE} compared to normal APP [39, 49, 52]. Subsites $S_5 - S_7$ localise in the vicinity of the insertion helix, a region also absent in other A1 aspartyl proteases, and is believed to contribute to substrate recognition and transition state binding [50]. The flexible flaps which cover the active sites of all A1 aspartic protease contribute to hydrolytic specificity and substrate access, believed to open to allow substrate/ inhibitor access, close when substrate/ inhibitor is bound, and open to release hydrolytic products. However, the details of this mechanism are unclear. The BACE1 flap position can differ by 4.5Å -7Å at the tip when comparing free unbound enzyme (apo) with enzyme bound to transition state inhibitor [47, 53]. A conserved aspartic protease Tyr^{71} residue in the tip of the flap forms hydrogen bonds with substrates /inhibitors at the P_1 and P_2' positions of BACE1, thereby mechanistically sealing the flap shut [47]. The open position is narrow and stabilised by intra-flap hydrogen bonds and a hydrogen bond with Tyr^{71}. A parallel side chain region in BACE1 (and also in BACE2), known as the third strand, (Fig. 4,5) forms hydrogen bonds with residues in the flap and active site residues, thereby influencing and stabilising the open or closed state of the enzyme [47]. Another region of flexibility shared between BACE1 and BACE2, most likely important in recognition and processing of APP substrate, is known as the 10s loop [39], which forms part of the hydrophobic S_3 binding pocket (Fig. 4, 5). This region can also display flexible conformations in BACE1 when comparing Apo and inhibitor complexed structures [45, 47–49]. and displays subtle differences in amino acid composition between BACE1 and BACE2 (Fig. 5) and may be involved in their substrate discrimination [39].

Examination of the interaction of the $P_4 - P_4'$ peptide inhibitor based on the mutant amino acid composition of APP_{SWE} (Glu-Val-*Asn-Leu**Ala-Ala-Glu-Phe) with BACE1's active site gives information about why this substrate displays a 60-fold increase in K_{CAT}/Km of APP_{SWE} over that of normal wild-type APP to cause AD [49]. Firstly, mutant P_1-Leu is closely crammed against P_3-Val and both have considerable hydrophobic contacts with BACE1, especially true for P_1-Leu, part of which encompasses its interaction the Tyr^{71} at the flap tip. This hydrophobic interaction would likely be much more unfavourable with P_1-Met in the normal APP substrate. Furthermore, the side-chain of mutant P_2-Asn is H-bonded to P_4-Glu and interacts strongly with Arg^{296} within the S_2 subsite, both interactions would be much less favourable with the positively charged P_2-Lys in normal APP. Together, the information gained on the unique structural features of BACE1 through investigating crystal structure is lending to the rational design of inhibitor drugs [54], incorporating such information on unique subsite-inhibitor interaction and flap control.

Towards understanding BACE1 function by BACE1 deletion

In 2001 the first reports describing mice deficient in BACE1 were published [16–18]. The mice were viable and fertile and displayed no overt phenotypic abnormalities, as judged by embryonic development, and by gross anatomy, morphology, physiology, clinical chemistry, and behaviour [16, 18, 55]. In addition, the production of all β-secretase products (Aβ, CTF-β (C99) and sAPPβ) was abolished when $BACE1^{-/-}$ mice were crossed with mice overexpressing APP_{SWE} (Tg2576 mice) [18], which normally produce high levels of these β-secretase products. Secretion of Aβ peptides was also eradicated in BACE1-deficient embryonic cortical neurons [16, 17], and endogenous C99 (CTFβ) production was completely blocked in BACE1 knockout mice developed subsequently [56]. Furthermore, later studies showed $BACE1^{-/-}$ -Tg2576 mice did not develop Aβ plaque deposits with age [57]. These results confirmed definitively that BACE1 is the major β-secretase in the brain, and highlighted its importance as a therapeutic target to block Aβ formation and plaque production in AD. Following from this, it was shown that BACE1 deficiency could rescue memory impairments and cholinergic dysfunction that are apparent in young Tg2576 mice and in APP/PS-1 (Tg6799) mouse models of AD, most likely due to inhibition of the accumulation of soluble Aβ assemblies [58, 59].

Subsequent studies have been unanimous in showing BACE1 deletion blocks the enhanced production of Aβ in several transgenic mouse models of AD, including: APPswePS1ΔE9 [60], PDAPP [61, 62] and 5 x FAD APP/PS-1 mice [59]. Age-associated cognitive abnormalities in these mouse models are also blocked by BACE1 deletion in some [59, 60], but not all [61] mice models. Moreover, BACE1 deletion prevented neuronal loss in 5 x FAD APP/PS-1 mice [59], and lowering BACE1 levels using lentiviral vectors expressing siRNAs reduced amyloid production and neurodegenerative and behavioural impairments in APP transgenic mice [63]. Together, These findings further accentuated the relevance of targeting BACE1 inhibition as a therapeutic strategy to block Aβ production, neurodegeneration and cognitive decline in AD.

The first inkling that loss of BACE1 function was not totally without effect on neuronal function emanated from studies showing that $BACE1^{-/-}$ mice had subtle behavioural changes, being timid, anxious, and less prone to explore compared to mice overexpressing BACE1, which displayed bold, less anxious behaviour [56]. These $BACE1^{-/-}$ mice also revealed alterations in neurochemistry affecting levels of serotonin and dopamine, and dopamine turnover [56]. Other BACE1 knockout mice also demonstrated mild impairments in spatial learning and memory [58, 64]. Furthermore, a $BACE1^{-/-}$ mouse first described in 2005 displayed a complex phenotype of increased neonatal mortality in 40% of the mice, and decreased size and hyperactive behavior in mice surviving to old age compared to litter mate controls [65]. A $BACE1^{-/-}$ / $BACE2^{-/-}$ bigenic mouse increased this morbidity [65].

More recently the discovery that BACE1 deletion affects neuronal function has gone hand in hand with findings that BACE1 cleaves several other substrates besides APP. These substrates, along with APP, have key physiological roles in neuronal function and synaptic transmission, in particular neuregulin (NRG) and voltage-gated sodium channel (VGSC) β subunits (see section below on APP substrates). More detailed and specific analysis of BACE1 KO mice has also revealed that BACE1 deletion actually affects several aspects of cognitive, emotional and synaptic function [58, 60, 61, 64, 66] and significantly impairs the myelination of central and peripheral neurons [67 – 69]. The cognitive defects in these mice appear to link to loss of APP processing [60], whereas the hypomyelination and emotional deficits, which include schizophrenia-like phenotypes, appear to link to loss of neuregulin processing [66]. It has also been shown that BACE1, but not β-secretase activity, is necessary for a type of enhanced memory and

cognitive abilities associated with the production of the APP fragment AICD, but not with Aβ production [70]. Taking all the BACE1 deletion results together, it is clear that we most likely need a better understanding of the normal and pathological role of BACE1 in synaptic function, cognition and emotion before we can predict the impact of BACE1 inhibition on human brain. In this we should gain knowledge of the best approach to target BACE1 activity towards normalising, but not ablating, its function as a therapeutic strategy in AD.

BACE1 substrates: more that just APP

APP has been the most studied substrate of BACE1 due to its role in AD pathogenesis. However, normal APP and its homologue APLP proteins are processed rather inefficiently by BACE1 [13] as BACE1 prefers bulky hydrophobic residue (e.g. Leu) at the P_1 position as occurs in APP_{SWE} (see section above on catalysis). This hinted that more favourable BACE1 substrates with similar amino acid configuration to APP_{SWE} could exist [71 – 74], several of which have now been identified. These include: the β-subunits of voltage gated sodium channels (VGSCβ) subunits 1 – 4 [75, 76], neuregulin-1 (NRG-1) [67, 69], α-2,6 sialyltransferase (ST6Gal-1) [77], the cell adhesion protein P-selectin glycoprotein ligand-1 (PSGL-1) [78], LRP-1 [79], and the interleukin-1 receptor type-II [80]. Notably, alignment of the known cleavage sites in BACE1 substrates from $P_5 – P_5'$ shows there is no clear consensus sequence for BACE1 processing (Fig. 6). However, there is a high preference for substrates with Leu or Phe at P_1 (Fig. 6)

Taking APP and APLPs first, cleavage of APP by BACE1 at the β site occurs preferentially in early and late endosomes, which provide the acidic environment necessary for maximal protease activity, but this can also occur at the plasma membrane and in the ER [25, 44, 81–84], cleavage at β′-site occurs predominantly in TGN [82]. BACE2 can also cleave APP at the β-site but preferentially cleaves within the Aβ domain (between amino acid 19 and 20 of Aβ) of APP [21, 25, 85]. Overexpression studies of BACE1 and BACE2 *in vitro* show that they compete with each other for APP substrate, but it is unknown if this is the case *in vivo* [27]. The APP homologous proteins APLP-1 and APLP-2 are also α- [86] β [87–89] and γ-secretase substrates [88, 90] *in vitro* and *in vivo* [87]. APLP-1 and APLP-2 do not contain sequences homologous to Aβ [for review [91]], and the cleavage sequence at the β-sites in APP and APLP-1 and APLP-2 is not conserved. Thus despite lack of catalytic efficiency, BACE1 cleavage of APP and APLPs is a specific proteolytic controller of this family of proteins.

BACE1 cleaves β2 → β4 subunits of voltage-gated Na^+ channels (VGSC) [76], where cleavage occurs at the juxta-membrane position of these type I single transmembrane domain proteins, with subsequent cleavage by γ-secretase. VGSCs are central to CNS function, as they are responsible for neuronal excitability through initiation and propagation of the action potential. $BACE1^{-/-}$ mice display subtle changes in steady state inactivation of VGSCs [65], pointing to the physiological relevance of this BACE1 VGSC β−subunit cleavage *in vivo*. β−subunits are auxiliary subunits of VGSCs and associate with the principal pore-forming α subunit to regulate gating and cell surface expression of VGSCs. Recent evidence shows that BACE1 cleavage of β2 subunits regulates VGSC activity *in vivo*, where mice overexpressing BACE1, and AD patients with elevated BACE1 activity [75], show increased processing of the β2 subunit and increased levels of $Na_1 1.1 – \alpha$ subunits. The increase in $Na_1 1.1 – \alpha$ subunits was associated with retention of these subunits inside cells, with a significant decrease in their cell surface expression, and an associated decrease in Na^+ current densities [75]. Thus, increased BACE1 activity in AD brain may also adversely influence VGSC activity and action potential, and inhibiting BACE1 activity may re-establish this activity.

Phenotypic analysis of $BACE1^{-/-}$ mice led to the discovery that NRG-1 is a physiological substrate of BACE1 [69]. This and subsequent work found that axons in the peripheral and central nervous system of $BACE1^{-/-}$ mice are hypomyelinated [67–69]. The peripheral hypomyelination phenocopies heterozygous NRG-1 type III deficient mice [92], and mice lacking ErbB signalling in Schwann cells. NRG-1 is an epidermal growth factor-like ligand which exerts its function as the ligand for members of the ErbB receptor tyrosine kinase family [93]. The NRG-1 signalling pathway plays a vital role in CNS development and function, including myelination, peripheral nerve development, synapse formation and neuroplasticity [92, 93] and the *NRG-1* gene is linked to an increased risk of schizophrenia [94]. NRG-1 (Type III) adopts a two transmembrane structure, with the active EGF domain in the lumenal portion, which is believed to require endoproteolysis to signal effectively [93]. BACE1 deletion leads to defects in BACE1 processing of NRG-1 leading to accumulation of holo-NRG-1 and a corresponding reduction in N-terminal NRG-1, in addition to indicated defects in NRG-1-Akt signalling and decreased ErbB4 PSD-95 signalling [66 – 68]. These signalling changes are believed to underlie defects in both peripheral and

central nervous system myelination and synaptic function in the BACE1$^{-/-}$ mice. Defects in myelination, with decreasing myelin density, have been detected in AD brain [95, 96], and may thus be related to changes in BACE1 in AD.

ST6Gal-1, PSGL-1 and IL-1RII are further substrates of BACE1 and are all involved in inflammatory responses, although their physiological relevance to BACE1 function in the CNS or AD is unclear. ST6Gal-1 is a sialyltransferase residing in the TGN which is secreted upon proteolytic cleavage, an activity which is increased in the presence of BACE1 [77, 97, 98]. Plasma ST6Gal-1 levels and α2,6-sialylation of plasma glycoproteins are reduced in BACE1$^{-/-}$ mice [98]. The cell adhesion protein PSGL-1 binds the cell surface glycoprotein P-selectin to mediate cellular adhesion, leukocyte activation and inflammatory responses in brain and peripheral organs [for reviews [99, 100]. PSGL-1 fragments are secreted when either BACE1 or BACE2 are overexpressed, implicating both proteases in its processing at its juxtamembrane sequence [78]. Evidence suggests that LRP-1 is a substrate for BACE1, as both proteins co-immunoprecipitate and are in close proximity at the plasma membrane. LRP-1 C-terminal fragments are increased upon BACE1 overexpression [79]. Although this has yet to be addressed *in vivo*, altered processing of LRP-1 in the AD brain could be speculated to influence both cholesterol homeostasis and altered degradation of Aβ through LRP-1.

These findings of multiple BACE1 substrates, particularly those of physiological relevance to CNS function, support several roles for BACE1 in regulating CNS function. The mechanism used by BACE1 to select and target specific substrates and the potential co- or interdependence of substrate cleavage by BACE1 in CNS function is an important area of future research when considering inhibition of BACE1 in AD, and when understanding the broad biology and function of BACE1.

BACE1 levels and β-secretase activity in normal and AD brain

Importantly, all studies have consistently detected significant increases in β-secretase enzyme activity (13%–185%) in AD brain regions affected by pathology [73, 101–103], [for review see [104]], indicating increased BACE1 activity is most likely to be a major cause of Aβ overproduction is sporadic forms of AD. In addition, analysis of β-secretase enzyme kinetics revealed a significant increase in the Vmax for β-secretase in AD temporal cortex, inferring changes occur at levels of altered enzyme velocity in the disease [101]. Studies comparatively examining BACE1 levels have also, for the most part, detected increased BACE1 protein levels to varying degrees in AD cases compared to controls [103, 105–108]. However, some studies have detected unchanged BACE1 levels in AD [102, 109], with one study from our group detecting significantly decreased levels of the major mature BACE1 isoform, which correlated strongly with synaptophysin loss, and severity of neurofibrillary pathology in the disease [101]. A recent study found that increased BACE1 levels were only apparent in a subset (~ 30%) of AD cases [108]. Comparative immunohistochemical analysis in AD and control brain revealed no major changes in detectable BACE1 levels in the hippocampus [109–111], frontal cortex [110] or temporal cortex [109]. However, subtle changes in BACE1 immunoreactivity were apparent in AD, such as BACE1 increases in and around senile plaques, in reactive astrocytes, and in remaining neurons [109] with converse BACE1 decreases in the transentorhinal [111] and entorhinal cortices particularly evident in neurons with NFTs [110]. A more recent study has shown BACE1 elevation occurs predominantly within neurons surrounding plaques in both AD and animal models of AD, where it colocalises with synaptophysin [107].

The discrepancies and variation in BACE1 protein level changes reported in AD, may arise from differences: in antibodies, brain tissue preparation, assay methodologies and patient groups, or from the complexity of BACE1 protein changes as indicated by the immunohistochemical analysis. Regardless, it is clear from all studies that increased BACE1 enzyme activity in AD is not reflected by equal increases in BACE1 protein levels. In addition, with the caveat that mRNA splice variants have not been measured, most studies report no changes in BACE1 mRNA levels in AD brain [105, 108, 112 – 114]. Together this indicates that regulatory factors outside BACE1 protein levels cause increased activity of β-secretase activity in AD, and need to be understood to target the enzyme therapeutically to quell excess Aβ production in the disease. To understand mechanisms by which BACE1 changes in AD and how in particular the enzyme activity is increased, it is essential to delineate how BACE1 expression activity and function are regulated in the brain. It is emerging that the regulation of these events is multifaceted and complex, where mechanisms of enzyme regulation include: BACE1 trafficking, subcellular and membrane microdomain localisation; BACE1 protein interactions; transcriptional; translational and post-translational regulation. These are reviewed in sections below.

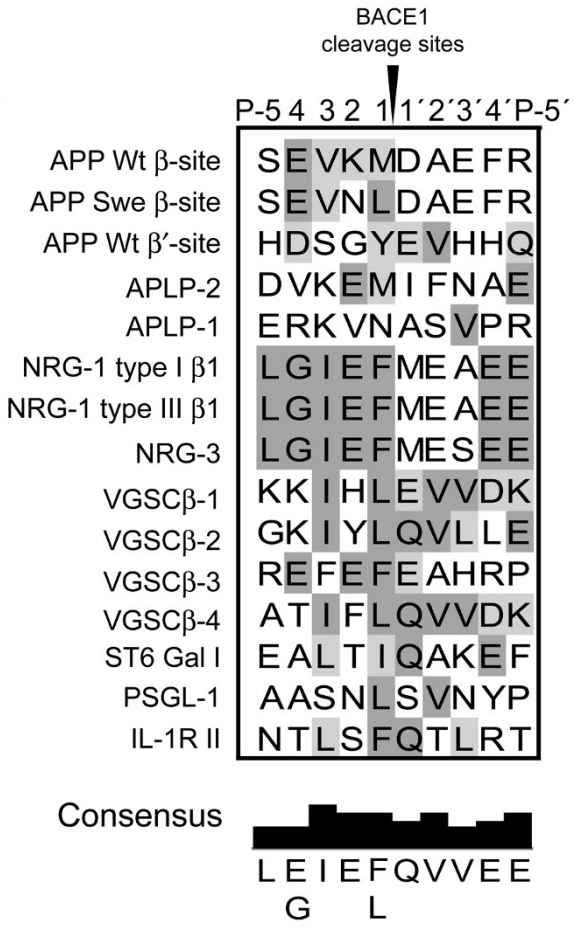

Figure 6. Sequence alignment of known BACE1 substrates. Amino acid sequences of known BACE1 substrates stretching from the N-terminal P5 to the C-terminal P5′ positions were aligned using the alignment editing program Jalview. Alignment was coloured according to BLOSUM62 score. A consensus sequence is shown below the list of substrates. Note the preference for hydrophobic Leu or Phe residues in the P1 position of BACE1 substrates.

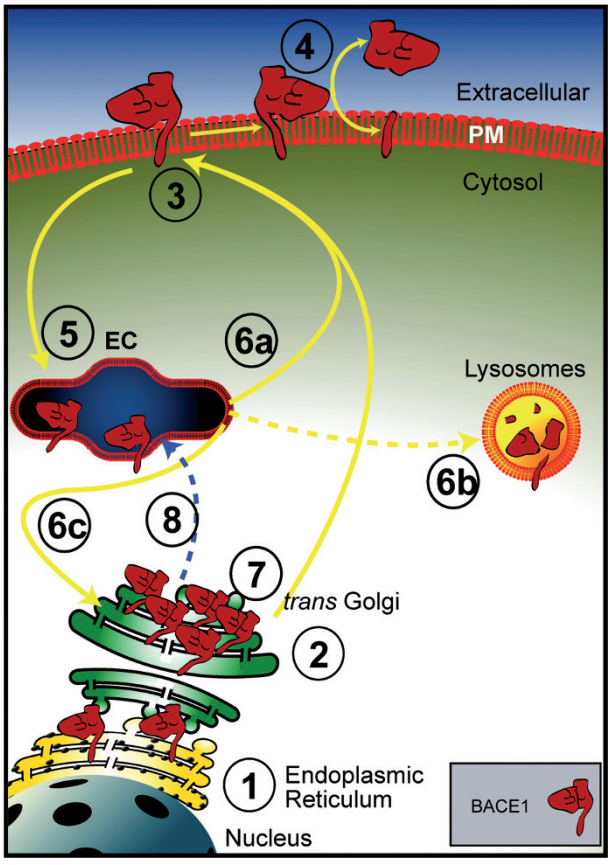

Figure 7. Intracellular trafficking of the BACE1 protein. After synthesis, BACE1 resides in the endoplasmic reticulum (**1**) and is transported to the trans-Golgi network (TGN) where the vast majority of it resides (**2**). From the TGN, BACE1 is transported to the plasma membrane (PM) (**3**) where a small proportion can undergo ectodomain shedding (**4**). The majority of BACE1 is internalised into endocytic compartments (EC) (**5**) where the acidic environment provides the optimal conditions for the proteolysis of APP. From the endocytic compartments BACE1 can be recycled directly back to the cell surface (**6a**), transit to lysosomes for degradation (**6b**) or retrogradely to the TGN (**6c**) from where it can be trafficked back to the PM (**7**). It is also possible that BACE1 can be transported directly from the TGN to endocytic compartments (**8**).

BACE2 in brain and in AD

The catalytic properties of BACE1 and BACE2 are very similar, yet distinct. As mentioned, BACE2 can also cleave at the β-site of APP [25, 52] but has a preference for cleaving APP within the Aβ site [25, 27, 52, 85, 115], thus as with α-secretase, precluding Aβ production [52, 85, 116]. One of the autosomal dominant APP mutations (the Flemish mutation $Ala^{692} \rightarrow Gly^{692}$) which causes cerebral amyloid angiopathy (CAA), is adjacent to the BACE2 cleavage site. It has been postulated that BACE2 may contribute to Aβ generation and CAA pathology in individuals with this mutation, by pushing APP cleavage towards the β-site [52]. However, in general it is more likely that, as with α-secretase, BACE2 activity counteracts Aβ production by competing with BACE1 for APP [27, 115, 117].

It should be noted that despite their low levels *BACE2* mRNA and protein are detectable in human and rodent brain [12, 25, 101, 118]. BACE2 knockout mice display an overall healthy phenotype [65]. However, a combined deletion of BACE1 and BACE2 caused enhanced morbidity over that shown with BACE1 deletion alone [65]. This study also confirmed that BACE1 is the major β-secretase *in vivo* and the only β-secretase active in neurons, but suggested that BACE2 could produce Aβ in glia [65]. Examination of BACE2 protein levels in AD temporal cortex has shown these to be decreased compared to control levels [101]. In contrast, consistent with the triplication of the *BACE2* gene in individuals with Downs syndrome (DS) (trisomy-21), *BACE2* mRNA and

protein levels are increased in cells from individuals with DS [119]. In addition, adults with DS, and also diagnosed with AD, have increased levels of brain BACE2 protein [120], and also increased BACE1 activity and levels of mature BACE1 [115, 121]. It summary, it is unclear how or whether BACE2 contributes to Aβ production in AD or DS, but if so, the consensus would be that it acts primarily as an anti-amyloidogenic protease.

BACE1 regulation by trafficking, post-translational modification and maturation

APP cleavage by BACE1 at the β site occurs preferentially in early or late endosomes, which provide the acidic environment necessary for maximal BACE1 activity, but can also occur in the ER and at the plasma membrane. Cleavage at β′-site occurs predominantly in TGN. Subsequent cleavage by γ-secretase results in Aβ production which is brought to the cell surface for extracellular release. Thus, cell trafficking of BACE1 is closely related to the mechanism and regulation of its activation/inactivation and Aβ production, and has received substantial focus in understanding the regulation of BACE1 activity (Fig. 7). BACE1 trafficking occurs along the constitutive secretory pathway to the cell surface. After translation, the signal sequences in newly synthesised pro-BACE1 localise it to the ER, where it is acetylated on seven possible Lys residues [55] and glycosylated on three of four potential N-glycosylation sites [122]. Acetylation of BACE1 and elements in the transmembrane domain assists its folding stability and correct maturation through the ER to the trans-Golgi network (TGN) [55]. In the TGN BACE1's pro-domain is removed by proteolytic cleavage using furin or furin-like convertases [12, 43, 44]. Then BACE1 is deacetylated [55], and complex N-glycosylation completed [11, 34, 83, 117, 122], which increases the mass of BACE1 I-501 from ~50 kDa to ~70 kDa. Splicing modifications in exon-3, in BACE1 isoforms I-476 and I-457 removes two glycosylation sites ($Asn^{153}Asn^{172}$), causing retention of a pro-enzymatic non-active BACE1 form in the ER [29, 123, 124]. The preponderance of BACE1 I-501 in brain is thus of importance as it favours enhanced enzyme activity in contrast to peripheral organs where the smaller non-enzymatic isoforms are reported to predominate [44, 123]. The mechanism by which N-glycosylation enhances BACE1 activity is poorly understood but is most likely due to folding, stability, solubility, or substrate recognition. BACE1 also contains 3 intramolecular disulphide bonds which are important in maintaining the correct folding orientation for BACE1 necessary for its efficient maturation.

From the TGN, BACE1 traffics to the plasma membrane where a small proportion can undergo ectodomain shedding, induced by metalloprotease ADAM-10 cleavage [125], to release the large BACE1 catalytic domains into the extracellular milieu (Fig. 7). This process is blocked by palmitoylation of Cys residues in the BACE1 C-terminal tail [126], which may also regulate BACE1 localisation to membrane microdomains. Ectodomain shedding may represent a mechanism of down-regulating BACE1 activity [125], but has conversely been described also to increase Aβ generation [126]. The majority of BACE1 at the plasma membrane is re-internalised into endosomal compartments [44, 83, 117, 127] (Fig. 7). The C-terminal di-leucine motif in an acid **c**luster dileucine (ACDL) sorting motif DXXLL (D^{496}–I^{497}-S^{498}-L^{499}-L-^{500} in BACE1) is a critical recognition signal for BACE1 endocytosis [83, 128], and also for further trafficking of BACE1 intracellularly [129–133]. Phosphorylation of Ser^{498} preceding the dileucines [128, 134, 135] is also mechanistically important in the intracellular trafficking of BACE1, as is the interaction of the D^{496}–I^{497}-S^{498}-L^{499}-L-^{500} motif with GGA (Golgi-localised, γ-ear containing, ADP ribosylation factor-binding) proteins, which depends on BACE1 Ser^{498} phosphorylation [130–133, 136, 137], (see section below for further details on GGA interacting proteins).

Endosomes are important sorting stations in further intracellular BACE1 trafficking, where BACE1 has been described to: transit from late endosomes toward the lysosome for degradation [129], to follow the recycling pathway back to the cell surface [83, 133, 134], to be retrogradely transported to the TGN [133, 137] for transit back to the cell surface through the secretory pathway [133, 137], or possibly to transit directly back to the endosomal compartment from the TGN [133, 137, 138] (Fig. 7). BACE1 can be internalised by pathways that do not require the ACDL motif, where the interaction of the N-terminal ectodomain of BACE1 interacts with substrate APP to increase BACE1 internalisation to endosomes [139]. The half-life of BACE1 is long, greater than 12 hours [34, 83, 140], allowing for substantial trafficking and activation / inactivation cycles between the TGN, plasma membrane, and endosomal-lysosomal pathway. The final degradation of BACE1 is proposed to occur either via transit to the late endosomal-lysosomal system [129], or via the ubiquitin proteasomal system [141]. BACE1 can also form dimers which demonstrate increased catalytic activity [142, 143]. In summary, signalling pathways and interacting proteins, which traffic both APP and BACE1 to and from the

endosomal compartments, such as those controlling phosphorylation at Ser^{498} BACE1 and GGA-BACE1 interaction (see section on interacting proteins) may be important in the increased aberrant activity of BACE1 in AD. Likewise, pathways that maintain APP at the plasma membrane, where it will be processed by α-secretase, will also block Aβ production.

BACE1 activity and membrane lipid microdomains

BACE1 activity and access to substrates is regulated by the composition of membranes in the bilayer where lipid raft domains are important in the processing of APP (for review see [144]). These detergent resistant membrane (DRM) domains are enriched in cholesterol and sphingomyelin. A portion of BACE1 resides in DRMs in cultured cells [79, 145–51] and in brain [150, 152, 153], as do a portion of many BACE1 substrates [76, 152–155] and some BACE1 interacting proteins [145, 147, 156, 157]. β-secretase activity [149] is present in DRM domains when BACE1 is overexpressed, and artificial targeting of overexpressed BACE1 to DRM domains by the addition of a GPI anchor increases β-secretase processing of APP [151]. Two contrasting models currently exist regarding the biological function of BACE1 in DRMs. One indicates that DRM domains are the main sites for Aβ production [146, 156, 158] and another indicates that BACE1 residence in DRM domains segregates it from the majority of APP which is not in DRMs [for reviews [144, 159]].

Although the above models do not agree, understanding the localisation of endogenous APP and BACE1 (rather than overexpressed forms) in membrane microdomains, at the cell surface and in organelles, and how this regulates BACE1 activity, and β-secretase processing of APP is important [159]. Endosomes in particular have high lipid raft and cholesterol content, and cholesterol dependent clustering of APP can regulate APP endocytosis with increased amyloidogenic processing [160]. The inheritance of ApoEε4 allele is a significant risk factor for the development of AD, and perturbations in cholesterol homeostasis in AD brain membranes would be likely to link to ApoE metabolism, which delivers cholesterol to synaptic membranes. Interestingly, a BACE1 allele combined with ApoEε4 allele may increase the risk for sporadic AD [40, 161–163]. Moreover, preliminary findings also detected a significantly higher Vmax for β-secretase in cases with the ApoEε4/ε3 genotype compared to ApoEε3/ε3 [101, 114], and increased CSF BACE1 activity is associated with ApoEε4 genotype in individuals with mild cognitive impairment and AD [164].

Control of BACE1 by interacting proteins partners

Some proteins that interact with BACE1 are emerging as key regulators of BACE1 activity and function, and thus far appear to converge on both trafficking and lipid microdomain protein function, as well as cell-death pathways. Importantly, many of these interacting proteins, and the ones we will focus upon here, decrease BACE1 activity and the production of Aβ, highlighting the significance of understanding their control of BACE1 both normally and as therapeutic targets in AD. BACE1 interacting proteins include: the gamma-ear-containing, ADP ribosylation factor-binding (GGA) proteins [130, 132, 133, 165]; reticulon (RTN) proteins [166–168]; Sorting protein related receptor/lipoprotein receptor L (SorLA) [169]; prion protein [170]; flotillin [145]; prostate apoptosis response factor-4 (Par-4) [171]; PS-1 [172, 173]; phospholipid scramblase-1 (PLSCR-1) [147], copper chaperone for superoxide dismutase-1 (CCS) [174], and brain specific type-II membrane protein (BRI-3) [175].

Key trafficking proteins that interact with and regulate BACE1 include GGA proteins; RTNs and SorLA. GGA proteins, mentioned in the trafficking section above, are monomeric adaptor proteins that function in intracellular sorting of cargo proteins, particularly from the TGN to the endosomal compartment [for review [176]]. The N-terminal VHS domains of GGA proteins bind to BACE1 through its ACDL C-terminal sorting signal (D^{496}–I^{497}-S^{498}-L^{499}-L-^{500}) [130–132, 136–138]. GGA-BACE1 binding affinity is substantially enhanced when BACE1-Ser^{498} is phosphorylated [131, 132, 136, 137]. This is hypothesized to work as a molecular switch, where interaction of phosphorylated Ser^{498}-BACE1 with GGA-1/2 in early endosomal compartments traffics BACE1 retrogradely to the TGN [133, 137]. On the other hand, non phosphorylated BACE1 recycles back to the cell surface either directly from endosomes [133] or indirectly via the TGN [133, 137]. There is also a possibility that BACE1 traffics from the TGN back to the endosomal compartment, although this needs further study [133, 137, 138]. More recently it has been shown that GGA-3 interaction with BACE1 regulates BACE1 trafficking to lysosomes for degradation, and that GGA-3 depletion, which can occur by apoptosis induced caspase 3 activation, stabilises BACE1, increasing its half life and enhancing β−secretase activity [165]. Levels of GGA-3 [165] and GGA-1 [133] are significantly reduced in AD brain, which would be predicted to increase BACE1's half life and its retention in the endosomal compartment. This may be significant in aberrant BACE1 activation and Aβ processing in AD.

RTNs 1 → 4 are expressed throughout the secretory pathway but mainly in the ER [177]. All four RTNs interact with BACE1 [166] through the RTN C-terminus, an interaction that negatively modulates BACE1 activity [166–168, 178]. A short C-terminal stretch in immediate proximity to the transmembrane domain of BACE1 is necessary for BACE1 binding to RTN's C-terminal [178]. Slight changes in RTN3 membrane topology, an RTN expressed to high levels in neurons, disrupt its binding to BACE1 [178]. The exact mechanism or subcellular localisation of interaction of RTNs with BACE1 causing negative modulation of BACE1 activity is unclear. However, the finding that aggregation of RTN3 occurs in dystrophic neurites in AD brain [179] indicates that RTN3 may not bind BACE1 appropriately in AD, thereby failing to negatively modulate its activity. Finally, SorLA is a highly conserved member of the transmembrane vacuolar protein sorting 10 (Vps10p) receptors and interacts with both BACE1 and APP, controlling the APP trafficking, causing its retention in the TGN [180]. The interaction domain between SorLA and BACE1 has not been defined, but this interaction significantly reduces BACE1-APP interaction in cells, and reduces β-secretase processing of APP [169]. SorLA levels are highly reduced in AD brain, and SorLA null mice have accelerated Aβ production [180, 181], deficiencies that may impact on Aβ production by BACE1 [180]. Of note, however, is the finding that sorLA interaction with BACE1 was not reproduced in another study reporting that the neuronal *SorLA* gene is genetically associated with AD [182]. Interestingly, the interaction of the γ-secretase components PS-1 with BACE1 is believed to be important for trafficking and maturation of BACE1. PS-1 and APP colocalise and are transported anterogradely in the same vesicle compartment in axons [183]. PS-1-BACE1 interaction is postulated to regulate BACE1 maturation through the secretory pathway as PS-1 preferentially binds immature BACE1 [172, 173], and PS-1 deletion significantly reduces the levels of mature BACE1 [172].

Together the interaction of normal prion protein (PrP^c), flotillin and phospholipid scramblase-(PLSCR-1) with BACE1 draws further attention to understanding the function of BACE1 in lipid raft microdomains. PrP^c is a GPI anchor protein present at high concentrations in synaptic membranes and astrocytes, whose function in the CNS is still unclear [184]. PrP^c interacts with BACE1 in DRM domains [170], where PrP^c is enriched, and this interaction requires a region of the PrP^C N-terminal [170]. The interaction of BACE1 with PrP^c inhibits sAPPβ and Aβ secretion *in vitro,* but does not appear to directly modulate β-secretase enzyme activity. This may have functional ramifications for AD pathogenesis [184] where PrP^C levels have been described to be altered [185–188]. Flotillin-1 is a major protein component of DRM domains [189] and co-immunoprecipitate with BACE1, where overexpression of flotillin-1 increases BACE1 levels in DRMs, and β-secretase enzyme activity [145]. The interaction of BACE1 with PLSCR-1, a type II integral membrane protein, is mediated via the ACDL dileucine residues and is described to be potentially important in BACE1 incorporation into DRM domains [147].

Interestingly, the interaction of the copper chaperone for superoxide dismutase-1 (CCS) with BACE1's C-terminal, which occurs through the CCS N-terminal, indicates a non-proteolytic role for BACE1. BACE1-CCS interaction leads to decreased availability of CCS for the anti-oxidant enzyme SOD-1, reducing SOD-1 activity and diminishing oxidative injury [174, 190], thus implicating BACE1 function in redox control and copper metabolism. Polysaccharide interaction with BACE1 are also implicated in the control of BACE1, where heparin sulphate (HS) polysaccharides (GAGs) are described to be naturally occurring inhibitors of β-secretase activity. Heparin can bind to pro-BACE1 and HS proteoglycans inhibit β-secretase catalysis [191, 192]. It is suggested that high concentrations of HS inhibit BACE1, while low concentrations promote BACE1 activity and autocatalysis of the pro-zymogen form of BACE1 [191]. The significance of this to AD is not clear.

Transcriptional and translational control of BACE1

Despite the unchanged levels of *BACE1* mRNA in AD [105, 108, 112–14] many studies have shown clear transcriptional regulation of BACE1, mostly in response to cell stress. Most of these cell stressors increase total *BACE1* mRNA, coincident with similar increases in BACE1 protein levels and β-secretase activity, whereas in AD changes predominantly effect the BACE1 protein and particularly increase enzyme activity. Thus, it is as yet unclear which of these many transcriptional regulators of BACE1 are physiologically relevant to BACE1 dysfunction in AD. However, studies in this area should shed light on mechanisms that could possibly cause aberrant BACE1 activation in AD. As mentioned above, characterisation of the BACE1 promoter shows strong similarity to housekeeping genes [19–21] with features common to both constitutive and inducible expression [193]. Furthermore, the BACE1 promoter is differentially regulated according to cell type, with highest activity in neurons compared to non-neuronal cells [23] contrasting to the BACE2 promoter [20, 22]. Putative specific tran-

scription sites have been identified in the BACE1 promoter, and are implicated in the regulation of *BACE1* expression [for review see [124]]. These include Sp-1 [19, 21, 193, 194] YinYang-1 [195], PPAR-γ [196], NFκB [197, 198], STAT6 [199], STAT3 [200] and HIF1α [201 – 203]. Several of these transcription factors have been implicated in the induction of BACE1 expression, including: Sp-I, HIF1α, YinYang-1, STATs and NFκB. NFκB can both stimulate and repress BACE1 transcription [197, 198], and BACE1 transcription is also repressed by PPARγ [196], possibly linking to the ability of non-steroidal anti-inflammatory drugs to reduce Aβ.

Many of the transcription factors that control *BACE1* mRNA expression respond to physiological or pathological stressors that have been broadly implicated in AD pathogenesis [204]. These include: hypoxia/ oxidative stress/ mitochondrial dysfunction [202, 203, 205–07], altered calcium homeostasis [208], gliosis [209], ischemia [118], cholesterol content [210], brain injury [211] and Aβ itself [197, 198]. Cell signal transduction pathways which impact on these various transcription factors have also been shown to regulate BACE1, and are known to be altered in AD. These include: PKC [212], ERK1/2 [206, 213], p25/CDK2 [200] Cjun/JNK [207, 213] and intracellular calcium [208]. If all of these stressors and signalling mechanisms shown to control BACE1 *in vitro* also occurred *in vivo* in the adult human brain, up-regulation of BACE1 levels and Aβ production would be rather rapid and non-specific. In addition, pathological Aβ production would be predicted to occur following diverse brain injuries and not just specifically in AD. Nevertheless, the housekeeping / stress response role of BACE1 is most likely important in AD pathogenesis and further studies need to delineate those BACE-1 responses that are physiologically relevant *in vivo* in the adult brain and in AD.

Another possible area deserved of attention is the role of BACE1 splice variants in AD pathogenesis. As mentioned, these differ significantly in their enzymatic activities, and their physiological and possible pathological functions are relatively unexplored. Although all splice variants of BACE1 are detectable in the human frontal cortex [29, 214, 215] the largest I-501 variant, which is by far the most enzymatically active, is the predominant species and increases during aging in mice. Interestingly, splice variants are reported to be expressed in a brain region and brain species-specific manner [214]. Differential expression of BACE1 splice variants may sway APP production towards Aβ production in AD or during cell stress. In line with this switching, the balance between BACE1 I-501 to the less enzymatic active forms in brain would help to decrease BACE1 activity in AD. To add further complexity *BACE1* mRNA is regulated in its translation, where the extra long 5′ untranslated region (5′-UTR) of *BACE1* mRNA (446 nucleotides long), present in at least 3 *BACE1* mRNAs [216] can suppress *BACE1* translation. This 5'UTR contains unusual features often found in mRNA showing tight translational control, and shows a high degree of secondary structure. Leaky scanning of the 5′-UTR and re-initiation at the physiological *BACE1* AUG site [217] or ribosomal shunting of upstream open reading frames (uORFs) have been described as possible mechanisms for regulation of BACE1 translation. Translation of BACE1 may thus be constitutively repressed in some cell types. In addition, physiological microRNAs have recognition sites in the 3' UTR region of BACE1 mRNA [108, 218]. These may be important controllers of BACE1 post-transcriptional gene regulation in AD, where decreased levels of BACE1 recognising microRNAs have been recently described in AD brain [108, 218] and these have been found to correlate with increased BACE1 protein levels in a subset (~30%) of AD patients [108].

BACE1 synaptic function and AD pathogenesis

Synaptic loss is a critical element in AD pathogenesis [219–221] that is closely associated with the duration and severity of cognitive impairment in the disease [222]. Thus, understanding the relationship between BACE1 activity, Aβ generation and synaptic function, normally and in AD, is vital. APP is located at the synapse and the synapse is a major site for Aβ deposition [223]. In the context of BACE1 activity it is important to reiterate that monomeric soluble Aβ has a normal physiological synaptic function, particularly at glutamatergic synapses, where it has been hypothesised to reduce excitotoxicity and synaptic transmission by controlling the number of functional synapses, thus limiting neuronal excitability [224]. This is in agreement with recent findings, reviewed above, showing that a complete block of BACE1 cleavage of APP in *BACE1* null mice causes alterations in synaptic plasticity, neuronal excitability and cognitive function. Significantly, BACE1 processing of APP to yield monomeric soluble synaptic Aβ is closely associated with neuronal activity, where action potential, synaptic vesicle release and synaptic vesicle endocytosis all positively modulate Aβ production [224–226]. Importantly, this indicates that the dynamics of the neurotransmitter release cycle may be mechanistically linked to BACE1 activation / inactivation cycles and Aβ production.

The tipping point from a physiological to a pathological function for BACE1 in Aβ production is unknown. This transition may result in loss of the normal physiological BACE1 / Aβ function in synaptic transmisssion, with a gain of an aberrant function for both protease and peptide. This is most likely to be coincident with abnormal increases in BACE1 activity and overproduction of oligomeric assemblies of Aβ, and insoluble amyloid plaques, both of which have been demonstrated to be neurotoxic, and to induce cognitive defects [221, 227]. In this process, accumulating evidence suggests that soluble oligomeric forms of Aβ are more synapto-toxic than fibrils [221, 228]. Importantly, oligomeric species of Aβ aberrrantly modulate various aspects of glutamateric synaptic transmission, including the induction of: dendritic spine loss and AMPA receptor removal, altered NMDA receptor trafficking, calcium regulation and disruption of phosphatidylinositol-4,5 bisphosphate, a key component in both exocytosis and endocytosis of synaptic vesicles [229–232]. Thus, oligomeric Aβ may promote a feedback-loop disrupting components of the synaptic release and endocytic machinery to induce increasingly abnormal BACE1 function. It is notable that rather severe changes in the synaptic endocytic machinery, axonal transport and pre-synaptic neuronal function occur in AD brain, some of which implicate defects in the autophagic machinery and which also impact on tau function [233, 234]. Together these changes may cause increased BACE1 activity by, for example, increasing its time localised to acidic endosomal compartments where its activity is maximal.

Together the above data indicate that a detailed exploration of BACE1 activity and function at the synapse, and particularly within synaptic endosomal compartments, is an important future task in gaining an understanding of increased BACE1 activity in AD, and for designing treatment strategies that target BACE1. It is known that APP, BACE1 and PS-1 are anterogradely transported together in the same vesicle-like compartment to CNS nerve terminal presynapses [183] and that lesions of axon fibers, blocking transport to the synaptic terminal, substantially reduce Aβ accumulation in APP transgenic mice [235, 236]. A localisation of BACE1 to synaptic terminals is supported by findings showing BACE1 is enriched in presynaptic terminals in mouse hippocampus [60, 107], and in the plexiform layers in retina and synaptic olfactory terminals of rat [205, 237]. Although detailed examination of BACE1 at the synapse in normal human brain or in AD is lacking, a study from our lab has shown decreased levels of BACE1 protein correlate strongly with synaptophysin loss and the severity of NFT deposition in AD cases, even though BACE1 activity is significantly increased [101]. In addition, BACE1 elevation has been found to colocalise predominantly with synaptophysin within neurons surrounding plaques in AD, and in animal models of the disease [107]. It will thus be critical to further establish within which subcellular compartments and membrane microdomains BACE1 resides at the synapse, and how BACE1 trafficking, activity and substrate interaction is regulated during the synaptic vesicle release cycle, and during trafficking of BACE1 between the somatodendritic compartment and the synapse. Defects in the exocytosis or endocytosis of synaptic vesicles linked to impaired neurotransmission may turn out to be mechanistically associated with defective BACE1 activation and increased Aβ production in AD, leading in turn to impaired long lasting structural and functional synaptic changes that underly progressive cognitive dysfunction in the disease.

BACE inhibitors, conclusions and future pespectives

Since its discovery over eight years ago much information has been gained on the structure, function and regulation of BACE1. The enzmye remains a prime target for the development of inhibitory drugs to block excess Aβ production in AD. The area of drug development for BACE1 inhibition has been addressed in several excellent recent reviews [54, 238, 239] and is thus not adressed in detail here. We will but summarise briefly the progress in this area. The first generation of BACE1 transition state inhibitors using peptide-mimetics based on APP_{SWE} with non-clevable isoteres have been employed to determine the crystal stucture of the enzyme, and to understand BACE1 substrate and inhibitor interaction at the active site. These are reviewed in sections above which discuss the structure of BACE1. Although these inhibitors potently inhibit BACE1 activity and provide information for inhibitor design, they are unfortunately too large to penetrate the blood brain barrier (BBB) and to be functional as drug candidates. Thus, ideally β-secretase inhibitors should be 700 kDa or smaller, in addition to having high lipophillicity, in order to penetrate the blood brain barrier [49] and to access neuronal membranes, in particular the membranes of subcellular organelles where BACE1 resides. Improvements in the design of the above inhibitors have reduced their mass and increased their specificity, but they remain inneffective in permeating cell membranes. To this end cell penetrable BACE1 inhibitors [240–242] bearing penetratin [243] or carrier peptide [244] sequences are being explored. Recently, a membrane anchored β-secretase transi-

tion state inhibitor has been synthesised by linking the inhibitor to a sterol moiety [245]. This inhibitor efficiently targeted β−secretase in endosomes via endocytosis, and this targeted approach significantly enhanced inhibitor efficacy, both in cell culture and in APPsw/PSΔE9 mice. Future investigations of these sterol-linked membrane anchored inhibitor types may thus enable selective inhibitory targeting of BACE1 in AD, with the caveat of transit across the BBB, effective endocytosis in the disease, and selective inhibitor targeting to appropriate neuronal populations. The feasibility of BACE1 inhibition for treatment of AD has been confirmed through the reduction of APP β-secretase catabolites after oral administration of BACE1 inhibitor to APP_{SWE} mice [246] or direct injection of BACE1 inhibitor into cerebral ventricles of non-transgenic mice [247]. Microinjection of BACE1 inhibitor into the brain of APP_{WT} transgenic mice has also reduced the β-secretase processing of APP, without significant effects on NRG-1 processing [248].

From the detailed investigations of BACE1 biology reviewed above, it is increasingly clear that BACE1 has vital normal functions in the CNS in the production of normal levels of Aβ and in NRG-1 and VGSC subunit processing, with indicated associated regulation of cognitive and emotional processes. More CNS and synaptic function relevant substrates of BACE1 may emerge in the future. We thus need to develop a more detailed understanding of the physiological function of BACE1 activity and how this relates to cognition and emotion. In addition, it will be important to decipher what tips BACE1 from a normal functioning protease to induce its pathological overactivation with age in AD, with excess Aβ production and associated cognitive and emotional dysfuntion. As reviewed in detail above, the control of BACE1 activity and function in the CNS is multifactorial and complex. It is obvious that in time only some of these mechanisms will emerge to be critical primary regulators of BACE1 activity in human brain and in AD. Presently, factors that regulate BACE1 activity appear to converge on systems that control enzyme trafficking through different neuronal cellular compartments and membrane microdomains, which synchronise with its activation, inactivation and degradation cycles. As BACE1 is maximally activated at acidic pH, acidification of vesicles, particulary in the endosomal compartments, will be central to the correct activation and inactivation cycles for the enzyme. In addition, inappropriate acidification of other organelles in AD would also overactivate BACE1.

Despite the complexity and multiplicity of mechanisms that are emerging to regulate BACE1 activity, it is worth bearing in mind that something as simple as a double missense mutation in APP, which works be providing a more attractive APP substrate to BACE1, is the sole cause of increased Aβ production and rare familial forms of AD. Thus a critical single control element of BACE1 function which normally works to activate and deactivate its processing of APP during its life time in the neuron, may become slowly impaired with age in AD. The simplest explanation for increased BACE1 activity in AD is its increased domicile in the endosomal compartment, where it is maximally active. In addition, changes in the lipid environment of the AD endosome and plasma membrane may further enhance APP / BACE1 interaction in this domain. Thus, further understanding of the protein, lipid and signalling dynamics that control BACE1 activity with respect to its trafficking in neurons and synapses and how this relates to the development of AD pathology holds much promise for the effective targeting of BACE1 in order to normalise its function and block excess Aβ production in the disease.

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1 Hardy, J. and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353 – 356.
2 Mullan, M., Houlden, H., Windelspecht, M., Fidani, L., Lombardi, C., Diaz, P., Rossor, M., Crook, R., Hardy, J., Duff, K. and et al. (1992) A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the alpha 1-antichymotrypsin gene. Nat. Genet. 2, 340 – 342.
3 Haass, C., Lemere, C.A., Capell, A., Citron, M., Seubert, P., Schenk, D., Lannfelt, L. and Selkoe, D. J. (1995) The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway. Nat. Med. 1, 1291 – 1296.
4 Sinha, S., Anderson, J.P., Barbour, R., Basi, G.S., Caccavello, R., Davis, D., Doan, M., Dovey, H.F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Schenk, D., Seubert, P., Suomensaari, S.M., Wang, S., Walker, D., John, V. and et al. (1999) Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 402, 537 – 540.
5 Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Louis, J.C., Collins, F., Treanor, J., Rogers, G. and Citron, M. (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286, 735 – 741.
6 Yan, R., Bienkowski, M. J., Shuck, M. E., Miao, H., Tory, M. C., Pauley, A. M., Brashier, J. R., Stratman, N. C., Mathews, W.R., Buhl, A. E., Carter, D.B., Tomasselli, A.G., Parodi, L. A., Heinrikson, R. L. and Gurney, M. E. (1999) Membrane-

anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature 402, 533 – 537.
7 Hussain, I., Powell, D., Howlett, D.R., Tew, D. G., Meek, T. D., Chapman, C., Gloger, I. S., Murphy, K. E., Southan, C. D., Ryan, D. M., Smith, T. S., Simmons, D. L., Walsh, F. S., Dingwall, C. and Christie, G. (1999) Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol. Cell. Neurosci. 14, 419 – 427.
8 Lin, X., Koelsch, G., Wu, S., Downs, D., Dashti, A. and Tang, J. (2000) Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. Proc Natl Acad Sci U S A 97, 1456 – 1460.
9 Saunders, A. J., Kim, T.-W., Tanzi;, R. E., Fan, W., Bennett, B. D., Babu-Kahn, S., Luo, Y., Louis, J.-C., McCaleb, M., Citron, M., Vassar, R. and Richards;, W. G. (1999) BACE Maps to Chromosome 11;and a BACE Homolog, BACE2, Reside in the Obligate Down Syndrome Region of Chromosome 21. Science 286, 1255a-.
10 Solans, A., Estivill, X. and de La Luna, S. (2000) A new aspartyl protease on 21q22.3, BACE2, is highly similar to Alzheimer's amyloid precursor protein beta-secretase. Cytogenet. Cell. Genet. 89, 177 – 184.
11 Acquati, F., Accarino, M., Nucci, C., Fumagalli, P., Jovine, L., Ottolenghi, S. and Taramelli, R. (2000) The gene encoding DRAP (BACE2), a glycosylated transmembrane protein of the aspartic protease family, maps to the down critical region. FEBS Lett 468, 59 – 64.
12 Bennett, B. D., Babu-Khan, S., Loeloff, R., Louis, J. C., Curran, E., Citron, M. and Vassar, R. (2000) Expression analysis of BACE2 in brain and peripheral tissues. J. Biol. Chem. 275, 20647 – 20651.
13 Gruninger-Leitch, F., Schlatter, D., Kung, E., Nelbock, P. and Dobeli, H. (2002) Substrate and inhibitor profile of BACE (beta-secretase) and comparison with other mammalian aspartic proteases. J. Biol. Chem. 277, 4687 – 4693.
14 Haass, C., Hung, A. Y., Schlossmacher, M. G., Oltersdorf, T., Teplow, D. B. and Selkoe, D. J. (1993) Normal cellular processing of the beta-amyloid precursor protein results in the secretion of the amyloid beta peptide and related molecules. Ann. N. Y. Acad. Sci. 695, 109 – 116.
15 Koo, E. H. and Squazzo, S. L. (1994) Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J. Biol. Chem. 269, 17386 – 17389.
16 Roberds, S. L., Anderson, J., Basi, G., Bienkowski, M. J., Branstetter, D. G., Chen, K. S., Freedman, S. B., Frigon, N. L., Games, D., Hu, K., Johnson-Wood, K., Kappenman, K.E., Kawabe, T. T., Kola, I., Kuehn, R., Lee, M., Liu, W., Motter, R., Nichols, N. F., Power, M., Robertson, D. W., Schenk, D., Schoor, M., Shopp, G. M., Shuck, M. E., Sinha, S., Svensson, K. A., Tatsuno, G., Tintrup, H., Wijsman, J., Wright, S. and McConlogue, L. (2001) BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. Hum. Mol. Genet. 10, 1317 – 1324.
17 Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D. R., Price, D. L. and Wong, P.C. (2001) BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. Nat. Neurosci. 4, 233 – 234.
18 Luo, Y., Bolon, B., Kahn, S., Bennett, B. D., Babu-Khan, S., Denis, P., Fan, W., Kha, H., Zhang, J., Gong, Y., Martin, L., Louis, J. C., Yan, Q., Richards, W. G., Citron, M. and Vassar, R. (2001) Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. Nat. Neurosci. 4, 231 – 232.
19 Christensen, M. A., Zhou, W., Qing, H., Lehman, A., Philipsen, S. and Song, W. (2004) Transcriptional regulation of BACE1, the beta-amyloid precursor protein beta-secretase, by Sp1. Mol. Cell. Biol. 24, 865 – 874.
20 Maloney, B., Ge, Y. W., Greig, N. H. and Lahiri, D. K. (2006) Characterization of the human beta-secretase 2 (BACE2) 5'-flanking region: identification of a 268-bp region as the basal BACE2 promoter. J. Mol. Neurosci. 29, 81 – 99.
21 Sun, X., Wang, Y., Qing, H., Christensen, M. A., Liu, Y., Zhou, W., Tong, Y., Xiao, C., Huang, Y., Zhang, S., Liu, X. and Song, W. (2005) Distinct transcriptional regulation and function of the human BACE2 and BACE1 genes. Faseb. J. 19, 739 – 749.
22 Lahiri, D. K., Maloney, B. and Ge, Y. W. (2006) Functional domains of the BACE1 and BACE2 promoters and mechanisms of transcriptional suppression of the BACE2 promoter in normal neuronal cells. J. Mol. Neurosci. 29, 65 – 80.
23 Lahiri, D. K., Maloney, B. and Ge, Y. W. (2006) BACE1 gene promoter is differentially regulated: detection of a novel promoter region for its cell type-specific regulation. J. Mol. Neurosci. 28, 193 – 210.
24 Lange-Dohna, C., Zeitschel, U., Gaunitz, F., Perez-Polo, J. R., Bigl, V. and Rossner, S. (2003) Cloning and expression of the rat BACE1 promoter. J. Neurosci. Res. 73, 73 – 80.
25 Hussain, I., Powell, D. J., Howlett, D. R., Chapman, G. A., Gilmour, L., Murdock, P. R., Tew, D. G., Meek, T. D., Chapman, C., Schneider, K., Ratcliffe, S. J., Tattersall, D., Testa, T. T., Southan, C., Ryan, D. M., Simmons, D. L., Walsh, F. S., Dingwall, C. and Christie, G. (2000) ASP1 (BACE2) cleaves the amyloid precursor protein at the beta- secretase site. Mol. Cell. Neurosci. 16, 609 – 619.
26 Marcinkiewicz, M. and Seidah, N. G. (2000) Coordinated expression of beta-amyloid precursor protein and the putative beta-secretase BACE and alpha-secretase ADAM10 in mouse and human brain. J. Neurochem. 75, 2133 – 2143.
27 Basi, G., Frigon, N., Barbour, R., Doan, T., Gordon, G., McConlogue, L., Sinha, S. and Zeller, M. (2003) Antagonistic effects of beta-site amyloid precursor protein-cleaving enzymes 1 and 2 on beta-amyloid peptide production in cells. J. Biol. Chem. 278, 31512 – 31520.
28 Xin, H., Stephans, J. C., Duan, X., Harrowe, G., Kim, E., Grieshammer, U., Kingsley, C. and Giese, K. (2000) Identification of a novel aspartic-like protease differentially expressed in human breast cancer cell lines. Biochim. Biophys. Acta. 1501, 125 – 137.
29 Tanahashi, H. and Tabira, T. (2001) Three novel alternatively spliced isoforms of the human beta-site amyloid precursor protein cleaving enzyme (BACE) and their effect on amyloid beta-peptide production. Neurosci. Lett. 307, 9 – 12.
30 Keane, T. M., Creevey, C. J., Pentony, M. M., Naughton, T. J. and McLnerney, J. O. (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evol. Biol. 6, 29.
31 Panopoulou, G. and Poustka, A. J. (2005) Timing and mechanism of ancient vertebrate genome duplications – the adventure of a hypothesis. Trends Genet. 21, 559 – 567.
32 McLysaght, A., Hokamp, K. and Wolfe, K. H. (2002) Extensive genomic duplication during early chordate evolution. Nat. Genet. 31, 200 – 204.
33 Fischer, F., Molinari, M., Bodendorf, U. and Paganetti, P. (2002) The disulphide bonds in the catalytic domain of BACE are critical but not essential for amyloid precursor protein processing activity. J. Neurochem. 80, 1079 – 1088.
34 Haniu, M., Denis, P., Young, Y., Mendiaz, E. A., Fuller, J., Hui, J. O., Bennett, B. D., Kahn, S., Ross, S., Burgess, T., Katta, V., Rogers, G., Vassar, R. and Citron, M. (2000) Characterization of Alzheimer's beta -secretase protein BACE. A pepsin family member with unusual properties. J. Biol. Chem. 275, 21099 – 21106.
35 Lin, X. L., Lin, Y. Z. and Tang, J. (1994) Relationships of human immunodeficiency virus protease with eukaryotic aspartic proteases. Methods Enzymol. 241, 195 – 224.
36 Holm, I., Ollo, R., Panthier, J. J. and Rougeon, F. (1984) Evolution of aspartyl proteases by gene duplication: the mouse renin gene is organized in two homologous clusters of four exons. EMBO J. 3, 557 – 562.
37 Tang, J. (2004) Pepsin A. In: Handbook of Proteolytic Enzymes Ed. Eds.), Vol. p. pp. 19 - 28. Elsevier, London.

38 James, N. M. G. (2004) Catalytic pathway of aspartic peptidases. In: Handbook of Proteolytic Enzymes Ed. Eds.), Elsevier, London.12 – 19.

39 Ostermann, N., Eder, J., Eidhoff, U., Zink, F., Hassiepen, U., Worpenberg, S., Maibaum, J., Simic, O., Hommel, U. and Gerhartz, B. (2006) Crystal structure of human BACE2 in complex with a hydroxyethylamine transition-state inhibitor. J. Mol. Biol. 355, 249 – 261.

40 Shi, J., Zhang, S., Tang, M., Liu, X., Li, T., Wang, Y., Han, H., Guo, Y., Hao, Y., Zheng, K., Kong, X., Su, Z., Tong, Y. and Ma, C. (2004) The 1239G/C polymorphism in exon 5 of BACE1 gene may be associated with sporadic Alzheimer's disease in Chinese Hans. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 124, 54 – 57.

41 Vassar, R. (2002) Beta-secretase (BACE) as a drug target for Alzheimer's disease. Adv. Drug Deliv. Rev. 4, 1589 – 1602.

42 Shi, X. P., Chen, E., Yin, K. C., Na, S., Garsky, V. M., Lai, M. T., Li, Y. M., Platchek, M., Register, R. B., Sardana, M. K., Tang, M. J., Thiebeau, J., Wood, T., Shafer, J.A. and Gardell, S. J. (2001) The pro domain of beta-secretase does not confer strict zymogen-like properties but does assist proper folding of the protease domain. J. Biol. Chem. 276, 10366 – 10373.

43 Creemers, J. W., Ines Dominguez, D., Plets, E., Serneels, L., Taylor, N. A., Multhaup, G., Craessaerts, K., Annaert, W. and De Strooper, B. (2001) Processing of beta-secretase by furin and other members of the proprotein convertase family. J. Biol. Chem. 276, 4211 – 4217.

44 Capell, A., Steiner, H., Willem, M., Kaiser, H., Meyer, C., Walter, J., Lammich, S., Multhaup, G. and Haass, C. (2000) Maturation and pro-peptide cleavage of beta-secretase. J. Biol. Chem. 275, 30849 – 30854.

45 Patel, S., Vuillard, L., Cleasby, A., Murray, C. W. and Yon, J. (2004) Apo and inhibitor complex structures of BACE (beta-secretase). J. Mol. Biol. 343, 407 – 416.

46 Hong, L., He, X., Huang, X., Chang, W. and Tang, J. (2004) Structural features of human memapsin 2 (beta-secretase) and their biological and pathological implications. Acta. Biochim. Biophys. Sin. (Shanghai) 36, 787 – 792.

47 Hong, L. and Tang, J. (2004) Flap position of free memapsin 2 (beta-secretase), a model for flap opening in aspartic protease catalysis. Biochemistry 43, 4689 – 4695.

48 Hong, L., Turner, R. T., 3rd, Koelsch, G., Shin, D., Ghosh, A. K. and Tang, J. (2002) Crystal structure of memapsin 2 (beta-secretase) in complex with an inhibitor OM00-3. Biochemistry 41, 10963 – 10967.

49 Hong, L., Koelsch, G., Lin, X., Wu, S., Terzyan, S., Ghosh, A. K., Zhang, X. C. and Tang, J. (2000) Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. Science 290, 150 – 153.

50 Turner, R. T., 3rd, Hong, L., Koelsch, G., Ghosh, A. K. and Tang, J. (2005) Structural locations and functional roles of new subsites S5, S6, and S7 in memapsin 2 (beta-secretase). Biochemistry 44, 105 – 112.

51 Durham, T. B. and Shepherd, T. A. (2006) Progress toward the discovery and development of efficacious BACE inhibitors. Curr. Opin. Drug Discov. Devel. 9, 776 – 791.

52 Farzan, M., Schnitzler, C. E., Vasilieva, N., Leung, D. and Choe, H. (2000) BACE2, a beta -secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. Proc. Natl. Acad. Sci. U S A 97, 9712 – 9717.

53 Gorfe, A.A. and Caflisch, A. (2005) Functional plasticity in the substrate binding site of beta-secretase. Structure 13, 1487 – 1498.

54 Ghosh, A. K., Kumaragurubaran, N., Hong, L., Koelsh, G. and Tang, J. (2008) Memapsin 2 (beta-secretase) inhibitors: drug development. Curr. Alzheimer Res. 5, 121 – 131.

55 Costantini, C., Ko, M. H., Jonas, M. C. and Puglielli, L. (2007) A reversible form of lysine acetylation in the ER and Golgi lumen controls the molecular stabilization of BACE1. Biochem. J.

56 Harrison, S. M., Harper, A. J., Hawkins, J., Duddy, G., Grau, E., Pugh, P. L., Winter, P.H., Shilliam, C. S., Hughes, Z. A., Dawson, L. A., Gonzalez, M. I., Upton, N., Pangalos, M. N. and Dingwall, C. (2003) BACE1 (beta-secretase) transgenic and knockout mice: identification of neurochemical deficits and behavioral changes. Mol. Cell. Neurosci. 24, 646 – 655.

57 Luo, Y., Bolon, B., Damore, M. A., Fitzpatrick, D., Liu, H., Zhang, J., Yan, Q., Vassar, R. and Citron, M. (2003) BACE1 (beta-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time. Neurobiol. Dis. 14, 81 – 88.

58 Ohno, M., Sametsky, E. A., Younkin, L. H., Oakley, H., Younkin, S.G., Citron, M., Vassar, R. and Disterhoft, J. F. (2004) BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron. 41, 27 – 33.

59 Ohno, M., Cole, S. L., Yasvoina, M., Zhao, J., Citron, M., Berry, R., Disterhoft, J. F. and Vassar, R. (2007) BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mice. Neurobiol. Dis. 26, 134 – 145.

60 Laird, F. M., Cai, H., Savonenko, A. V., Farah, M. H., He, K., Melnikova, T., Wen, H., Chiang, H. C., Xu, G., Koliatsos, V. E., Borchelt, D. R., Price, D. L., Lee, H. K. and Wong, P. C. (2005) BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. J. Neurosci. 25, 11693 – 11709.

61 Kobayashi, D., Zeller, M., Cole, T., Buttini, M., McConlogue, L., Sinha, S., Freedman, S., Morris, R. G. and Chen, K. S. (2008) BACE1 gene deletion: impact on behavioral function in a model of Alzheimer's disease. Neurobiol. Aging 29, 861 – 873.

62 McConlogue, L., Buttini, M., Anderson, J. P., Brigham, E. F., Chen, K. S., Freedman, S. B., Games, D., Johnson-Wood, K., Lee, M., Zeller, M., Liu, W., Motter, R. and Sinha, S. (2007) Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice. J. Biol. Chem. 282, 26326 – 26334.

63 Singer, O., Marr, R. A., Rockenstein, E., Crews, L., Coufal, N. G., Gage, F. H., Verma, I. M. and Masliah, E. (2005) Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. Nat. Neurosci. 8, 1343 – 1349.

64 Ohno, M., Chang, L., Tseng, W., Oakley, H., Citron, M., Klein, W. L., Vassar, R. and Disterhoft, J. F. (2006) Temporal memory deficits in Alzheimer's mouse models: rescue by genetic deletion of BACE1. Eur. J. Neurosci. 23, 251 – 260.

65 Dominguez, D., Tournoy, J., Hartmann, D., Huth, T., Cryns, K., Deforce, S., Serneels, L., Camacho, I. E., Marjaux, E., Craessaerts, K., Roebroek, A. J., Schwake, M., D'Hooge, R., Bach, P., Kalinke, U., Moechars, D., Alzheimer, C., Reiss, K., Saftig, P. and De Strooper, B. (2005) Phenotypic and biochemical analyses of BACE1- and BACE2-deficient mice. J. Biol. Chem. 280, 30797 – 30806.

66 Savonenko, A. V., Melnikova, T., Laird, F. M., Stewart, K. A., Price, D. L. and Wong, P.C. (2008) Alteration of BACE1-dependent NRG1/ErbB4 signaling and schizophrenia-like phenotypes in BACE1-null mice. Proc. Natl. Acad. Sci. U S A 105, 5585 – 5590.

67 Hu, X., Hicks, C. W., He, W., Wong, P., Macklin, W. B., Trapp, B. D. and Yan, R. (2006) Bace1 modulates myelination in the central and peripheral nervous system. Nat. Neurosci. 9, 1520 – 1525.

68 Hu, X., He, W., Diaconu, C., Tang, X., Kidd, G. J., Macklin, W. B., Trapp, B. D. and Yan, R. (2008) Genetic deletion of BACE1 in mice affects remyelination of sciatic nerves. Faseb J.

69 Willem, M., Garratt, A. N., Novak, B., Citron, M., Kaufmann, S., Rittger, A., Destrooper, B., Saftig, P., Birchmeier, C. and Haass, C. (2006) Control of Peripheral Nerve Myelination by the {beta}-Secretase BACE1. Science.

70 Ma, H., Lesne, S., Kotilinek, L., Steidl-Nichols, J. V., Sherman, M., Younkin, L., Younkin, S., Forster, C., Sergeant, N., Delacourte, A., Vassar, R., Citron, M., Kofuji, P., Boland, L. M. and Ashe, K. H. (2007) Involvement of beta-site APP cleaving enzyme 1 (BACE1) in amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity. Proc. Natl. Acad. Sci. U S A 104, 8167 – 8172.

71 Turner, R. T., 3rd, Koelsch, G., Hong, L., Castanheira, P., Ermolieff, J., Ghosh, A. K. and Tang, J. (2001) Subsite specificity of memapsin 2 (beta-secretase): implications for inhibitor design. Biochemistry 40, 10001 – 10006.

72 Turner, R. T., 3rd, Loy, J. A., Nguyen, C., Devasamudram, T., Ghosh, A. K., Koelsch, G. and Tang, J. (2002) Specificity of memapsin 1 and its implications on the design of memapsin 2 (beta-secretase) inhibitor selectivity. Biochemistry 41, 8742 – 8746.

73 Yang, H. C., Chai, X., Mosior, M., Kohn, W., Boggs, L. N., Erickson, J. A., McClure, D. B., Yeh, W. K., Zhang, L., Gonzalez-DeWhitt, P., Mayer, J. P., Martin, J. A., Hu, J., Chen, S. H., Bueno, A. B., Little, S. P., McCarthy, J. R. and May, P. C. (2004) Biochemical and kinetic characterization of BACE1: investigation into the putative species-specificity for beta- and beta'-cleavage sites by human and murine BACE1. J. Neurochem. 91, 1249 – 1259.

74 Toulokhonova, L., Metzler, W. J., Witmer, M. R., Copeland, R. A. and Marcinkeviciene, J. (2003) Kinetic studies on beta-site amyloid precursor protein-cleaving enzyme (BACE). Confirmation of an iso mechanism. J. Biol. Chem. 278, 4582 – 4589.

75 Kim, D. Y., Carey, B. W., Wang, H., Ingano, L. A., Binshtok, A. M., Wertz, M. H., Pettingell, W. H., He, P., Lee, V. M., Woolf, C. J. and Kovacs, D. M. (2007) BACE1 regulates voltage-gated sodium channels and neuronal activity. Nat. Cell. Biol. 9, 755 – 764.

76 Wong, H. K., Sakurai, T., Oyama, F., Kaneko, K., Wada, K., Miyazaki, H., Kurosawa, M., De Strooper, B., Saftig, P. and Nukina, N. (2005) beta subunits of voltage-gated sodium channels are novel substrates of BACE1 and gamma - secretase. J. Biol. Chem.

77 Kitazume, S., Tachida, Y., Oka, R., Shirotani, K., Saido, T. C. and Hashimoto, Y. (2001) Alzheimer's beta-secretase, beta-site amyloid precursor protein- cleaving enzyme, is responsible for cleavage secretion of a Golgi- resident sialyltransferase. Proc. Natl. Acad. Sci. U S A 98, 13554 – 13559.

78 Lichtenthaler, S. F., Dominguez, D. I., Westmeyer, G. G., Reiss, K., Haass, C., Saftig, P., De Strooper, B. and Seed, B. (2003) The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1. J. Biol. Chem. 278, 48713 – 48719.

79 von Arnim, C. A., Kinoshita, A., Peltan, I. D., Tangredi, M. M., Herl, L., Lee, B. M., Spoelgen, R., Hshieh, T. T., Ranganathan, S., Battey, F. D., Liu, C. X., Bacskai, B. J., Sever, S., Irizarry, M. C., Strickland, D. K. and Hyman, B. T. (2005) The low density lipoprotein receptor-related protein (LRP) is a novel beta-secretase (BACE1) substrate. J. Biol. Chem. 280, 17777 – 17785.

80 Kuhn, P. H., Marjaux, E., Imhof, A., De Strooper, B., Haass, C. and Lichtenthaler, S. F. (2007) Regulated intramembrane proteolysis of the interleukin-1 receptor II by alpha-, beta-, and gamma-secretase. J. Biol. Chem. 282, 11982 – 11995.

81 Skovronsky, D. M., Moore, D. B., Milla, M. E., Doms, R. W. and Lee, V. M. (2000) Protein kinase C-dependent alpha-secretase competes with beta-secretase for cleavage of amyloid-beta precursor protein in the trans-golgi network. J. Biol. Chem. 275, 2568 – 2575.

82 Huse, J. T., Liu, K., Pijak, D. S., Carlin, D., Lee, V. M. and Doms, R. W. (2002) Beta-secretase processing in the trans-Golgi network preferentially generates truncated amyloid species that accumulate in Alzheimer's disease brain. J. Biol. Chem. 277, 16278 – 16284.

83 Huse, J. T., Pijak, D. S., Leslie, G. J., Lee, V. M. and Doms, R. W. (2000) Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. J. Biol. Chem. 275, 33729 – 33737.

84 Chyung, J. H. and Selkoe, D. J. (2003) Inhibition of receptor-mediated endocytosis demonstrates generation of amyloid beta-protein at the cell surface. J. Biol. Chem. 278, 51035 – 51043.

85 Yan, R., Munzner, J. B., Shuck, M. E. and Bienkowski, M. J. (2001) BACE2 functions as an alternative alpha-secretase in cells. J. Biol. Chem. 276, 34019 – 34027.

86 Endres, K., Postina, R., Schroeder, A., Mueller, U. and Fahrenholz, F. (2005) Shedding of the amyloid precursor protein-like protein APLP2 by disintegrin-metalloproteinases. Febs J. 272, 5808 – 5820.

87 Pastorino, L., Ikin, A. F., Lamprianou, S., Vacaresse, N., Revelli, J. P., Platt, K., Paganetti, P., Mathews, P. M., Harroch, S. and Buxbaum, J. D. (2004) BACE (beta-secretase) modulates the processing of APLP2 in vivo. Mol. Cell. Neurosci. 25, 642 – 649.

88 Eggert, S., Paliga, K., Soba, P., Evin, G., Masters, C. L., Weidemann, A. and Beyreuther, K. (2004) The proteolytic processing of the amyloid precursor protein gene family members APLP-1 and APLP-2 involves alpha-, beta-, gamma-, and epsilon-like cleavages: modulation of APLP-1 processing by n-glycosylation. J. Biol. Chem. 279, 18146 – 18156.

89 Li, Q. and Sudhof, T.C. (2004) Cleavage of amyloid-beta precursor protein and amyloid-beta precursor-like protein by BACE 1. J. Biol. Chem. 279, 10542 – 10550.

90 Scheinfeld, M. H., Ghersi, E., Laky, K., Fowlkes, B. J. and D'Adamio, L. (2002) Processing of beta-amyloid precursor-like protein-1 and -2 by gamma-secretase regulates transcription. J. Biol. Chem. 277, 44195 – 44201.

91 Walsh, D. M., Minogue, A. M., Sala Frigerio, C., Fadeeva, J. V., Wasco, W. and Selkoe, D.J. (2007) The APP family of proteins: similarities and differences. Biochem. Soc. Trans. 35, 416 – 420.

92 Michailov, G. V., Sereda, M. W., Brinkmann, B. G., Fischer, T. M., Haug, B., Birchmeier, C., Role, L., Lai, C., Schwab, M. H. and Nave, K. A. (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304, 700 – 703.

93 Falls, D.L. (2003) Neuregulins: functions, forms, and signaling strategies. Exp. Cell. Res. 284, 14 – 30.

94 Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S., Ivarsson, O., Chou, T. T., Hjaltason, O., Birgisdottir, B., Jonsson, H., Gudnadottir, V. G., Gudmundsdottir, E., Bjornsson, A., Ingvarsson, B., Ingason, A., Sigfusson, S., Hardardottir, H., Harvey, R.P., Lai, D., Zhou, M., Brunner, D., Mutel, V., Gonzalo, A., Lemke, G., Sainz, J., Johannesson, G., Andresson, T., Gudbjartsson, D., Manolescu, A., Frigge, M. L., Gurney, M. E., Kong, A., Gulcher, J. R., Petursson, H. and Stefansson, K. (2002) Neuregulin 1 and susceptibility to schizophrenia. Am. J. Hum. Genet. 71, 877 – 892.

95 Bartzokis, G. (2004) Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. Neurobiol. Aging 25, 5 – 18; author reply 49 – 62.

96 Braak, H., Del Tredici, K., Schultz, C. and Braak, E. (2000) Vulnerability of select neuronal types to Alzheimer's disease. Ann. N. Y. Acad. Sci. 924, 53 – 61.

97 Kitazume, S., Suzuki, M., Saido, T. C. and Hashimoto, Y. (2004) Involvement of proteases in glycosyltransferase secretion: Alzheimer's beta-secretase-dependent cleavage and a following processing by an aminopeptidase. Glycoconj. J. 21, 25 – 29.

98 Kitazume, S., Nakagawa, K., Oka, R., Tachida, Y., Ogawa, K., Luo, Y., Citron, M., Shitara, H., Taya, C., Yonekawa, H., Paulson, J. C., Miyoshi, E., Taniguchi, N. and Hashimoto, Y. (2005) In vivo cleavage of alpha2,6-sialyltransferase by Alzheimer beta-secretase. J. Biol. Chem. 280, 8589 – 8595.

99 Chen, M. and Geng, J. G. (2006) P-selectin mediates adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis. Arch. Immunol. Ther. Exp. (Warsz) 54, 75 – 84.

100 McEver, R. P. and Cummings, R. D. (1997) Perspectives series: cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment. J. Clin. Invest. 100, 485 – 491.

101 Stockley, J. H., Ravid, R. and O'Neill, C. (2006) Altered beta-secretase enzyme kinetics and levels of both BACE1 and BACE2 in the Alzheimer's disease brain. FEBS Lett 580, 6550 – 6560.

102 Tyler, S. J., Dawbarn, D., Wilcock, G. K. and Allen, S. J. (2002) alpha- and beta-secretase: profound changes in Alzheimer's disease. Biochem. Biophys. Res. Commun. 299, 373 – 376.

103 Fukumoto, H., Cheung, B. S., Hyman, B. T. and Irizarry, M. C. (2002) Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease. Arch. Neurol. 59, 1381 – 1389.

104 Stockley, J. H. and O'Neill, C. (2007) The proteins BACE1 and BACE2 and beta-secretase activity in normal and Alzheimer's disease brain. Biochem. Soc. Trans. 35, 574 – 576.

105 Holsinger, R. M., McLean, C. A., Beyreuther, K., Masters, C. L. and Evin, G. (2002) Increased expression of the amyloid precursor beta-secretase in Alzheimer's disease. Ann. Neurol. 51, 783 – 786.

106 Li, R., Lindholm, K., Yang, L. B., Yue, X., Citron, M., Yan, R., Beach, T., Sue, L., Sabbagh, M., Cai, H., Wong, P., Price, D. and Shen, Y. (2004) Amyloid beta peptide load is correlated with increased beta-secretase activity in sporadic Alzheimer's disease patients. Proc. Natl. Acad. Sci. U S A 101, 3632 – 3637.

107 Zhao, J., Fu, Y., Yasvoina, M., Shao, P., Hitt, B., O'Connor, T., Logan, S., Maus, E., Citron, M., Berry, R., Binder, L. and Vassar, R. (2007) Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: implications for Alzheimer's disease pathogenesis. J. Neurosci. 27, 3639 – 3649.

108 Hebert, S. S., Horre, K., Nicolai, L., Papadopoulou, A. S., Mandemakers, W., Silahtaroglu, A. N., Kauppinen, S., Delacourte, A. and De Strooper, B. (2008) Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. Proc. Natl. Acad. Sci. U S A 105, 6415 – 6420.

109 Harada, H., Tamaoka, A., Ishii, K., Shoji, S., Kametaka, S., Kametani, F., Saito, Y. and Murayama, S. (2006) Beta-site APP cleaving enzyme 1 (BACE1) is increased in remaining neurons in Alzheimer's disease brains. Neurosci. Res. 54, 24 – 29.

110 Leuba, G., Wernli, G., Vernay, A., Kraftsik, R., Mohajeri, M. H. and Saini, K. D. (2005) Neuronal and Nonneuronal Quantitative BACE Immunocytochemical Expression in the Entorhinohippocampal and Frontal Regions in Alzheimer's Disease. Dement. Geriatr. Cogn. Disord. 19, 171 – 183.

111 Sun, A., Koelsch, G., Tang, J. and Bing, G. (2002) Localization of beta-secretase memapsin 2 in the brain of Alzheimer's patients and normal aged controls. Exp. Neurol. 175, 10 – 22.

112 Preece, P., Virley, D. J., Costandi, M., Coombes, R., Moss, S. J., Mudge, A. W., Jazin, E. and Cairns, N. J. (2003) Beta-secretase (BACE) and GSK-3 mRNA levels in Alzheimer's disease. Brain Res. Mol. Brain Res. 116, 155 – 158.

113 Yasojima, K., McGeer, E. G. and McGeer, P. L. (2001) Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. Brain Res. 919, 115 – 121.

114 Matsui, T., Ingelsson, M., Fukumoto, H., Ramasamy, K., Kowa, H., Frosch, M. P., Irizarry, M. C. and Hyman, B. T. (2007) Expression of APP pathway mRNAs and proteins in Alzheimer's disease. Brain Res. 1161, 116 – 123.

115 Sun, X., Tong, Y., Qing, H., Chen, C. H. and Song, W. (2006) Increased BACE1 maturation contributes to the pathogenesis of Alzheimer's disease in Down syndrome. Faseb J. 20, 1361 – 1368.

116 Andrau, D., Dumanchin-Njock, C., Ayral, E., Vizzavona, J., Farzan, M., Boisbrun, M., Fulcrand, P., Hernandez, J. F., Martinez, J., Lefranc-Jullien, S. and Checler, F. (2003) BACE 1 and BACE 2 expressing human cells: Characterization of bAPP- derived catabolites, design of a novel fluorimetric assay and identification of new vitro inhibitors. J. Biol. Chem. 7, 7.

117 Fluhrer, R., Capell, A., Westmeyer, G., Willem, M., Hartung, B., Condron, M. M., Teplow, D. B., Haass, C. and Walter, J. (2002) A non-amyloidogenic function of BACE-2 in the secretory pathway. J. Neurochem. 81, 1011 – 1020.

118 Wen, Y., Onyewuchi, O., Yang, S., Liu, R. and Simpkins, J. W. (2004) Increased beta-secretase activity and expression in rats following transient cerebral ischemia. Brain Res. 1009, 1 – 8.

119 Barbiero, L., Benussi, L., Ghidoni, R., Alberici, A., Russo, C., Schettini, G., Pagano, S. F., Parati, E. A., Mazzoli, F., Nicosia, F., Signorini, S., Feudatari, E. and Binetti, G. (2003) BACE-2 is overexpressed in Down's syndrome. Exp. Neurol. 182, 335 – 345.

120 Motonaga, K., Itoh, M., Becker, L. E., Goto, Y. and Takashima, S. (2002) Elevated expression of beta-site amyloid precursor protein cleaving enzyme 2 in brains of patients with Down syndrome. Neurosci. Lett. 326, 64 – 66.

121 Nistor, M., Don, M., Parekh, M., Sarsoza, F., Goodus, M., Lopez, G. E., Kawas, C., Leverenz, J., Doran, E., Lott, I. T., Hill, M. and Head, E. (2006) Alpha- and beta-secretase activity as a function of age and beta-amyloid in Down syndrome and normal brain. Neurobiol. Aging.

122 Charlwood, J., Dingwall, C., Matico, R., Hussain, I., Johanson, K., Moore, S., Powell, D. J., Skehel, J. M., Ratcliffe, S., Clarke, B., Trill, J., Sweitzer, S. and Camilleri, P. (2001) Characterization of the glycosylation profiles of Alzheimer's beta - secretase protein Asp-2 expressed in a variety of cell lines. J. Biol. Chem. 276, 16739 – 16748.

123 Bodendorf, U., Fischer, F., Bodian, D., Multhaup, G. and Paganetti, P. (2001) A splice variant of beta-secretase deficient in the amyloidogenic processing of the amyloid precursor protein. J. Biol. Chem. 276, 12019 – 12023.

124 Rossner, S., Sastre, M., Bourne, K. and Lichtenthaler, S. F. (2006) Transcriptional and translational regulation of BACE1 expression–implications for Alzheimer's disease. Prog. Neurobiol. 79, 95 – 111.

125 Hussain, I., Hawkins, J., Shikotra, A., Riddell, D. R., Faller, A. and Dingwall, C. (2003) Characterization of the ectodomain shedding of the beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). J. Biol. Chem. 278, 36264 – 36268.

126 Benjannet, S., Elagoz, A., Wickham, L., Mamarbachi, M., Munzer, J. S., Basak, A., Lazure, C., Cromlish, J. A., Sisodia, S., Checler, F., Chretien, M. and Seidah, N.G. (2001) Post-translational processing of beta-secretase (beta-amyloid-converting enzyme) and its ectodomain shedding. The pro- and transmembrane/cytosolic domains affect its cellular activity and amyloid-beta production. J. Biol. Chem. 276, 10879 – 10887.

127 Yan, R., Han, P., Miao, H., Greengard, P. and Xu, H. (2001) The transmembrane domain of the Alzheimer's beta-secretase (BACE1) determines its late Golgi localization and access to beta -amyloid precursor protein (APP) substrate. J. Biol. Chem. 276, 36788 – 36796.

128 Pastorino, L., Ikin, A. F., Nairn, A. C., Pursnani, A. and Buxbaum, J. D. (2002) The carboxyl-terminus of BACE contains a sorting signal that regulates BACE trafficking but not the formation of total A(beta). Mol. Cell. Neurosci. 19, 175 – 185.

129 Koh, Y. H., von Arnim, C. A., Hyman, B. T., Tanzi, R. E. and Tesco, G. (2005) BACE is degraded via the lysosomal pathway. J. Biol. Chem. 280, 32499 – 32504.

130 He, X., Chang, W. P., Koelsch, G. and Tang, J. (2002) Memapsin 2 (beta-secretase) cytosolic domain binds to the

VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2. FEBS Lett 524, 183 – 187.
131 Shiba, T., Kametaka, S., Kawasaki, M., Shibata, M., Waguri, S., Uchiyama, Y. and Wakatsuki, S. (2004) Insights into the phosphoregulation of beta-secretase sorting signal by the VHS domain of GGA1. Traffic 5, 437 – 448.
132 von Arnim, C. A., Tangredi, M. M., Peltan, I. D., Lee, B. M., Irizarry, M. C., Kinoshita, A. and Hyman, B. T. (2004) Demonstration of BACE (beta-secretase) phosphorylation and its interaction with GGA1 in cells by fluorescence-lifetime imaging microscopy. J. Cell. Sci. 117, 5437 – 5445.
133 Wahle, T., Prager, K., Raffler, N., Haass, C., Famulok, M. and Walter, J. (2005) GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network. Mol. Cell. Neurosci.
134 Walter, J., Fluhrer, R., Hartung, B., Willem, M., Kaether, C., Capell, A., Lammich, S., Multhaup, G. and Haass, C. (2001) Phosphorylation regulates intracellular trafficking of beta-secretase. J. Biol. Chem. 276, 14634 – 14641.
135 Walter, J. (2006) Control of amyloid-beta-peptide generation by subcellular trafficking of the beta-amyloid precursor protein and beta-secretase. Neurodegener. Dis. 3, 247 – 254.
136 He, X., Zhu, G., Koelsch, G., Rodgers, K. K., Zhang, X. C. and Tang, J. (2003) Biochemical and structural characterization of the interaction of memapsin 2 (beta-secretase) cytosolic domain with the VHS domain of GGA proteins. Biochemistry 42, 12174 – 12180.
137 He, X., Li, F., Chang, W.P. and Tang, J. (2005) GGA proteins mediate the recycling pathway of memapsin 2 (BACE). J. Biol. Chem. 280, 11696 – 11703.
138 Wahle, T., Thal, D. R., Sastre, M., Rentmeister, A., Bogdanovic, N., Famulok, M., Heneka, M. T. and Walter, J. (2006) GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide. J. Neurosci. 26, 12838 – 12846.
139 Huang, X. P., Chang, W. P., Koelsch, G., Turner, R. T., 3rd, Lupu, F. and Tang, J. (2004) Internalization of exogenously added memapsin 2 (beta-secretase) ectodomain by cells is mediated by amyloid precursor protein. J. Biol. Chem. 279, 37886 – 37894.
140 Puglielli, L., Ellis, B. C., Saunders, A. J. and Kovacs, D. M. (2003) Ceramide stabilizes beta-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid beta-peptide biogenesis. J. Biol. Chem. 278, 19777 – 19783.
141 Qing, H., Zhou, W., Christensen, M. A., Sun, X., Tong, Y. and Song, W. (2004) Degradation of BACE by the ubiquitin-proteasome pathway. Faseb J. 18, 1571 – 1573.
142 Schmechel, A., Strauss, M., Schlicksupp, A., Pipkorn, R., Haass, C., Bayer, T. A. and Multhaup, G. (2004) Human BACE forms dimers and colocalizes with APP. J. Biol. Chem. 279, 39710 – 39717.
143 Westmeyer, G. G., Willem, M., Lichtenthaler, S. F., Lurman, G., Multhaup, G., Assfalg-Machleidt, I., Reiss, K., Saftig, P. and Haass, C. (2004) Dimerization of beta-site beta-amyloid precursor protein-cleaving enzyme. J. Biol. Chem. 279, 53205 – 53212.
144 Kaether, C. and Haass, C. (2004) A lipid boundary separates APP and secretases and limits amyloid beta-peptide generation. J. Cell. Biol. 167, 809 – 812.
145 Hattori, C., Asai, M., Onishi, H., Sasagawa, N., Hashimoto, Y., Saido, T. C., Maruyama, K., Mizutani, S. and Ishiura, S. (2006) BACE1 interacts with lipid raft proteins. J. Neurosci. Res. 84, 912 – 917.
146 Ehehalt, R., Keller, P., Haass, C., Thiele, C. and Simons, K. (2003) Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. J. Cell. Biol. 160, 113 – 123.
147 Kametaka, S., Shibata, M., Moroe, K., Kanamori, S., Ohsawa, Y., Waguri, S., Sims, P. J., Emoto, K., Umeda, M. and Uchiyama, Y. (2003) Identification of phospholipid scramblase 1 as a novel interacting molecule with beta -secretase (beta -site amyloid precursor protein (APP) cleaving enzyme (BACE)). J. Biol. Chem. 278, 15239 – 15245.
148 Tun, H., Marlow, L., Pinnix, I., Kinsey, R. and Sambamurti, K. (2002) Lipid rafts play an important role in A beta biogenesis by regulating the beta-secretase pathway. J. Mol. Neurosci. 19, 31 – 35.
149 Riddell, D. R., Christie, G., Hussain, I. and Dingwall, C. (2001) Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts. Curr. Biol. 11, 1288 – 1293.
150 Crameri, A., Biondi, E., Kuehnle, K., Lutjohann, D., Thelen, K. M., Perga, S., Dotti, C. G., Nitsch, R. M., Ledesma, M. D. and Mohajeri, M. H. (2006) The role of seladin-1/DHCR24 in cholesterol biosynthesis, APP processing and Abeta generation in vivo. EMBO J. 25, 432 – 443.
151 Cordy, J. M., Hussain, I., Dingwall, C., Hooper, N. M. and Turner, A. J. (2003) Exclusively targeting beta-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. Proc. Natl. Acad. Sci. U S A 100, 11735 – 11740.
152 Marlow, L., Cain, M., Pappolla, M. A. and Sambamurti, K. (2003) Beta-secretase processing of the Alzheimer's amyloid protein precursor (APP). J. Mol. Neurosci. 20, 233 – 239.
153 Kawarabayashi, T., Shoji, M., Younkin, L. H., Wen-Lang, L., Dickson, D. W., Murakami, T., Matsubara, E., Abe, K., Ashe, K. H. and Younkin, S. G. (2004) Dimeric amyloid beta protein rapidly accumulates in lipid rafts followed by apolipoprotein E and phosphorylated tau accumulation in the Tg2576 mouse model of Alzheimer's disease. J. Neurosci. 24, 3801 – 3809.
154 Yoon, I. S., Chen, E., Busse, T., Repetto, E., Lakshmana, M. K., Koo, E. H. and Kang, D. E. (2007) Low-density lipoprotein receptor-related protein promotes amyloid precursor protein trafficking to lipid rafts in the endocytic pathway. Faseb J. 21, 2742 – 2752.
155 Frenzel, K. E. and Falls, D. L. (2001) Neuregulin-1 proteins in rat brain and transfected cells are localized to lipid rafts. J. Neurochem. 77, 1 – 12.
156 Vetrivel, K. S., Cheng, H., Lin, W., Sakurai, T., Li, T., Nukina, N., Wong, P. C., Xu, H. and Thinakaran, G. (2004) Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. J. Biol. Chem. 279, 44945 – 44954.
157 Botto, L., Masserini, M., Cassetti, A. and Palestini, P. (2004) Immunoseparation of Prion protein-enriched domains from other detergent-resistant membrane fractions, isolated from neuronal cells. FEBS Lett 557, 143 – 147.
158 Fassbender, K., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., Runz, H., Kuhl, S., Bertsch, T., von Bergmann, K., Hennerici, M., Beyreuther, K. and Hartmann, T. (2001) Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. Proc. Natl. Acad. Sci. U S A 98, 5856 – 5861.
159 Ledesma, M. D. and Dotti, C. G. (2006) Amyloid excess in Alzheimer's disease: what is cholesterol to be blamed for? FEBS Lett 580, 5525 – 5532.
160 Schneider, A., Rajendran, L., Honsho, M., Gralle, M., Donnert, G., Wouters, F., Hell, S. W. and Simons, M. (2008) Flotillin-dependent clustering of the amyloid precursor protein regulates its endocytosis and amyloidogenic processing in neurons. J. Neurosci. 28, 2874 – 2882.
161 Nowotny, P., Kwon, J. M., Chakraverty, S., Nowotny, V., Morris, J. C. and Goate, A. M. (2001) Association studies using novel polymorphisms in BACE1 and BACE2. Neuroreport 12, 1799 – 1802.
162 Gold, G., Blouin, J. L., Herrmann, F. R., Michon, A., Mulligan, R., Duriaux Sail, G., Bouras, C., Giannakopoulos, P. and Antonarakis, S. E. (2003) Specific BACE1 genotypes provide additional risk for late-onset alzheimer disease in APOE varepsilon 4 carriers. Am. J. Med. Genet. 119B, 44 – 47.
163 Kan, R., Wang, B., Zhang, C., Jin, F., Yang, Z., Ji, S., Lu, Z., Zheng, C. and Wang, L. (2005) Genetic association of BACE1

gene polymorphism C786G with late-onset Alzheimer's disease in Chinese. J. Mol. Neurosci. 25, 127 – 131.
164 Ewers, M., Zhong, Z., Burger, K., Wallin, A., Blennow, K., Teipel, S. J., Shen, Y. and Hampel, H. (2008) Increased CSF-BACE 1 activity is associated with ApoE-epsilon 4 genotype in subjects with mild cognitive impairment and Alzheimer's disease. Brain 131, 1252 – 1258.
165 Tesco, G., Koh, Y. H., Kang, E. L., Cameron, A. N., Das, S., Sena-Esteves, M., Hiltunen, M., Yang, S. H., Zhong, Z., Shen, Y., Simpkins, J. W. and Tanzi, R. E. (2007) Depletion of GGA3 stabilizes BACE and enhances beta-secretase activity. Neuron 54, 721 – 737.
166 He, W., Lu, Y., Qahwash, I., Hu, X. Y., Chang, A. and Yan, R. (2004) Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. Nat. Med. 10, 959 – 965.
167 He, W., Hu, X., Shi, Q., Zhou, X., Lu, Y., Fisher, C. and Yan, R. (2006) Mapping of interaction domains mediating binding between BACE1 and RTN/Nogo proteins. J. Mol. Biol. 363, 625 – 634.
168 Murayama, K. S., Kametani, F., Saito, S., Kume, H., Akiyama, H. and Araki, W. (2006) Reticulons RTN3 and RTN4-B/C interact with BACE1 and inhibit its ability to produce amyloid beta-protein. Eur. J. Neurosci. 24, 1237 – 1244.
169 Spoelgen, R., von Arnim, C. A., Thomas, A. V., Peltan, I. D., Koker, M., Deng, A., Irizarry, M. C., Andersen, O. M., Willnow, T. E. and Hyman, B. T. (2006) Interaction of the cytosolic domains of sorLA/LR11 with the amyloid precursor protein (APP) and beta-secretase beta-site APP-cleaving enzyme. J. Neurosci. 26, 418 – 428.
170 Parkin, E. T., Watt, N. T., Hussain, I., Eckman, E. A., Eckman, C. B., Manson, J. C., Baybutt, H. N., Turner, A. J. and Hooper, N.M. (2007) Cellular prion protein regulates beta-secretase cleavage of the Alzheimer's amyloid precursor protein. Proc. Natl. Acad. Sci. U S A 104, 11062 – 11067.
171 Xie, J. and Guo, Q. (2005) Par-4 Is involved in regulation of beta -secretase cleavage of the alzheimer's amyloid precursor protein. J. Biol. Chem.
172 Kuzuya, A., Uemura, K., Kitagawa, N., Aoyagi, N., Kihara, T., Ninomiya, H., Ishiura, S., Takahashi, R. and Shimohama, S. (2007) Presenilin 1 is involved in the maturation of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). J Neurosci Res 85, 153 – 165.
173 Hebert, S. S., Bourdages, V., Godin, C., Ferland, M., Carreau, M. and Levesque, G. (2003) Presenilin-1 interacts directly with the beta-site amyloid protein precursor cleaving enzyme (BACE1). Neurobiol. Dis. 13, 238 – 245.
174 Angeletti, B., Waldron, K. J., Freeman, K. B., Bawagan, H., Hussain, I., Miller, C. C., Lau, K. F., Tennant, M. E., Dennison, C., Robinson, N. J. and Dingwall, C. (2005) BACE1 cytoplasmic domain interacts with the copper chaperone for superoxide dismutase-1 and binds copper. J. Biol. Chem. 280, 17930 – 17937.
175 Wickham, L., Benjannet, S., Marcinkiewicz, E., Chretien, M. and Seidah, N. G. (2005) Beta-amyloid protein converting enzyme 1 and brain-specific type II membrane protein BRI3: binding partners processed by furin. J. Neurochem. 92, 93 – 102.
176 Nakayama, K. and Wakatsuki, S. (2003) The structure and function of GGAs, the traffic controllers at the TGN sorting crossroads. Cell Struct. Funct. 28, 431 – 442.
177 Yan, R., Shi, Q., Hu, X. and Zhou, X. (2006) Reticulon proteins: emerging players in neurodegenerative diseases. Cell. Mol. Life Sci. 63, 877 – 889.
178 He, W., Shi, Q., Hu, X. and Yan, R. (2007) The membrane topology of RTN3 and its effect on binding of RTN3 to BACE1. J. Biol. Chem.
179 Hu, X., Shi, Q., Zhou, X., He, W., Yi, H., Yin, X., Gearing, M., Levey, A. and Yan, R. (2007) Transgenic mice over-expressing reticulon 3 develop neuritic abnormalities. EMBO J. 26, 2755 – 2767.
180 Andersen, O. M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., von Arnim, C. A., Breiderhoff, T., Jansen, P., Wu, X., Bales, K. R., Cappai, R., Masters, C.L., Gliemann, J., Mufson, E. J., Hyman, B. T., Paul, S. M., Nykjaer, A. and Willnow, T.E. (2005) Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc. Natl. Acad. Sci. U S A 102, 13461 – 13466.
181 Scherzer, C. R., Offe, K., Gearing, M., Rees, H. D., Fang, G., Heilman, C. J., Schaller, C., Bujo, H., Levey, A. I. and Lah, J. J. (2004) Loss of apolipoprotein E receptor LR11 in Alzheimer disease. Arch. Neurol. 61, 1200 – 1205.
182 Rogaeva, E., Meng, Y., Lee, J. H., Gu, Y., Kawarai, T., Zou, F., Katayama, T., Baldwin, C. T., Cheng, R., Hasegawa, H., Chen, F., Shibata, N., Lunetta, K. L., Pardossi-Piquard, R., Bohm, C., Wakutani, Y., Cupples, L. A., Cuenco, K. T., Green, R. C., Pinessi, L., Rainero, I., Sorbi, S., Bruni, A., Duara, R., Friedland, R. P., Inzelberg, R., Hampe, W., Bujo, H., Song, Y.Q., Andersen, O. M., Willnow, T. E., Graff-Radford, N., Petersen, R. C., Dickson, D., Der, S. D., Fraser, P. E., Schmitt-Ulms, G., Younkin, S., Mayeux, R., Farrer, L. A. and St George-Hyslop, P. (2007) The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat. Genet. 39, 168 – 177.
183 Kamal, A., Almenar-Queralt, A., LeBlanc, J. F., Roberts, E. A. and Goldstein, L. S. (2001) Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. Nature 414, 643 – 648.
184 Hooper, N. M. and Turner, A. J. (2008) A new take on prions: preventing Alzheimer's disease. Trends Biochem Sci 33, 151 – 155.
185 Ferrer, I., Blanco, R., Carmona, M., Puig, B., Ribera, R., Rey, M. J. and Ribalta, T. (2001) Prion protein expression in senile plaques in Alzheimer's disease. Acta. Neuropathol. 101, 49 – 56.
186 Kovacs, G. G., Head, M. W., Hegyi, I., Bunn, T. J., Flicker, H., Hainfellner, J. A., McCardle, L., Laszlo, L., Jarius, C., Ironside, J. W. and Budka, H. (2002) Immunohistochemistry for the prion protein: comparison of different monoclonal antibodies in human prion disease subtypes. Brain Pathol. 12, 1 – 11.
187 Rezaie, P., Pontikis, C. C., Hudson, L., Cairns, N. J. and Lantos, P. L. (2005) Expression of cellular prion protein in the frontal and occipital lobe in Alzheimer's disease, diffuse Lewy body disease, and in normal brain: an immunohistochemical study. J. Histochem. Cytochem. 53, 929 – 940.
188 Voigtlander, T., Kloppel, S., Birner, P., Jarius, C., Flicker, H., Verghese-Nikolakaki, S., Sklaviadis, T., Guentchev, M. and Budka, H. (2001) Marked increase of neuronal prion protein immunoreactivity in Alzheimer's disease and human prion diseases. Acta. Neuropathol. 101, 417 – 423.
189 Bickel, P. E., Scherer, P. E., Schnitzer, J. E., Oh, P., Lisanti, M. P. and Lodish, H. F. (1997) Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. J. Biol. Chem. 272, 13793 – 13802.
190 Furukawa, Y., Torres, A. S. and O'Halloran, T. V. (2004) Oxygen-induced maturation of SOD1: a key role for disulfide formation by the copper chaperone CCS. EMBO J. 23, 2872 – 2881.
191 Beckman, M., Holsinger, R. M. and Small, D. H. (2006) Heparin Activates beta-Secretase (BACE1) of Alzheimer's Disease and Increases Autocatalysis of the Enzyme. Biochemistry 45, 6703 – 6714.
192 Scholefield, Z., Yates, E. A., Wayne, G., Amour, A., McDowell, W. and Turnbull, J. E. (2003) Heparan sulfate regulates amyloid precursor protein processing by BACE1, the Alzheimer's beta-secretase. J. Cell. Biol. 163, 97 – 107.
193 Ge, Y. W., Maloney, B., Sambamurti, K. and Lahiri, D. K. (2004) Functional characterization of the 5' flanking region of the BACE gene: identification of a 91 bp fragment involved in

basal level of BACE promoter expression. Faseb J. 18, 1037 – 1039.
194 Zhou, W., Qing, H., Tong, Y. and Song, W. (2004) BACE1 Gene Expression and Protein Degradation. Ann. N. Y. Acad. Sci. 1035, 49 – 67.
195 Nowak, K., Lange-Dohna, C., Zeitschel, U., Gunther, A., Luscher, B., Robitzki, A., Perez-Polo, R. and Rossner, S. (2006) The transcription factor Yin Yang 1 is an activator of BACE1 expression. J. Neurochem. 96, 1696 – 1707.
196 Sastre, M., Dewachter, I., Rossner, S., Bogdanovic, N., Rosen, E., Borghgraef, P., Evert, B. O., Dumitrescu-Ozimek, L., Thal, D. R., Landreth, G., Walter, J., Klockgether, T., van Leuven, F. and Heneka, M. T. (2006) Nonsteroidal anti-inflammatory drugs repress beta-secretase gene promoter activity by the activation of PPARgamma. Proc. Natl. Acad. Sci. U S A 103, 443 – 448.
197 Buggia-Prevot, V., Sevalle, J., Rossner, S. and Checler, F. (2008) NFkappaB-dependent control of BACE1 promoter transactivation by Abeta42. J. Biol. Chem. 283, 10037 – 10047.
198 Bourne, K. Z., Ferrari, D. C., Lange-Dohna, C., Rossner, S., Wood, T. G. and Perez-Polo, J. R. (2007) Differential regulation of BACE1 promoter activity by nuclear factor-kappaB in neurons and glia upon exposure to beta-amyloid peptides. J. Neurosci. Res. 85, 1194 – 1204.
199 Sambamurti, K., Kinsey, R., Maloney, B., Ge, Y. W. and Lahiri, D. K. (2004) Gene structure and organization of the human beta-secretase (BACE) promoter. Faseb J. 18, 1034 – 1036.
200 Wen, Y., Yu, W. H., Maloney, B., Bailey, J., Ma, J., Marie, I., Maurin, T., Wang, L., Figueroa, H., Herman, M., Krishnamurthy, P., Liu, L., Planel, E., Lau, L. F., Lahiri, D. K. and Duff, K. (2008) Transcriptional regulation of beta-secretase by p25/cdk5 leads to enhanced amyloidogenic processing. Neuron 57, 680 – 690.
201 Xue, S., Jia, L. and Jia, J. (2006) Hypoxia and reoxygenation increased BACE1 mRNA and protein levels in human neuroblastoma SH-SY5Y cells. Neurosci. Lett. 405, 231 – 235.
202 Zhang, X., Zhou, K., Wang, R., Cui, J., Lipton, S. A., Liao, F. F., Xu, H. and Zhang, Y. W. (2007) Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. J. Biol. Chem. 282, 10873 – 10880.
203 Sun, X., He, G., Qing, H., Zhou, W., Dobie, F., Cai, F., Staufenbiel, M., Huang, L.E. and Song, W. (2006) Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression. Proc. Natl. Acad. Sci. U S A 103, 18727 – 18732.
204 Cole, S.L. and Vassar, R. (2007) The Alzheimer's disease beta-secretase enzyme, BACE1. Mol. Neurodegener. 2, 22.
205 Xiong, K., Cai, H., Luo, X. G., Struble, R. G., Clough, R. W. and Yan, X. X. (2007) Mitochondrial respiratory inhibition and oxidative stress elevate beta-secretase (BACE1) proteins and activity in vivo in the rat retina. Exp. Brain. Res. 181, 435 – 446.
206 Tamagno, E., Parola, M., Bardini, P., Piccini, A., Borghi, R., Guglielmotto, M., Santoro, G., Davit, A., Danni, O., Smith, M. A., Perry, G. and Tabaton, M. (2005) Beta-site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways. J. Neurochem. 92, 628 – 636.
207 Tamagno, E., Guglielmotto, M., Aragno, M., Borghi, R., Autelli, R., Giliberto, L., Muraca, G., Danni, O., Zhu, X., Smith, M. A., Perry, G., Jo, D. G., Mattson, M. P. and Tabaton, M. (2008) Oxidative stress activates a positive feedback between the gamma- and beta-secretase cleavages of the beta-amyloid precursor protein. J. Neurochem. 104, 683 – 695.
208 Cho, H. J., Jin, S. M., Youn, H. D., Huh, K. and Mook-Jung, I. (2008) Disrupted intracellular calcium regulates BACE1 gene expression via nuclear factor of activated T cells 1 (NFAT 1) signaling. Aging Cell. 7, 137 – 147.
209 Hartlage-Rubsamen, M., Zeitschel, U., Apelt, J., Gartner, U., Franke, H., Stahl, T., Gunther, A., Schliebs, R., Penkowa, M., Bigl, V. and Rossner, S. (2003) Astrocytic expression of the Alzheimer's disease beta-secretase (BACE1) is stimulus-dependent. Glia. 41, 169 – 179.
210 Ghribi, O., Larsen, B., Schrag, M. and Herman, M. M. (2006) High cholesterol content in neurons increases BACE, beta-amyloid, and phosphorylated tau levels in rabbit hippocampus. Exp. Neurol. 200, 460 – 467.
211 Blasko, I., Beer, R., Bigl, M., Apelt, J., Franz, G., Rudzki, D., Ransmayr, G., Kampfl, A. and Schliebs, R. (2004) Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease beta-secretase (BACE-1). J. Neural. Transm. 111, 523 – 536.
212 Wang, L., Shim, H., Xie, C. and Cai, H. (2008) Activation of protein kinase C modulates BACE1-mediated beta-secretase activity. Neurobiol. Aging 29, 357 – 367.
213 Tamagno, E., Guglielmotto, M., Giliberto, L., Vitali, A., Borghi, R., Autelli, R., Danni, O. and Tabaton, M. (2008) JNK and ERK1/2 pathways have a dual opposite effect on the expression of BACE1. Neurobiol. Aging.
214 Zohar, O., Cavallaro, S., D'Agata, V. and Alkon, D. L. (2003) Quantification and distribution of beta-secretase alternative splice variants in the rat and human brain. Brain Res. Mol. Brain Res. 115, 63 – 68.
215 Ehehalt, R., Michel, B., De Pietri Tonelli, D., Zacchetti, D., Simons, K. and Keller, P. (2002) Splice variants of the beta-site APP-cleaving enzyme BACE1 in human brain and pancreas. Biochem. Biophys. Res. Commun. 293, 30 – 37.
216 Lammich, S., Schobel, S., Zimmer, A. K., Lichtenthaler, S. F. and Haass, C. (2004) Expression of the Alzheimer protease BACE1 is suppressed via its 5'-untranslated region. EMBO Rep. 5, 620 – 625.
217 Zhou, W. and Song, W. (2006) Leaky scanning and reinitiation regulate BACE1 gene expression. Mol. Cell. Biol. 26, 3353 – 3364.
218 Wang, W. X., Rajeev, B. W., Stromberg, A. J., Ren, N., Tang, G., Huang, Q., Rigoutsos, I. and Nelson, P. T. (2008) The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J. Neurosci. 28, 1213 – 1223.
219 Selkoe, D. J. (2002) Alzheimer's disease is a synaptic failure. Science 298, 789 – 791.
220 Walsh, D. M. and Selkoe, D. J. (2007) A beta oligomers - a decade of discovery. J. Neurochem. 101, 1172 – 1184.
221 Selkoe, D. J. (2008) Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav. Brain Res.
222 Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A. and Katzman, R. (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann. Neurol. 30, 572 – 580.
223 Takahashi, R. H., Milner, T. A., Li, F., Nam, E. E., Edgar, M. A., Yamaguchi, H., Beal, M.F., Xu, H., Greengard, P. and Gouras, G.K. (2002) Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am. J. Pathol. 161, 1869 – 1879.
224 Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., Sisodia, S. and Malinow, R. (2003) APP processing and synaptic function. Neuron 37, 925 – 937.
225 Cirrito, J. R., Yamada, K. A., Finn, M. B., Sloviter, R. S., Bales, K. R., May, P. C., Schoepp, D. D., Paul, S. M., Mennerick, S. and Holtzman, D. M. (2005) Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. Neuron 48, 913 – 922.
226 Cirrito, J. R., Kang, J. E., Lee, J., Stewart, F. R., Verges, D. K., Silverio, L. M., Bu, G., Mennerick, S. and Holtzman, D. M. (2008) Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. Neuron 58, 42 – 51.
227 Haass, C. and Selkoe, D. J. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat. Rev. Mol. Cell. Biol. 8, 101 – 112.

228 Lue, L. F., Kuo, Y. M., Roher, A. E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J. H., Rydel, R. E. and Rogers, J. (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. 155, 853 – 862.
229 Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S. and Malinow, R. (2006) AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron 52, 831 – 843.
230 Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., Nairn, A. C., Salter, M. W., Lombroso, P. J., Gouras, G. K. and Greengard, P. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. Nat. Neurosci. 8, 1051 – 1058.
231 Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I. and Glabe, C. G. (2005) Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. J. Biol. Chem. 280, 17294 – 17300.
232 Berman, D. E., Dall'armi, C., Voronov, S. V., McIntire, L. B., Zhang, H., Moore, A. Z., Staniszewski, A., Arancio, O., Kim, T. W. and Di Paolo, G. (2008) Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. Nat. Neurosci. 11, 547 – 554.
233 Stokin, G. B. and Goldstein, L. S. (2006) Axonal transport and Alzheimer's disease. Annu. Rev. Biochem. 75, 607 – 627.
234 Nixon, R.A. (2007) Autophagy, amyloidogenesis and Alzheimer disease. J. Cell. Sci. 120, 4081 – 4091.
235 Lazarov, O., Lee, M., Peterson, D. A. and Sisodia, S. S. (2002) Evidence that synaptically released beta-amyloid accumulates as extracellular deposits in the hippocampus of transgenic mice. J. Neurosci. 22, 9785 – 9793.
236 Sheng, J. G., Price, D. L. and Koliatsos, V. E. (2003) The beta-amyloid-related proteins presenilin 1 and BACE1 are axonally transported to nerve terminals in the brain. Exp. Neurol. 184, 1053 – 1057.
237 Yan, X. X., Xiong, K., Luo, X. G., Struble, R. G. and Clough, R. W. (2007) beta-Secretase expression in normal and functionally deprived rat olfactory bulbs: inverse correlation with oxidative metabolic activity. J. Comp. Neurol. 501, 52 – 69.
238 Ghosh, A. K., Bilcer, G., Hong, L., Koelsch, G. and Tang, J. (2007) Memapsin 2 (beta-secretase) inhibitor drug, between fantasy and reality. Curr. Alzheimer Res. 4, 418 – 422.
239 John, V. (2006) Human beta-secretase (BACE) and BACE inhibitors: progress report. Curr. Top Med. Chem. 6, 569 – 578.
240 Hom, R. K., Fang, L. Y., Mamo, S., Tung, J. S., Guinn, A. C., Walker, D. E., Davis, D. L., Gailunas, A. F., Thorsett, E. D., Sinha, S., Knops, J. E., Jewett, N. E., Anderson, J. P. and John, V. (2003) Design and synthesis of statine-based cell-permeable peptidomimetic inhibitors of human beta-secretase. J. Med. Chem. 46, 1799 – 1802.
241 Huang, D., Luthi, U., Kolb, P., Edler, K., Cecchini, M., Audetat, S., Barberis, A. and Caflisch, A. (2005) Discovery of cell-permeable non-peptide inhibitors of beta-secretase by high-throughput docking and continuum electrostatics calculations. J. Med. Chem. 48, 5108 – 5111.
242 Stachel, S. J., Coburn, C. A., Steele, T. G., Jones, K. G., Loutzenhiser, E. F., Gregro, A. R., Rajapakse, H. A., Lai, M. T., Crouthamel, M. C., Xu, M., Tugusheva, K., Lineberger, J. E., Pietrak, B. L., Espeseth, A. S., Shi, X. P., Chen-Dodson, E., Holloway, M.K., Munshi, S., Simon, A. J., Kuo, L. and Vacca, J. P. (2004) Structure-based design of potent and selective cell-permeable inhibitors of human beta-secretase (BACE-1). J. Med. Chem. 47, 6447 – 6450.
243 Lefranc-Jullien, S., Lisowski, V., Hernandez, J. F., Martinez, J. and Checler, F. (2005) Design and characterization of a new cell-permeant inhibitor of the beta-secretase BACE1. Br. J. Pharmacol. 145, 228 – 235.
244 Chang, W. P., Koelsch, G., Wong, S., Downs, D., Da, H., Weerasena, V., Gordon, B., Devasamudram, T., Bilcer, G., Ghosh, A. K. and Tang, J. (2004) In vivo inhibition of Abeta production by memapsin 2 (beta-secretase) inhibitors. J. Neurochem. 89, 1409 – 1416.
245 Rajendran, L., Schneider, A., Schlechtingen, G., Weidlich, S., Ries, J., Braxmeier, T., Schwille, P., Schulz, J. B., Schroeder, C., Simons, M., Jennings, G., Knolker, H. J. and Simons, K. (2008) Efficient inhibition of the Alzheimer's disease beta-secretase by membrane targeting. Science 320, 520 – 523.
246 Hussain, I., Hawkins, J., Harrison, D., Hille, C., Wayne, G., Cutler, L., Buck, T., Walter, D., Demont, E., Howes, C., Naylor, A., Jeffrey, P., Gonzalez, M. I., Dingwall, C., Michel, A., Redshaw, S. and Davis, J.B. (2007) Oral administration of a potent and selective non-peptidic BACE-1 inhibitor decreases beta-cleavage of amyloid precursor protein and amyloid-beta production in vivo. J. Neurochem. 100, 802 – 809.
247 Nishitomi, K., Sakaguchi, G., Horikoshi, Y., Gray, A. J., Maeda, M., Hirata-Fukae, C., Becker, A. G., Hosono, M., Sakaguchi, I., Minami, S. S., Nakajima, Y., Li, H.F., Takeyama, C., Kihara, T., Ota, A., Wong, P. C., Aisen, P. S., Kato, A., Kinoshita, N. and Matsuoka, Y. (2006) BACE1 inhibition reduces endogenous Abeta and alters APP processing in wild-type mice. J. Neurochem. 99, 1555 – 1563.
248 Sankaranarayanan, S., Price, E. A., Wu, G., Crouthamel, M. C., Shi, X.P., Tugusheva, K., Tyler, K. X., Kahana, J., Ellis, J., Jin, L., Steele, T., Stachel, S., Coburn, C. and Simon, A. J. (2008) In vivo beta-secretase 1 inhibition leads to brain Abeta lowering and increased alpha-secretase processing of amyloid precursor protein without effect on neuregulin-1. J. Pharmacol. Exp. Ther. 324, 957 – 969.