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Alpha, beta-and gamma-secretases in alzheimer's disease

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1. ABSTRACT

Generation of Amyloid peptide (Abeta) is at the beginning of a cascade that leads to Alzheimer's disease. Currenty, the mechanisms of Abeta generation and Abeta prevention are subject of intensive research. Amyloid precursor protein (APP), as well as beta- and gamma-secretases are the principal players involved in Abeta production, while alpha-secretase cleavage on APP prevents Abeta deposition. Inhibitors or modulators that target beta- and gamma-secretases as well as alpha-secretase activators are promising candidates for treatment of Alzheimer's disease. A deep knowledge of the secretases is required to develop disease modifying drugs that target them. The most challenging quest is to translate the growing knowledge about the cell biology of secretases and their mechanisms of action into effective therapeutics. Here, we review the main features of the secretases.

2. INTRODUCTION

Although the etiology of Alzheimer's disease (AD) is not completely understood, the study of disease genes that cause AD has revealed important clues about the pathogenesis of this disorder. Familial AD (FAD) cases are caused by autosomal dominant mutations in the genes for APP and the presenilins (PS1 and PS2) (1). These mutations increase production of the 42-aminoacids (aa)-long, fibrillogenic form of Abeta, relative to the 40-aa-long Abeta. Amyloid plaques, also called senile plaques, one of the neuropathological feature of AD, result from the deposition of aggregated Abeta peptides (2). In addition, patients with APP gene duplications or individuals with Down's syndrome (trisomy 21), who have increased dosage of the APP gene (located on chromosome 21), develop early-onset AD and overproduce $Abeta_{42}$ (3). These findings, along with a large body of evidence from other

sources (4), strongly suggest that Abeta plays a central, early role in AD pathogenesis.

Where does Abeta come from? APP, beta- and gamma-secretases are the three principal players involved in Abeta production. Abeta derives from amyloidogenic metabolism of APP and is released in a stepwise fashion.

Amyloidogenic processing of APP is initiated by beta-secretase cleavage, which results in the release of the large soluble ectodomain, sAPPbeta, and in a membrane-tethered C-terminal fragment, beta-CTF. The second cleavage is mediated by gamma-secretase, which cuts beta-CTF within the transmembrane domain to release Abeta into the extracellular milieu and APP intracellular domain (AICD) into the cytoplasm. gamma-secretase cleaves at multiple sites within the transmembrane segment of APP due to its heterogeneous site preference, thus generating variable length (39-42 aa long) Abeta peptides. As stated before, the longest forms of Abeta are prone to rapid aggregation, oligomerization and fibril formation, events that are considered critical for the development of AD pathology.

Abeta production turned out to be one paradigmatic example of a more general biological process called regulated intramembrane proteolysis: membrane proteins, as APP or Notch, firstly undergo a shedding process leading to the release of ectodomains in the extracellular fluids and secondly the membrane-retained stubs can be cleaved within their transmembrane domains giving rise to small hydrophobic peptides released into extracellular fluids as well as to intracellular domains into the cytoplasm. These small intracytoplasmic peptides may possess different functions including activation of nuclear signalling (5).

On the other hand, the non-amyloidogenic processing of APP is initiated by alpha-secretase, which cuts APP at the lumenal domain 16 aa downstream of beta-secretase cleavage site, also releasing a soluble ectodomain of APP, sAPPalpha, and generating a truncated CTF (alpha-CTF) that is then cleaved by gamma-secretase. Because alpha-secretase cleavage truncates the N-terminus of Abeta, non-amyloidogenic processing results in the generation of N-terminally truncated Abeta peptides, referred to as p3 peptides.

Although initial investigation defined alpha-secretase as integral to the physiological pathway, while Abeta production was considered pathological, subsequent studies discovered that $Abeta_{42}$ is found normally in biological fluids (approximately 5-10%).

Proteolytic processing of APP *per se* is a highly regulated event and several regulatory components of secretases have been identified. Commitment of APP into amyloidogenic and non-amyloidogenic processing depends on the cellular levels of alpha- and beta-secretases. Therefore, regulators of intracellular trafficking and subcellular localization of APP and of the secretases have been extensively examined. Interestingly, the two processes mediated by alpha and beta-secretase are differentially segregated within the cells, being alpha-secretase activity localized in the Trans-Golgi Network (TGN) or at the plasma membrane (6). This gives new importance to the studies addressing mechanisms of trafficking of alpha-secretase to the membrane (7). On the other hand, beta-secretase activity is mainly confined to the endoplasmic reticulum and the endosomal/lysosomal system (8). Moreover, while alpha-secretase processing is predominant in non-neuronal cells, APP is mainly channeled into the amyloidogenic pathway in neurons as a consequence of high abundance of beta-secretase in neuronal cells (9). Given that alpha- and beta-secretases are supposed to compete with each other for APP processing and have opposite effects on Abeta generation, deciphering the signaling pathways and molecular events involved in the commitment of APP to these pathways has high therapeutic potential.

3. BACE1 (BETA-SITE APP CLEAVING ENZYME)

The identity of beta-secretase had long been sought. In 1999, five groups reported the molecular cloning of beta-secretase, variously naming the enzyme BACE1 (10), beta-secretase (11), Asp2 (12, 13) or memapsin 2 (14) (here, beta-secretase will be referred to primarily as BACE1). The groups used very different isolation methods (i.e., expression cloning, protein purification, genomics), y*et al* identified the same enzyme and concurred that it possessed all the known characteristics of beta-secretase (15). For example, BACE1 cleaves APP with the Swedish familiar AD-causing mutation (APPswe;) 10- to 100-fold more efficiently than wild-type APP, as expected for beta-secretase. In addition, the specific activity of recombinant BACE1 on wild-type and mutant APP substrates is consistent with beta-secretase. Importantly, Abeta generation, amyloid pathology, electrophysiological dysfunction and cognitive deficits are abrogated when $BACE1^{-/-}$ mice are bred to APP transgenic mice (16-18). $BACE1^{-/-}$ mice are devoid of cerebral Abeta production, demonstrating that BACE1 is the major -if not unique- beta-secretase enzyme in the brain. This notion is further supported by reports of lentiviral delivery of BACE1 RNA interference (RNAi) that can attenuate amyloidosis and cognitive deficits in APP transgenic mice (12, 18, 19). The rescue of memory deficits in $BACE1^{-/-}$; *APP* bigenic mice suggests that therapeutic BACE1 inhibition should improve Abeta-dependent cognitive impairment even in humans with AD. Interestingly, several studies have determined that levels of BACE1 protein and activity are approximately twofold elevated in AD brain (20), suggesting the possibility that BACE1 increase might initiate or accelerate AD pathogenesis

Soon after BACE1 was discovered, a homologue was identified, BACE2. BACE1 and BACE2 may have arisen by ancestral gene duplication and share 64% aa sequence similarity, which raised the possibility that BACE2 was also a beta-secretase. Both BACE1 and BACE2 can process APP at the betasite, but BACE2 has a preference to cleave between amino acids 19 and 20 of Abeta sequence, thus precluding its formation. Although

BACE-2 is interesting in comparative terms and its precise physiological roles are unclear, it is expressed at low levels in neurons and there is no compelling evidence that it plays a direct role in the beta-secretase processing of APP. Moreover, BACE1 characterization and validation studies have unequivocally demonstrated that BACE1 is the authentic beta-secretase in the brain. Therefore in the following, we will focus on BACE1.

3.1. BACE1: molecular cell biology

Since its discovery, 12 years ago, much has been learnt about BACE1. BACE1 gene is on chromosome-11 at 11q23.2-q23.3 and contains nine exons. A total of 4 splice variants can be synthesized from BACE1 gene, encoding proteins of 501, 476, 457 and 432 aa in size respectively (21). The most common normally spliced variant is the largest 501 aa form (I-501), coding for a type 1 transmembrane protein with a 21-residue cleavable signal sequence, a large ectodomain of 434 aa, a single transmembrane domain of 22 aa and a short cytoplasmic tail of 24 residues.

The highest expression levels of BACE are found in neurons, as expected for beta-secretase. BACE1 is also expressed in pancreas but its activity here is low due to the generation of alternatively spliced transcripts, which produce BACE1 variants with reduced proteolytic activity (22). In brain tissue of mice and humans BACE1 forms homodimers and interestingly, such homodimers exert a higher enzymatic activity than the monomeric enzyme (23).

BACE1 structure is related to the pepsin and retroviral aspartic proteases. The key feature of aspartic proteases is their bilobar globular structure, with an essential catalytic aspartic dyad located at the interface of the homologous N- and C-terminal lobes. These aspartic residues activate water molecules to mediate nucleophilic attack on the substrate's peptide bond and mutation of the catalytic active site aspartic residues abolishes enzyme activity. Although sharing similarities with the pepsin and retroviral aspartic protease families, BACE proteins represent a novel subgroup, being the first reported aspartic proteases to contain a transmembrane domain and carboxyl terminal extension (24) and also possessing unique disulphide bridge distribution (25). The unique transmembrane regions of BACE1 and BACE2 confer an evolutionary specialization, allowing their sequestration to membranes of specific organelles and the plasma membrane. This exposes their catalytic lobes to the luminal 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 modifications and protein-protein interactions.

Purified BACE1 has an optimal enzymatic activity at an acidic pH of approximately 4.5, which reflects its primary site of action inside the cell (26).

BACE1, like all the other aspartic proteases, is initially synthesized as a zymogen (containing a short prodomain) in the endoplasmic reticulum (ER). ProBACE1 does not spontaneously convert to mature BACE1 like some aspartic protease zymogens, but requires other proteases to eliminate the pro-peptide. The efficient exit of the enzyme from the ER is determined by the prodomain (27), which is subsequently removed by proprotein convertase, by furin or by a furin-like protease (27, 28). This process is not required for its activation as pro-BACE can still cleave APP (29). However, removal of its prodomain increases BACE1 activity by approximately twofold (30). Molecular dynamics simulation studies have suggested that the partial catalytic activity of the zymogen could be explained by the high mobility of the prosegment in comparison with that of other zymogens, resulting in the occasional exposure of the catalytic site for access of its substrate, APP (31).

During its transport through the cell, BACE1 undergoes a number of post-translational modifications. Within the lumen of the ER, BACE1 is subject to simple glycosylation on four asparagine residues (32) and transient acetylation on seven arginine residues (33). While deletion of the prodomain does not alter the enzymatic activity of BACE1 towards its substrate APP to a great extent (34), removal of the glycosylation sites has a significant impact on BACE1 activity, as site-directed mutagenesis of these asparagine residues significantly reduces the proteolytic activity.

The simple carbohydrates added in the ER produce an immature BACE1 protein of approximately 65kDa. These sugars are then processed by an endoglycosidase H-resistant complex form producing the mature 75kDa species. Further addition of complex carbohydrates and removal of BACE1 prodomain occur in the Golgi compartment (35, 36). BACE1 also contains three disulphide bonds in the catalytic domain between cysteines 216–420, 278–443 and 330–380 (32), which are important for the correct folding, and hence proteolytic activity, of the enzyme.

After its maturation in the ER and the Golgi apparatus, BACE1 is transported to the plasma membrane. Similar to APP, BACE1 is internalized from the plasma membrane to early endosomes, the main compartment of Abeta generation (37). The internalization is primarily driven by a conserved di-leucine sorting signal within the C-terminus (38, 39). Mutation of the di-leucine motif (40) resulted in increased levels of BACE1 at the cell surface, consistent with decreased internalization to endosomes.

BACE1 cycles between cell surface and endosomes and at steady-state, the majority of BACE1 is found in the late Golgi–TGN compartments and in endosomes (41).

Also phosphorylation influences BACE1. Serine 498 is phosphorylated by casein kinase1 (42): while both the wild-type, phosphorylated BACE1 and a non-phosphorylable mutant localize to early endosomes, only the phosphorylated form is recycled back to the membrane (42).

The cytoplasmic domain contains several cysteine residues which are subject to palmitoylation. This modification may function as an anchor for the protein to the membrane, as mutations of these cysteine residues increase the release of BACE1 ectodomain into the medium (43).

BACE1 stability and turnover is regulated also by reversible acetylation of seven lysine residues in its lumenal (N-terminal) domain, this event occurring in the ER and serving as a 'quality control' step in protein maturation (33). Acetylated BACE1 traffics to the Golgi, where deacetylation of the mature protein can occur. Non-acetylated, immature BACE1 is degraded in a non-proteasomal, post-ER compartment. BACE1 can undergo proteolytic degradation by the proteasome although the physiological relevance of BACE1 processing by the proteasome is unclear.

3.2. BACE1: different levels of regulation

Apart from post-translational modifications, BACE1 is tightly regulated inside the cells at several levels. From intracellular localization to translation, from synaptic activity to microglial activation, all these mechanisms contribute to control enzyme's activity.

We will now go through the most relevant among these regulatory factors. It is well known that binding of BACE1 to other partner proteins modulates its localization and activity. Intracellular localization of BACE1 can be altered by increased cellular expression of reticulon/Nogo proteins, identified as BACE1 interacting proteins (44, 45), which significantly increase retention of BACE1 within the ER (46). While intracellular trafficking of BACE1 to the more acidic endosomes clearly enhances Abeta production, increased localization of BACE1 within the ER reduces Abeta generation.

On the other hand, BACE1 recycling step depends on Golgi-localized gamma-ear containing ADP (GGA) ribosylation factor binding proteins (47, 48). GGA1 mediates rerouting from endosomes to the TGN and recycling to the plasma membrane leads to a longer half-life of BACE1. In addition, it was reported that phosphorylated BACE1 could be transported in a GGA3 dependent manner from endosomes to lysosomes where BACE1 can be degraded (47). Depletion of GGA proteins by RNAi or disruption of phosphorylation of BACE1 on serine 498 increase accumulation of BACE1 in early endosomes (39, 47).

Also SORL1, a member of the vacuolar protein sorting 10 receptors, interacts with BACE1 in the Golgi and the interaction directly inhibits the formation of BACE1–APP complex (49). Mice lacking SORL1 show accelerated amyloidosis highlighting its importance in the disease. Polymorphisms in the gene are linked to AD and SORL1 levels are reduced in AD brains, facilitating amyloid deposition (50).

Since BACE1 is active in neurons, an influence of neuronal activity on BACE1 was postulated. Indeed, recently, Kamenetz and coworkers described an upregulation of BACE1 by synaptic activity and they put forward a role for Abeta, which, in a feedback loop, might regulate BACE1 levels via modulation of synaptic activity. Moreover, *in vivo* work indicated that BACE1-mediated cleavage of APP can facilitate learning, memory, and synaptic plasticity (52).

Furthermore, activated microglial cells producing TNFalpha and INFgamma could mediate BACE1 upregulation Other results suggest that BACE1 is a stress-response protein: levels of the enzyme are increased by oxidative stress (54), hypoxia (55), ischemia (56), apoptosis (47) and traumatic brain injury (57).

Considering BACE1 gene regulation, several researchers independently demonstrated that BACE1 expression is restricted via its 5′UTR (24). In general, complex secondary structures, presence of upstream open reading frames and the binding of transactivating factors to the 5′UTR and/or the 3′UTR are shown to influence translational efficiency. So far regulation of BACE1 at transcriptional level was mainly studied *in vitro* (53, 58). Several promoter elements important for BACE1 transcriptional regulation: transcription factor binding sites for signal transducer and activator of transcription (STAT1/3 and STAT6) have been identified in BACE1 promoter (58).

Following the publication of epidemiological studies that correlated cellular cholesterol and AD pathogenesis, the role of cholesterol in amyloidogenic processing of APP came under close scrutiny. Given that cholesterol is one of the major constituent of lipid rafts, the involvement of raft membrane microdomains in APP processing was investigated. Indeed, multiple lines of evidence suggest that amyloidogenic processing of APP is associated with membrane raft microdomains. A subset of BACE1 and full length APP associates with lipid raft domains. Targeting BACE1 lumenal domain to lipid rafts by addition of a glycophosphatidylinositol anchor increases APP processing at beta-cleavage site (59).

Post-translational acyl modifications of proteins, such as *S*-palmitoylation and *N*-myristoylation, target variety of cytosolic and transmembrane proteins to lipid raft microdomains due to the high affinity of acyl chains for the ordered lipid environment within raft domains. BACE1 undergoes *S*-palmitoylation at four cysteine residues near the transmembrane and cytoplasmic boundary. Experimental mutation of these residues completely abolishes palmitoylation of BACE1 and prevents raft association of BACE1. Importantly, unlike the case in many other proteins, the lack of palmitoylation does not affect protein stability. Surprisingly however, displacement of BACE1 by abolishing *S*-palmitoylation neither affected BACE1 processing of APP nor the secretion of Abeta in cultured cell lines. These results indicate that *S*-palmitoylation-dependent raft targeting of BACE1 is dispensable for APP processing and is confirmed by palmitoylation-deficient mutant of BACE1, able of processing APP as efficiently as wild type BACE1 (60).

Regulation of BACE1 can occur also by shedding. As stated before, one of the unique structural features of BACE1 among aspartic proteases is the presence of a type I transmembrane domain. This membrane domain contributes to the retention of BACE1 within the late Golgi and TGN compartments. Removal of this transmembrane domain creates a soluble form of BACE1 that is rapidly secreted into the extracellular medium (61). Hence, increased shedding of BACE1 likely represents an alternative strategy to reduce BACE1 processing of APP, because soluble BACE1 fails to form an optimal spatial interaction with APP in cells (61). BACE1 is shed from cells through cleavage at its membrane anchor between alanine 429 and valine 430 (62) by an as yet unidentified proteinase activity. Metalloproteinase inhibitors block BACE1 shedding from cells overexpressing BACE1, from which it was concluded that the BACE1 'sheddase' is likely to be a member of the 'a disintegrin and metalloprotease' (ADAM) family of proteins. Activation of protein kinase C, which is known to upregulate the shedding of BACE1, has been shown by a number of groups to decrease Abeta production in cell lines, primary cells and mouse brain (41). In contrast, Hussain and coworkers showed that inhibition of BACE1 shedding using metalloprotease inhibitors had no effect on the beta-cleavage of APP (63). The physiological role of BACE1 shedding, of the soluble enzyme and of its potential to modulate the amyloidogenic processing of APP still remain contentious. In this frame, Benjannet (43) showed that the overexpression of soluble BACE1 resulted in a dramatic increase in the production of Abeta and that membrane anchorage in the vicinity of its substrate is not essential.

Further studies are necessary not only to identify all regulatory mechanisms and partners controlling BACE1 activity but also to clarify the role of soluble BACE1 in APP processing.

3.3. BACE1 substrates

The most known substrate of BACE1, for which it was identified in 1999, is APP. BACE1 acts precisely at the +1 site of Abeta to release a CTF99 and a soluble residual fragment, sAPPbeta, or secondarily at tyrosine 10–glutamate 11 (beta1 site) to yield an N-truncated CTF, suggesting that beta-secretase is a site-specific protease. BACE1 cleaves wild-type APP during transit in the endocytic pathway. Interestingly, APPswe; is more readily cleaved by BACE1 in the secretory pathway, as early as during transit of nascent APP though the Golgi apparatus (64). Shortening or twisting APP in the region between the membrane and BACE1 cleavage site disrupts BACE1 processing (65), suggesting that the secondary structure of BACE1 substrates affects enzyme-substrate interaction. Moreover, when cells were transfected with APP lacking the transmembrane domain, it was not cleaved by BACE1, suggesting that the enzyme is a membrane bound protease which only cleaves APP when membrane bound.

It is also important to note that the enzyme can cleave soluble substrates *in vitro* in a sequence specific manner. The failure to observe *in vivo* cleavage is likely related to the relatively high Km of the enzyme, which may be compensated by the high local concentration of enzyme and substrate in a single molecular layer in cellular membranes. However, weak affinity for wild-type APP and low yield of BACE1 cleavage products suggest that APP is not the primary endogenous substrate for BACE1.

In addition to APP, BACE1 has a variety of other substrates, preferring acidic or polar residues in contrast to other known aspartyl proteases. All reported BACE1 substrates are type 1 transmembrane proteins and, interestingly, most of these substrates are involved in contact-dependent intercellular communications, such as Golgi-localized membrane-bound alpha 2,6-sialyltransferase (9), P-selectin glycoprotein ligand-1 (66), the APP homolog proteins APLP1 and APLP2 (67, 68), low-density lipoprotein receptor-related protein (LRP), the voltage-gated sodium channel (Na_v1) beta2 subunit ($Na_v beta_2$) (69), neuregulin-1 (70, 71), and neuregulin-3. Of the known substrates of BACE1 in addition to APP, $Na_v beta_2$ appears to be the major BACE1 substrate in the CNS.

The existence of multiple BACE1 substrates suggests a variety of BACE1 physiological functions.

3.4. BACE1 functions

Apart from its involvement in APP processing as beta-secretase, BACE1 has a number of other functions. Since BACE1 is enriched in neurons and transported to axons, its potential role in axonal growth and brain development was investigated by examining BACE1-null mice. Significant reduction in myelin sheath thickness of both central and peripheral nerves was found and was correlated with a reduction in myelin proteins (70). Further, mice deficient in BACE1 have altered hippocampal synaptic plasticity, decreased cognitive performance and reduced lifespan. It has been suggested that high expression of BACE1 in neurons at birth could be linked to the onset of myelination by Schwann cells and depends on signaling from the accompanying axons. Interestingly, this function must be tightly regulated and independent of Abeta as overexpression of BACE1 also causes neurodegeneration despite reducing levels of Abeta (72).

In addition to the role of BACE1 in myelination, there is also evidence regarding its expression, along with APP, in platelets, where it might play a role in inflammation (73). Of course, all these different functions have to be reminded while trying to inhibit BACE1 for therapeutic purposes.

4. GAMMA-SECRETASE

The idea that a hydrolytic reaction could cleave transmembrane domains of proteins in the cell membrane was long considered heretical. Now, intramembrane proteolysis is considered a pivotal biochemical process in many physiological reactions in all living species. The basic and recurrent theme is simple: proteolysis of an integral membrane protein separates its extracellular from its intracellular domain, both of which can exert novel functions, e.g., as a ligand, transmitting signals to other

cells or as a transcriptional regulator, modulating gene expression in a cell-autonomous way. Intramembrane proteolysis provides direction to the physiological processes that are under its control because the signaling is irreversible. Thousands of candidates have been identified in the genomes of various species, illustrating the fundamental and evolutionarily conserved nature of this process (74). Abeta generation was shown to be a normal physiological process occurring continuously in various cell types and tissues. This implied the existence of a physiological proteolytic activity able to cleave the C terminus of Abeta in the membrane. This unknown protease was termed gamma-secretase (5).

Despite this, several years passed before the field realized that gamma-secretase was a complex made of several proteins (75). The puzzle of the protein composition of gamma-secretase complex is now largely resolved (76). gamma-secretase needs four proteins to become operational and functional: presenilins (PS), presenilin enhancer-2 (PEN-2), anterior pharynx defective 1 (APH1) and Nicastrin (NCT). Extensive gain- and loss-of-function genetic experiments (77) and reconstitution of gamma-secretase complexes in mammalian (78) and yeast cells (79) have convincingly demonstrated that the four proteins are necessary and sufficient to generate active gamma-secretase.

4.1. The gamma-secretase complex

With two PS and two APH1 genes and assuming a 1:1:1:1 stoichiometry of the individual components (80, 81), at least four different gamma-secretase complexes can theoretically be built. Alternative splicing of PS1 and APH1 further adds to the complexity (76). The different complexes have been identified using specific antibodies against the individual components and they appear to coexist in the same cell lines (82, 83). Different gamma-secretase complexes containing different Presenilin or APH1 protein subunits are present in various tissues and have heterogeneous biochemical and physiological properties. Targeting specifically the APH1B containing complexes results, for example, in significant improvements of multiple severe AD-related phenotypes in a mouse model (84). The exploration of this diversity has only started.

Establishing the precise role of each subunit of the complex has not been possible in the absence of an *in vitro* system that allowed reconstitution of gamma-secretase activity from purified components. Technically, *in vitro* reconstitution of gamma-secretase activity is a challenge not only because of the topology of PS, with eight to nine transmembrane domains (TMD) (an issue that remains somewhat controversial), but also because the other three components have to be taken into account. If PS holoprotein alone possessed the enzymatic activity, this objective would perhaps not be as difficult. Recently, Ahn and coworkers took a creative route by using a highly purified bacterially expressed recombinant PS1, harboring the naturally occurring FAD mutation lacking exon 9 (DeltaE9) and incorporating the recombinant protein into artificial liposomes (85). The selection of this particular PS1 variant is one key to their success, because PS1DeltaE9 lacking endoproteolytic cleavage site remains constitutively active. The purified proteoliposomes were then tested with an artificial truncated APP substrate for *in vitro* gamma-secretase assay after detergent solubilization. Validity of the reconstituted system was confirmed by a number of control experiments and the results are strikingly clear: PS1DeltaE9 incorporated into liposomes together with substrate produced bona fide intrinsic gamma-secretase activity without the other three components of the gamma-secretase complex. Without mutated proteins, genetic ablation of only one component results in mislocalization, incomplete maturation and destabilisation of the remaining components, clearly indicating that inter- and intramolecular interactions are crucial in the course of assembly and activation of the gamma-secretase complex. Assembly is most likely to be initiated through the formation of an NCT-APH1 subcomplex to which the other components, either single or in subcomplexes, are added. Note also that interfering with these interactions often affects localization of other components, which suggests that complex assembly and activation is intrinsically coupled to transport. It also follows that the component that exhibits the lowest abundance in a cell acts as the limiting factor for gamma-secretase assembly and activity.

4.1.1. Presenilins

Presenilin1 (PS1), the dominant presenilin in brain, and presenilin2 (PS2) have been identified using classical genetic linkage studies in families with rare forms of FAD. More than 170 different mutations in the PS1 gene and additional 13 in PS2 have been found, establishing mutations in these two genes as the major causes of FAD. The first evidence that presenilins are critically linked to gamma-secretase activity was the finding that deficiency in PS1 resulted in a marked reduction of Abeta production (75). Subsequently, it was shown that two aspartate residues within two predicted transmembrane regions are critical for activity, suggesting that PS was a novel aspartyl protease and may be the catalytic unit of gamma-secretase (86). Finally, in 1999, a series of papers applied different reasoning to explain that PS provided the catalytic core of the protease (86-88). This was ultimately proven by the finding that photoactivable transition-state analog gamma-secretase inhibitors targeted PS.

The full-length PS proteins are similar in aa composition (<65%), contain nine TMD and are synthesized as 50-kDa proteins in the ER. They undergo a final proteolytic "maturation" step when incorporated into the gamma-secretase complex and are cleaved in the cytoplasmic loop between the 6th and 7th transmembrane region to generate an amino-terminal fragment (NTF) and a CTF. Both fragments are stable, they stay in close association as a heterodimer in the membrane and represent the active form of PS, whereas full-length PS is rapidly degraded. It was recognized that endoproteolytic processing of PS is required for activity (64). Intriguingly, the resulting NTF and CTF contain one of the two catalytic aspartyl residues (86). The PS are, therefore, aspartyl proteases, and apart from the two aspartyl residues embedded in the TMD, they share a few additional

conserved signatures with the signal peptide peptidases, the second group in the aspartyl-intramembrane protease family (89).

These two highly conserved aspartate residues within TMD6 and TMD7 constitute the core of the catalytic site. Mutation of either abolishes gamma-secretase activity (86). Together with surrounding residues, they mark a highly conserved YD/GxGD consensus motif (90). Residues at the luminal portion of TMD6 are predicted to form a subsite for substrate or inhibitor binding on the alpha-helix facing a hydrophilic milieu, whereas those around the GxGD catalytic motif within TMD7 are highly water accessible, suggesting formation of a hydrophilic structure within the pore (91). Tyrosine 389 may also contribute to the catalytic site (92). The conformation of the active site depends also on more remote sequences within PS1, such as the C-terminal PAL motif and cysteine residues in TMD1, whch dynamically moves during catalytic process (93); TMD8; TMD7 and TMD9 (94). It still remain unknown whether the residues other than catalytic aspartic and PAL motif are involved in the catalytic activity. These interactions between remote parts of the molecule fit with the idea that PS1 adopts a ring-like topology (95). On the whole, it is still unclear whether residues other than PAL and aspartates are involved in the catalytic site.

Many publications have claimed functions for PS outside the gamma-secretase complex, for instance, in vesicular trafficking, Ca^{2+} homeostasis, beta-catenin stabilization and cell adhesion. These claims of a nonproteolytic function for PS are intriguing and have gained considerable support from experiments in *Physcomitrella patens*. In this moss plant, the deletion of the *PS* homolog results in growth alterations that are caused by deficiencies in cytoskeleton function. Because *P. patens* does not express orthologs of APP and Notch and, more importantly, because the defect could be rescued by a mutated, proteolytic inactive form of human PS1, this clearly suggests evolutionarily conserved additional functions for PS orthologs apart from their well-understood role in proteolysis (96).

4.1.2. Nicastrin

NCT, in *Caenorhabditis elegans* also designated as anterior pharynx defective 2 (APH2), is a 130-kDa type I integral membrane glycoprotein (97, 98). NCT itself is not catalytically active but instead promotes the maturation and proper trafficking of the other proteins in the complex. In particular, the assembly and activity of gamma-secretase complex requires the integrity of the complete NCT ectodomain (98, 99). NCT also binds carboxy-terminal derivatives of APP and modulates the production of Abeta from these derivatives. Missense mutations in a conserved hydrophilic domain of NCT increase $Abeta_{42}$ and $Abeta_{40}$ peptide secretion. Deletions in this domain inhibit Abeta production (98). The DAP domain of NCT must play a crucial role, because deletions (98) and single point mutations (83) significantly reduce or even abolish the interaction with PS or PEN2, without influencing binding of NCT to APH1.

4.1.3. APH1

APH1 exists in two isoforms, APH1a and b, encoded by two genes in humans; mice have a third isoform, APH1c. APH1 is a 25-kDa, seven-TMD protein (100). Interactions involving APH1 are not well characterised. For instance, the APH1 TMD interacting with NCT has not been determined but is supposed to reside in the second half of APH1.

4.1.4. PEN-2

PEN-2 is a small, hairpin-like, integral membrane protein with a molecular weight of 12 kDa. It has a topology such that both the N-terminus and the C-terminus face first the lumen of the ER and later the extracellular environment. Biochemical studies have shown that a conserved sequence motif DYLSF at the C-terminus, as well as the overall length of the C-terminal tail, are required for the formation of an active gamma-secretase complex (101).

4.2. Building gamma-secretase

The four proteins responsible for gamma-secretase activity stabilize one another upon assembly and allow for the correct trafficking of the mature complex to the cell surface and to endocytic compartments where the complex exerts its activity.

The complex is thought to assemble and mature via proteolysis in the early ER under tight control of ER-Golgi recycling regulators, which allow defined quantities of the complex to reach post-Golgi compartments.

All studies agree on the initial formation of an NCT-APH1 subcomplex as the first step. This subcomplex is stable even in the absence of PS and PEN2 (83) and is more resistant to detergent dissociation compared with other subcomplexes (102).

The following steps in gamma-secretase assembly are less clear. There are basically two hypotheses. Full-length PS has been shown to migrate in a high-molecular-weight complex with both NCT and APH1, which suggests that PEN2 joins a pre-existing trimeric intermediate and subsequently causes PS endoproteolysis (103). The alternative hypothesis is based on identification of two additional intermediate complexes, NCT-APH1-PS1 CTF and PEN2-PS1 NTF. The fact that PEN2 can bind full-length PS independently of NCT and APH1 supports this view (102). The PEN2-PS1 intermediate should be formed before or during the endoproteolysis of PS.

From the moment the individual components are co-translationally inserted into the ER until they function in an active complex in distal, post-Golgi compartments, gamma-secretase-complex assembly encounters many hurdles. Since only a relatively small pool of endogenous PS1 is associated with active complexes, mechanisms controlling cellular levels of gamma-secretase activity must exist. The mutual dependency of the stability, maturation and transport of the components pins gamma-secretase assembly down to early biosynthetic compartments. Full assembly might even occur in the ER (104). Although we cannot exclude this, several findings indicate that ER-Golgi

recycling mechanisms play an active role in the stepwise assembly of gamma-secretase. The coupling of assembly to transport is reminiscent of secondary ER quality control in the assembly of several heteromultimeric membrane proteins. There is a model, called percolation-model that integrates gamma-secretase assembly into ER-Golgi transport regulation. Individual and newly synthesized complex components are co-translationally inserted in the ER. Assembly masks retention/retrieval signals, allowing only correctly formed complexes to pass the Golgi (105). Because no typical retention/retrieval motifs are present in individual gamma-secretase components, besides an ER retention motif in the PS1 CTF, recycling may be controlled by additional proteins bearing such motifs. At least one of such proteins was identified: Rer1p (106). In *S. cerevisiae*, Rer1p retrieves escaped ER proteins. Although two different gamma-secretase components – NCT and PEN2 – were shown to interact with Rer1p, functional implications for gamma-secretase assembly and activity were only demonstrated in the case of the Rer1p-NCT interaction (107). Moreover, Rer1p recognizes the same polar residues within the NCT TMD that are important for the interaction between NCT and APH1. Rer1p and APH1 thus compete for binding to NCT. Therefore, binding to Rer1p could selectively retrieve immature NCT from the intracellular compartment or cis-Golgi back to the ER, preventing it from premature escape and enhancing the probability of encountering APH1. Binding of APH1 would sterically mask the Rer1p interaction site, allowing the NCT-APH1 subcomplex to escape the Rer1p-dependent `percolation' mechanism. Subsequent binding of the PS1 C-terminus to the TMD of NCT could provide a molecular mechanism to `lock' the NCT-APH1 interaction into a maturing complex, preventing it from shifting back to the Rer1p interaction. Finally, full assembly of the complex may subsequently mask the PS1 ER-retention motif, providing a dual `hide and run' mechanism for gamma-secretase complex to reach distal compartments.

Once NCT-APH1 is formed, PS1 and PEN2 (either sequentially or together) join it to form a fully assembled complex. Again, this event does not need to be restricted to the ER but can also take place in the intracellular compartment. Full-length PS1 can exit the ER, which suggests that endoproteolysis (and PEN2 interaction) occurs later and maybe during additional retrieval events from Golgi to ER.

Hence, the ER-Golgi quality control system ensures that monomeric gamma-secretase components are selectively retrieved from the cis-Golgi to the ER through interaction with cargo-retrieval receptors. Conversely, full complex assembly leads to a masking of the interactions with these retrieval receptors, allowing escape from ER-Golgi recycling and transport of assembled complexes to their final destination in distal compartments, including cell surface and endosomes.

4.3. Gamma-secretase: Mechanism of action

In the current model of gamma-secretase activity, both the NTF and CTF of PS are required for the actual aspartyl protease activity, whereas NCT and APH1 are attributed a substrate recognition function. On the whole, little is known about the way the different subunits of gamma-secretase contribute to its activity and specificity and about how substrates are bound and hydrolyzed in the catalytic site.

The canonical picture of how substrates are cleaved by gamma-secretase is derived from studies with APP and the Notch-1 receptor. Most of the available information is deduced from indirect experiments, using site-directed mutagenesis and inhibitor studies, building analogies with what is known from classical protease biochemistry and from crystal structures made with other intramembrane cleaving proteases. APP CTFs or helical peptide inhibitors that mimic the TMD of APP bind to a distinct substrate-binding site (exosite) in PS1 (95, 108). Cysteine scanning experiments, which replace individual aa residues in PS1 with cysteine residues (80, 91-94), have suggested that this substrate-binding site is located in TMD9 (94, 109).

The main feature of PS/gamma-secretase is the hydrolysis of a peptide bond within TMDs. This requires access of the catalytic site to water molecules, which is difficult to understand given the hydrophobic environment of the lipid bilayer. Lateral gating has been proposed as a possible mechanism: the substrate docks on the outer surface of the complex and is then transported into the active cleft where hydrolysis occurs. The lateral gating by transmembrane helices and the entrance of water molecules in the active site through a hydrophilic path in the membrane seem to be common features of intramembrane proteolysis and the cysteine scanning data obtained for PS1 support such a model for gamma-secretase. The recent success in crystallising GlpG, a rhomboid protease in *E. coli*, has resolved this biochemical conundrum. The reaction actually takes place in a V-shaped cavity separated from the lipid environment by six TMDs. The catalytic hystidine-serine dyad is buried at the base of this cavity, which opens at the extracellular site. In the closed conformation, the cleft is capped by a flexible loop 5. Lifting the capping loop and/or lateral displacement of helix 5 is suggested to be induced by substrate binding. This not only allows water to access the cavity but destabilizes hydrophobic side chain interactions, thereby opening a lateral gate for the substrate to reach the catalytic site.

3.4. Gamma-secretase: substrates and functions

Gamma-secretase substrates show low homologies at their cleavage sites (110), thus, requiring its activity to be tightly regulated.

The most studied among gamma-secretase substrates are Notch and APP. Apart from them, gamma-secretase cleaves various type I transmembrane proteins, including the adhesion molecules N-cadherin and E-cadherin (111), the neurotrophin receptor p75 (112), the regulatory beta2 subunit of voltage-gated sodium channels (113), the axon guidance molecule DCC and neuregulin.

Notch is a component of evolutionarily conserved signaling mechanisms that regulate development. Besides

its critical role in neuronal development, Notch pathway may also function in adult brain and may control processes such as neurogenesis, neuritic growth (114) and neural stem cell maintenance (115), as well as synaptic plasticity and long-term memory, which have been related to cognitive function.

Neuregulin has an important role in myelin formation and single nucleotide polymorphisms in the gene have been associated with an increased risk of neurodevelopmental disorders. A deficiency in a gamma-secretase subtype in mice causes disturbed neuregulin processing in Neuregulin-1 polymorphism brain, possibly causing prepulse inhibition, increased dopaminergic tonus and behavioral symptoms relevant to neurodevelopmental disorders such as schizophrenia (116).

Processing of gamma-secretase substrates other than APP is segregated from the APP processing, which occurs in the raft domains: several studies demonstrated that gamma-secretase and APP CTFs co-reside in raft microdomains of late endosomes, the TGN and TGN-derived vesicles (117). Absence or inhibition of gamma-secretase activity results in the accumulation of APP CTFs in raft domains (117), suggesting that gamma-secretase cleavage of alpha-CTF and beta-CTF occurs there. Potential raft targeting signals have been recently identified in gamma-secretase subunits: NCT and APH1 (118). NCT is *S*-palmitoylated at cysteine 689 in the TMD and APH1 undergoes *S*-palmitoylation at cysteine 182 and cysteine 245 that are oriented towards the cytosol. Unlike the case of BACE1, *S*-palmitoylation contributes to the protein stability of nascent NCT and APH1 but is not required for the assembly of NCT and APH1 into the gamma-secretase complex. Stability of palmitoylation-deficient NCT and APH1 that are already assembled in the gamma-secretase complex is indistinguishable from that of the wild type subunits. Furthermore, palmitoylation of NCT and APH1 is required for raft association of these nascent subunits but did not affect the raft localization of PS1 and PEN2 or of the assembled gamma-secretase complex. Therefore, these studies imply the presence of additional dominant signals or interacting proteins that may target the fully assembled gamma-secretase complex to rafts. Like in the case of BACE1, stable overexpression of these palmitoylation-deficient mutant subunits of NCT and APH1 did not affect amyloidogenic processing of APP as well as of other gamma-secretase substrates. On the whole, physiological significance of *S*-palmitoylation of gamma-secretase subunits has not been completely understood.

The vast functional diversity of its substrates, which are involved in cell fate decisions, adhesion, neurite outgrowth and synapse formation, highlights the important role that gamma-secretase plays in development, adulthood and neurogenesis.

5. ALPHA-SECRETASE

In the non-amyloidogenic pathway, APP is cleaved within Abeta domain by an alpha-secretase precluding deposition of intact Abeta. The initial protease cleaves between lysine 16 and leucine 17 of the Abeta sequence and appears to be followed by an as yet unidentified carboxypeptidase cleavage selectively removing lysine 16 (28). This APP alpha-secretase-mediated cleavage occurs constitutively (constitutive alpha-secretase) and can additionally be stimulated above its constitutive level by a heterogeneous group of molecules.

Alpha-secretase was first shown to have characteristics of a metalloprotease (119) and different metalloproteases were suggested as potential constitutive alpha-secretases, because they cleave APP-derived synthetic peptides *in vitro* and because their over-expression increased APP cleavage. The most frequently studied candidate alpha-secretases are members of the ADAM (a disintegrin and metalloprotease) family (6, 120). Studies with a range of specific proteinase inhibitors have shown that alpha-secretase is a zinc metalloproteinase, since hydroxamic acid-based compounds such as batimastat inhibit the appearance of sAPPalpha (119, 121) without affecting the levels of full-lenght APP or its beta-secretase cleavage. Although both the stimulated and the constitutive alpha-secretase activity in human neuroblastoma are completely blocked by batimastat (122), another hydroxamic acid-based compound is able to distinguish between these two activities (123). These data imply that at least two different but closely related zinc metalloproteinase are involved in the constitutive and regulated alpha-cleavage of APP. Therefore, it is likely that alpha-secretase activity is a property of several enzymes at the cell surface. Among the 33 known mammalian ADAMs, three members have been shown to exert alpha-secretase activity: 9, 10 and 17, also called Tumor necrosis factor-alpha convertase (TACE).

5.1. Who is alpha-secretase?

Molecular identity of alpha-secretase is controversially discussed. The three different metalloproteases mentioned above were suggested as potential alpha-secretases, since their overexpression increased APP cleavage. However, because overexpression of a protease may artificially or indirectly increase APP alpha-secretase cleavage, the physiological relevance of a candidate protease needs to be shown using the corresponding protease knockdown or knockout cells. The finding that APP shedding was never fully suppressed after RNAi-mediated knockdown in cultured cells and in deficient mice of the individual proteases has led to the conclusion that ADAM9, 10 and 17 may all together contribute to alpha-secretase activity and that in the absence of one of them, the other proteases can still mediate APP alpha-secretase cleavage. Only in a subset of ADAM10-deficient fibroblasts, APP alpha-secretase cleavage was altered to a variable degree (124). The reason for this variability is not yet clear. Importantly, because ADAM10 knockout mice die embryonically and ADAM17 knockout mice die perinatally, only embryonic fibroblasts, but not neurons, from these animals have been analysed for APP shedding. Perhaps certain cells, such as mouse embryonic fibroblast have the ability to partially compensate for the loss of ADAM10, while this was clearly not seen in primary neurons and several cell lines

investigated. The finding that APP shedding was never fully abolished was taken as evidence that all three proteases may have redundant functions with regard to APP alpha-secretase cleavage. Moreover, since alpha-secretase can cleave in a constitutive way as well as in a regulate way, the different proteases are supposed to cleave APP upon different stimuli. This assumption is in clear contrast to other ADAM protease substrates, many of which are predominantly cleaved by a single ADAM protease (125, 126).

Recently, it was shown that APP constitutive alpha-secretase cleavage requires ADAM10. However, the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) appears to stimulate the ADAM10 cleavage of APP (127), suggesting that ADAM10 is not only the constitutive alpha-secretase, but also contributes to the regulated alpha-secretase activity. In addition, ADAM17 can act as regulated APP alpha-secretase, at least upon stimulation with the phorbol ester PMA. Future studies need to address whether ADAM17 cleavage of APP also occurs under physiological or pathophysiological relevant conditions other than upon treatment with the synthetic phorbol ester PMA because PMA does not naturally occur in the body.

Additional metalloproteases of the ADAM or MMP family may also contribute to regulated alpha -secretase cleavage. Based on over-expression studies, candidates for regulated alpha -secretase are MMP9, membrane type-1 matrix metalloprotease (MT1-MMP), MT3-MMP, MT5-MMP and ADAM8 (128, 129). Several of these proteases do not cleave APP at the site of constitutive cleavage, but at multiple sites in the ectodomain. One site is the hystidine 14- glutamine 15 peptide bond within the Abeta domain, such that an increased expression or activity of these proteases can reduce Abeta generation and enhance alpha-secretase cleavage, leading to a slightly truncated sAPPalpha. Even if MMP9 does not produce sAPPalpha from full-length APP under physiological conditions, it might still be beneficial for AD by degrading Abeta.

On the whole, there is convincing evidence for ADAM10 being the only constitutive alpha-secretase, with a role, also in regulated pathways, whereas ADAM17 may be restricted to the regulated alpha-secretase pathway. The involvement of ADAM9 is less convincing but it is quite possible that under particular conditions and in certain cell type this, or indeed other members of the ADAMs family, may have a more significant role in alpha-secretase cleavage of APP.

5.2. ADAM10

ADAM10 was first isolated from bovine brain as an enzyme capable of degrading myelin basic protein (130). Subsequent isolation and sequencing of its cDNA revealed that it was a member of the ADAMs family (131).

In adult brain, ADAM10 is widely distributed and expressed in astrocytes, microglial cells and neurons. ADAM10 protein levels were found to be reduced significantly in platelets of sporadic AD patients. sAPPalpha levels in platelets and cerebrospinal fluid of AD patients were also found to be decreased (132). In contrast, ADAM10 mRNA levels were found to be two-fold increased in hippocampal and cerebellar sections of AD patients (133). These results were from the brains of severe AD patients and it is possible that, in later stages of the disease, ADAM10 expression is increased as a defense mechanism or as a secondary effect of inflammation and reactive gliosis.ADAM10 is a type I transmembrane protein with an N-terminal signal peptide, a propeptide domain containing a cysteine switch, a cleavage site for the pro-hormone convertases, a metalloprotease domain, a cysteine-rich region, a disintegrin domain, an epidermal growth factor (EGF)-like sequence, a transmembrane domain and a short cytoplasmic tail.

Intracellularly ADAM10 is predominantly found as proenzyme in the Golgi, presumably in an inactive form (6). After cleavage of the signal peptide, ADAM10 enters the secretory pathway to be processed and activated by the proprotein convertases furin or PC7 (134). ADAM10 prodomain exhibits a dual function: the separately expressed prodomain is capable of inactivating endogenous ADAM10 in cell cultures while overexpressed ADAM10 without its prodomain is inactive (134). By contrast, coexpression of the prodomain in trans rescues the activity of the deletion mutant of ADAM10 without the intramolecular prodomain. This implicates that the prodomain acts as a transient inhibitor but also as an internal chaperone in ADAM10 maturation. Activation of ADAM10 is regulated by a cysteine residue in the prodomain. According to the "cysteine-switch" mechanism (135), the conserved cysteine in the prodomain complexes at the fourth coordination site of the zinc ion in the catalytic center and inhibits the entrance of the water molecule responsible for hydrolysis. Only after proteolytic removal of the prodomain, the latent inhibition is released.

To better examine the effect of the prodomain on the alpha-secretase activity of ADAM10, a deletion mutant lacking the prodomain (DeltaPro) was used. After stable over-expression of the DeltaPro mutant in HEK cells, the same alpha-secretase activity was found as in control HEK cells. Stimulation of HEK cells and cells overexpressing the DeltaPro mutant with the phorbol ester PMA resulted in a sixfold increase of alpha-secretase activity due to stimulation of endogenous ADAM10 in HEK cells, with no significant further increase in cells overexpressing the DeltaPro mutant. Therefore, expression of ADAM10 as a proenzyme seems to be a prerequisite for the proteolytic activity of ADAM10.

The prodomain obviously plays an important role for the correct folding of the latent proenzyme in ER and/or the further transport in the secretory pathway (136). The prodomain could, therefore, act as an intramolecular or steric chaperone for the folding and activity of several proteases containing prosequences (137, 138). Interestingly, ADAM10 also undergoes ectodomain shedding by ADAM9 and 15, which is followed by gamma-secretase intramembrane proteolysis and

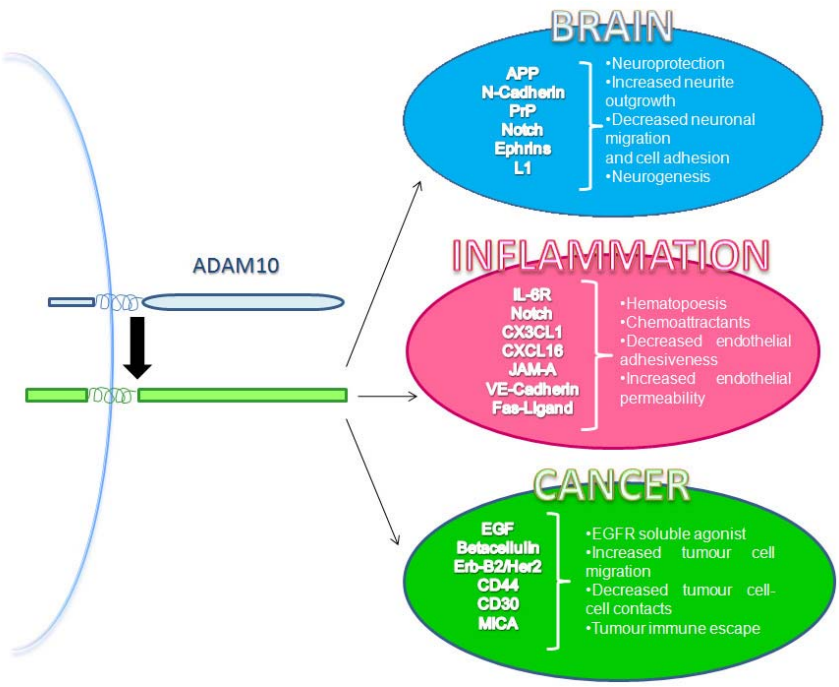

Figure 1. Schematic representation of ADAM10 and its substrates.

translocation of the ADAM10 intracellular domain into the nucleus, where it is found in nuclear speckles thought to be involved in gene regulation.

During transport through the secretory pathway, ADAM10 is N-glycosylated resulting in the active protease, which mediates proteolysis in the late compartments of the secretory pathway and at the plasma membrane. Cell-surface biotinylation experiments demonstrated that the proteolytically activated form of ADAM10 is mainly localized at the plasma membrane (6). This result supported the view that cleavage of the transmembrane protein APP occurs by a membrane-bound endoprotease at the cell surface (139, 140). On the other hand, several reports provided evidence that APP is cleaved by the alpha-secretase in a TGN compartment of the secretory pathway, where ADAM10 is also found (141, 142).

5.3. ADAM10: Substrates and functions

Substrate requirements for alpha-secretase activity are unusual. The enzyme does not have a strict sequence requirement for recognition of the site of cleavage, needing only an alpha-helical domain proximal to the cleavage site, but it cleaves substrates at a well-defined distance (16 residues) from the plasma membrane (139, 143). ADAM10 activity is dependent on the conformation of its substrate, as suggested by replacement of alanine 21 in $Abeta_{11–28}$ by glycine which reduced both the alpha-helical conformation and the velocity of cleavage by ADAM10 (6). This position corresponds to a naturally occurring alanine to glycine mutation at position 692 of APP_{770}, (144) which was identified in patients with cerebral hemorrhages due to amyloid angiopathy. ADAM-mediated shedding occurs for substrates on the same cell surface but can also happen in trans, when ADAM10 resides on one cell surface and cleaves a substrate residing on the neighboring cell surface as shown for ephrin-receptor signaling (145).

ADAM10 sheds over 30 different membrane proteins besides APP, such as Notch, betacellulin, klotho and N-cadherin (146, 147) and more substrates are likely to be identified in the future. In general, ADAM10 substrates belong to three different classes of membrane bound proteins (141). Most of them are type 1 transmembrane proteins such as APP (6), APLP2 or the receptor for glycosylation end products. Type II transmembrane proteins such as Fas ligand have also been reported. Additionally, at least three glycosylphosphatidylinositol-anchored proteins are candidate substrates for ADAM10.

Given the large number of its substrates and through their cleavage, ADAM10 is involved in different physiological and pathophysiological processes, such as embryonic development, cell adhesion, signal transduction, axon outgrowth, the immune system, cancer and AD (Figure 1) (147, 148).

To study ADAM10 functions, knock-out mice were generated but they die early (embryonic day E9.5) during embryonic development and show the typical loss-of-function phenotype of Notch signaling (124). This demonstrates that Notch, which is a cell surface receptor involved in cell differentiation, is a major ADAM10 substrate during development. Efforts have been made to generate conditional ADAM10 knock-out mice. These should elucidate functions of ADAM10 later during

development or in adulthood or in specific tissues but they die perinatally with a disrupted neocortex and a severely reduced ganglionic eminence, due to precocious neuronal differentiation resulting in an early depletion of progenitor cells. This suggests that ADAM10 plays a central role in the developing brain by controlling mainly Notch-dependent pathways but likely also by reducing surface shedding of other neuronal membrane proteins including APP (149).

5.4. Alpha-secretase: regulation

We are still lacking the complete picture of the cellular mechanisms that control alpha-secretase cleavage. However, like many other cellular processes, the alpha-secretase cleavage of APP is tightly regulated at transcriptional, translational and post-translational level.

This regulation was first shown for the activation of muscarinic acetylcholine receptors (150).

At transcriptional level, there appears to be a tight regulation of ADAM10 expression. ADAM10 promoter contains several transcription factor binding-sites, including a retinoic acid-responsive element, where retinoic acid receptors and retinoic X receptors bind (151). Accordingly, retinoic acid and other vitamin A derivatives increase ADAM10 expression suppressed through mechanisms involving the 5' untranslated region of ADAM10 mRNA (152). It remains to be tested whether this constitutes a permanent block of translation.

Alternatively, translation may be modulated by signaling pathways, as was demonstrated for a similar translational suppression of BACE1 (153, 154).

At post-translational level, different mechanisms control alpha-secretase cleavage. They can be broadly grouped into 3 different categories. First, activation of certain receptors increases alpha-secretase cleavage. This includes stimulation by growth factors, cytokines and neurotransmitters. Interestingly, several first messengers (including phorbol esters, cholinergic agonists and other neurotransmitters) activate the phospholipase-C (PLC)/protein-kinase-C (PKC) dependent pathway and enhance secretion of sAPPalpha into the conditioned medium of cultured cells (150, 155). Activation of muscarinic and epidermal growth factor receptors enhances the production of sAPPalpha by pathways involving PKC, tyrosine kinases, the mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (156). Direct activation of PKC with phorbol esters also upregulates sAPPalpha secretion (157). Second, mechanisms that control the intracellular trafficking of APP or ADAM10 tightly control the amount of alpha-secretase cleavage. Endocytic APP trafficking is a prime modulator of alpha-secretase cleavage. This is explained by the cellular localization of the secretases. alpha-Secretase cleavage occurs at the plasma membrane, whereas beta-secretase cleavage mostly occurs in endosomes. A reduction of APP endocytosis increases APP levels at the cell surface, resulting in enhanced APP cleavage by alpha-secretase and reduced Abeta levels. Conversely, enlarged endosomes, which are consistent with an increased APP endocytosis and beta-secretase cleavage, are associated with early neuropathological changes observed in AD brains (158). Trafficking of ADAM10 is also controlled, but less is known about the underlying mechanisms. A recent study identified tetraspanin 12 (TSPAN12) as a novel interaction partner of ADAM10. TSPAN12 is an integral membrane protein and was found to facilitate alpha-secretase cleavage of APP (159). TSPAN12 predominantly interacts with the mature form of ADAM10 and additionally promotes ADAM10 maturation, suggesting that it increases ADAM10 transport through the secretory pathway to the plasma membrane. Another protein implicated in ADAM10 trafficking and APP alpha-secretase cleavage is nardilysin (N-arginine dibasic convertase) (160). Although nardilysin is a metalloprotease, mutational studies demonstrated that the alpha-secretase enhancing activity did not require the proteolytic activity of nardilysin, suggesting that it acts as a scaffolding or transport protein. Another example links signaling pathways and changes in ADAM10 trafficking. Short-term activation of the NMDA receptor in primary neurons activates APP alpha-secretase cleavage. The underlying mechanism involves synapse associated protein-97 (SAP97) (161, 162). Third, a heterogeneous group of molecules regulates alpha-secretase cleavage, including natural inhibitors, such as tissue inhibitor of metalloproteases 3 (163), as well as changes in the membrane cholesterol concentration. alpha-secretase cleavage takes place outside the cholesterol-rich membrane microdomains (lipid rafts), such that an inhibition of cholesterol biosynthesis, for example by statins or the squalene synthase inhibitor zaragozic acid, stimulates the cleavage (164, 165).

6. BALANCE BETWEEN AMYLOIDOGENIC AND NON-AMYLOIDOGENIC APP METABOLISM

Alpha and beta-secretase are supposed to compete for APP. The molecular mechanisms underlying the competition between alpha and beta-secretase are not yet fully understood but may involve changes in the cellular compartments where the cleavage typically takes place. As mentioned in previous section, alpha-secretase cleavage occurs at the plasma membrane (139), but also in the TGN, at least upon stimulation with PMA (37). In contrast, beta-secretase cleavage of wild-type APP occurs mainly in the endosome and to a lower extent in the TGN. Competition between alpha and beta-secretase cleavage is seen for example upon over-expression of BACE1. This strongly increased beta-secretase cleavage and reduced alpha-secretase cleavage (10, 166), probably because over-expressed BACE1 artificially cleaves APP in early compartments of the secretory pathway, before APP reaches the plasma membrane and has access to alpha-secretase. A second condition with competition between alpha and beta-secretase cleavage is APPswe;, which is linked to an FAD. Compared with wild-type APP the APPswe; is more efficiently cleaved by beta-secretase and is processed to more Abeta and less sAPPalpha, presumably because APPswe; is already

cleaved by beta-secretase in the TGN, before encountering alpha-secretase.

Although this competition seems to be clearly the case for the regulated component of the alpha-secretase cleavage, it is not yet clear whether it also happens under constitutive cleavage conditions. A recent study using conditional ADAM10 knock-out mice reported that ADAM10 deficiency did not only reduce sAPPalpha levels, but also sAPPbeta and Abeta (149). The reason is not yet clear. Since conditional ADAM10 knock-out induced premature neuronal differentiation and defects in neuronal migration, it will be important to analyze neurons where ADAM10 knock-out starts at a later time point, when differentiation of neurons is complete. Ideally, these would be adult neurons. Interestingly, no competition between constitutive alpha and beta-secretase cleavage was observed in cell lines (HEK293, CHO and neuroblastoma SH-SY5Y) (166, 167), raising the possibility that the competition between both proteases under constitutive cleavage conditions depends on the cell type. This may potentially be due to the fact that expression of BACE1 is high in neurons, but much lower in cell lines.

On the whole, this shows that the regulated –but probably not the constitutive- component of alpha-secretase can compete with beta-secretase, in agreement with the idea that a pharmacological activation of alpha-secretase may be a therapeutic approach to AD, by increasing Abeta by reducing the neuroprotective sAPPalpha or by both mechanisms (168).

7. SECRETASES AS TARGETS FOR ALZHEIMER'S DISEASE TERAPHY

AD is a chronic neurodegenerative disorder in which protein misfolding of Abeta (plus Tau) leads to neuronal damage and destruction; as neurons die, they reduce their biosynthesis of multiple neurotransmitters (particularly acetylcholine), leading to a multiplicity of behavioral symptoms, including decreased memory and cognition. In line with this amyloid hypothesis of AD, there exists a cascade to neurotoxicity, starting with protein misfolding and ultimately culminating in behavioral symptoms. If a therapeutic target is "high" in this cascade (e.g., protein misfolding), the resulting agent may be disease-modifying or even curative; if a therapeutic target is "low" is this cascade (e.g., neurotransmitter deficit replacement), the resulting agent is more likely to offer "symptomatic" improvement rather than disease modification. Existing treatments for AD fail to address the underlying pathology of the disease; they merely provide short-lived symptomatic relief. Given AD large human, social and economic burden, effective disease-modifying treatments are greatly needed. How close we are to this goal is difficult to estimate, but available results from clinical research trials are not in line with previous optimistic predictions of an imminent break through (169). Apart from common problems in drug design as design of selective compounds without undesirable and potentially toxic side-effects or genetic polymorphism that might affect drug response, there are several problems specifically linked to AD. Patients with AD have various types of neuropathology (i.e., amyloid plaques, NFTs, infarcts, Lewy bodies) (170) and there are mixed causes of dementia in many patients, particularly those who are older than 80 years. Criteria for identifying subgroups with more homogeneous biomarker evidence of AD pathology are needed to facilitate drug design. Another problem is related to the measurement of the effects: many methods are developed according to the design of Acetylcholinesterase Inhibitors (AChEIs), an approach that has indicated the AChEI symptomatic effect, but is not sensitive in detecting the efficacy of disease-modifying drugs (171).

At the moment, drug design and development for AD are focused on the identification of small-molecule therapeutics: new chemical entity organic molecules with drug-like properties, with the ability to cross the blood–brain barrier (BBB) by either (most commonly) passive diffusion or active transport. In order to cross the BBB by passive diffusion, the drug molecule should be relatively small and have the right balance of lipophilicity and hydrophilicity. The druggable targets currently being exploited for the discovery of such curative agents may be summarized as follows: inhibitors of Abeta aggregation, inhibitors of Abeta production, inhibitors of Abeta-induced neurotoxic effects, inhibitors of Abeta-induced neurotransmitter effects, inhibitors of tau-induced neurotoxicity.

Here we will focus mainly on strategies to prevent the production of the Abeta peptide. This goal can optimally be achieved either by inhibiting beta or gamma-secretase enzymes implicated in the production of Abeta from APP or by activating alpha-secretase (172). Recently, research interest has focused on sorting and trafficking of the three secretases as potential targets for therapy, in order to segregate secretases from their target: APP.

7.1. BACE1 Inhibition

BACE1 is an attractive therapeutic target for prevention and treatment of AD, being the catalyst of the initial, rate-limiting step of Abeta production (173). Studies in animals have offered the promise to support the therapeutic value of BACE1 inhibition. RNAi targeting BACE1 has proved to effectively reduce amyloid production and ameliorate neurodegenerative and behavioral deficits in an APP transgenic mouse model (19). Further supporting its validity as a drug target is the observation that BACE1-knockout mice do not generate Abeta.

Since the three-dimensional structure of BACE1 has been determined, many groups have embarked in the search for BACE1 inhibitors (174). BACE1 crystal structures display the conserved general folding of aspartyl proteases and this might limitate inhibitors' specificity. However, co-crystallization studies with peptidomimetic inhibitors show that beta-secretase also has some very definite differences in structure compared with other aspartyl proteases. For instance, the beta-secretase active site is more open and solvent accessible and has S2 and S4 subsites that are relatively hydrophilic. These differences could be exploited in the design of selective beta-secretase inhibitors.

Table 1. BACE1 Inhibitors

Peptidomimetics (hispidin/ catechins)	**Peptidomimetics (OM99-1/OM99-2)**
Micromolar potency	Nanomolar potency
Poor specificity	Poor specificity
Small molecules	Large size
Non-competitive	Poor bioavailability
Challenge for the future	
Inhibition of BACE1 aggregation	
Partial BACE1 inhibition	
Higher selectivity	
Reduction of side effects	
Normalization of the deregulated BACE1 expression level	

Over the past decade, many BACE1 inhibitors have been reported and these inhibitors are mainly divided into two classes: peptidomimetic and non-peptidomimetic inhibitors (Table 1) (175).

To date, it has been difficult to design non-peptidomimetic small inhibitors that avidly bind the rather large catalytic site of BACE1. Naturally occurring small-molecule noncompetitive inhibitors, such as hispidin and the catechins, have only micromolar potency and poor specificity. However, a synthetic selective non-peptidic BACE-1 inhibitor, GSK188909, potently inhibits beta-cleavage of APP and reduces levels of secreted and intracellular Abeta in SHSY5Y cells expressing APP and *in vivo* (176).

On the contrary, rational drug design based on substrate analogues and peptidomimetics (based on the aa sequence at BACE1 cleavage site on APP), has provided many compounds that are potent BACE1 inhibitors *in vitro* . Many have failed preclinical trials due to insufficient pharmacological properties and to the difficulty in getting high-affinity inhibitors to cross the BBB. First-generation beta-secretase inhibitors such as OM99-1 and OM99-2 were designed as transition-state mimetics. Although these were very potent (having low-nanomolar affinities), they were essentially peptidic in nature and lacked sufficient drug-like properties. In addition, neither compound was specific to beta-secretase and both inhibited other aspartic proteases. Further refinement of OM99-2 has led to reductions in size that maintain nanomolar potency. Although peptidomimetic inhibitors showed potent activity against BACE1, their relatively large molecular size, low metabolic stability and poor bioavailability render their development into therapeutic drug candidates difficult.

Despite intensive efforts, it is still very challenging to find compounds which combine high potency and selectivity with good brain penetration (Table 1). Therefore, few compounds have entered early-stage clinical trials so far.

Development of BACE1 inhibitors is challenging also because, in addition to APP, this enzyme has many substrates. Identification of these substrates is useful not only for evaluation of potential mechanism-based toxicity arising from inhibition of BACE1 but also for designing potent and selective BACE1 inhibitors. The first inkling that total loss of BACE1 function was not totally without effect on neuronal function emanated from studies showing that BACE1-/- mice had subtle behavioral changes, being timid, anxious, and less prone to explore compared to mice overexpressing BACE1 [56]. These BACE1-/- mice also revealed alterations in neurochemistry affecting levels of serotonin and dopamine and dopamine turnover. Because of potential untoward side effects associated with strong inhibition or reduction of BACE1, investigators have tested whether a moderate decrease in BACE1 activity would provide benefits in the CNS while limiting mechanism-based toxicities.

New perspectives are now being evaluated to solve all these issues (Table 1).

First, because the processing of APP to generate Abeta requires both gamma-secretase and BACE1, and that modest reduction of gamma-secretase provides benefit to the brain without adverse side effects (177), it is possible that reductions of both enzymes would provide additive protection against Abeta amyloidosis. To assess the value of this novel anti-amyloid combination therapy, Chow and coworkers took advantage of availability of a mouse model lacking one allele each of APH1 and BACE1 that possesses 30% and 50% of gamma-secretase and BACE1, respectively, to model such a therapeutic strategy in APPswe;PS1DeltaE9 mice. Importantly, these investigators showed that the genetic reductions of both Aph-1a and BACE1 additively attenuate the amyloid burden and ameliorate cognitive deficits occurring in APPswe;PS1DeltaE9 mice. Obviously, given the fact that the list of BACE1 and gamma-secretase substrates continues to grow, potential strategies to target BACE1 or gamma-secretase activity will have to consider and incorporate rational means to avoid potential adverse consequences resulting from total inhibition of BACE1 and gamma-secretase processing of diverse substrates.

Second, since in brain tissue of mice and humans BACE1 forms homodimers and such homodimers exert a higher enzymatic activity than monomeric BACE1 (23, 178), the use of inhibitors of BACE1 aggregation is under evaluation.

Finally, beside a direct pharmaceutical inhibition of BACE1, alternative approaches, which address the normalization of the deregulated BACE1 expression level, could be employed in the future. However, since a pharmacological interference with regulatory mechanisms is not only very difficult to achieve but may also cause rather pleiotropic effects, it is still much too early to speculate about the potential of a therapeutic control of BACE1 levels.

7.2. Gamma-secretase: inhibition

Concerning gamma-secretase, the potentially most challenging and rewarding question is whether the knowledge of the complex composition and regulation will ever be translated into a workable therapeutic for AD.

Gamma-secretase was the first target in the amyloid pathway to be intensely pursued for drug development. Efforts began in the early 1990s, when it was

Table 2. Gamma-secretase Inhibitors

Inhibitors	**Modulators**
Side effects	Poor CNS penetration
Poor substrate specificity	Low potency
Toxicity	Less side effects
Challenge for the future	
Reduction of side effects	
Higher selectivity	
Cofactor binding to the complex	

Table 3. ADAM10 Activators

Advantages	**Drawbacks**
Reduction of Abeta release	Lack of specificity
Increase in sAPPalpha production	Tumor promoting

demonstrated that cultured cells have to express beta-secretase and gamma-secretase to constitutively generate Abeta peptide. This finding triggered screening campaigns to identify non-toxic inhibitors of cellular Abeta production. Several different classes of molecules were identified, and secondary assays demonstrated that these compounds inhibited the production of all Abeta isoforms via the gamma-secretase but not the beta-secretase pathway. Medicinal chemistry programmes ultimately led to drug-like molecules that could reduce plasma and soluble brain Abeta in mice after only a few hours and with only single administration. A pressing concern from the drug development perspective is the effects of gamma-secretase inhibition on substrates other than APP that may have significant drawbacks. gamma-Secretase inhibitors affect Notch signaling by blocking proteolysis of Notch-1 (another gamma-secretase substrate) through inhibition of cleavage at site 3 of the Notch receptor. The Notch signaling pathway plays a significant role in cell differentiation, both during development and in adulthood. Although a number of highly potent gamma-secretase inhibitors have been identified, interference with Notch signaling is a potential impediment to their ultimate successful implementation as therapeutic agents for the treatment of AD.

Consequently, gamma-secretase modulators (rather than inhibitors) are also being evaluated as potential therapeutic agents (Table 2). Selective $Abeta_{42}$-lowering agents, or SALAs, are being developed as potential disease-modifying therapeutic agents. SALAs do not inhibit gamma-secretase outright; rather, they allosterically modulate gamma-secretase to change the site of action on APP. In doing this, they cause a reduction in the proportion of the longer, more toxic $Abeta_{42}$ relative to $Abeta_{40}$. gamma-secretase modulators can selectively block APP proteolysis without any Notch-based adverse effects. A subset of non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, indomethacin and sulindac sulfide, bind to APP and act as gamma-secretase modulators, decreasing $Abeta_{40}$ and $Abeta_{42}$ production, with increased generation of $Abeta_{1–38}$ fragments (179, 180). Among these compounds, tarenflurbil was tested in phase 3 in patients with mild Alzheimer's disease, but did not show clinical effects. Negative results could be due to low gamma-secretase modulator potency, poor CNS penetration, and inhibition of microglia-mediated Abeta clearance by residual NSAID activity. Further studies with $Abeta_{42}$-selective modulators revealed that the $Abeta_{42}$ reduction is not mediated by cyclooxygenase (COX) inhibition or other known non-COX targets of NSAIDs, but by direct interaction of the compounds with gamma-secretase or its substrate. By not completely inhibiting gamma-secretase, SALAs may exert a beneficial effect without affecting Notch processing *in vivo*. This is an attractive alternative to gamma-secretase inhibitors, which have a tendency to produce gastrointestinal and immunological side effects. Although selective lowering of $Abeta_{42}$ has been shown to attenuate plaque formation and behavioral deficits in AD murine models, it remains to be seen whether these beneficial effects will be translated to humans. TMP21 (181) (also known as TMED10), orphan G-protein-coupled receptor 3 (182) and different APH1 isoforms have been reported to modulate Abeta production through gamma-secretase but to spare Notch cleavage. However, the underlying molecular mechanisms by which they impart their specificities were not elucidated in those studies.

Different studies demonstrated that it is possible to selectively regulate substrate specificity of this vitally important and potentially promiscuous enzyme by cofactors binding to the complex. GSAP seems to confer substrate specificity on gamma-secretase by forming a ternary complex with gamma-secretase and the substrate APP-CTF (183). The present results support the concept that appropriate cofactors impart substrate specificity on the gamma-secretase core enzyme complex, as they do on a number of other proteases (184).

Another caveat in developing secretase inhibitors for AD is the finding that the CTF99 fragment of APP generated by beta-secretase cleavage may be more toxic than Abeta. This suggests that blocking gamma-secretase could be detrimental, leading to the worsening of AD symptoms. This question will undoubtedly be answered as SALAs progress through clinical trials.

7.3. Alpha-secretase: activation

An activation of alpha-secretase cleavage may have two distinct beneficial effects for AD. First, it lowers the levels of the neurotoxic and pathogenic Abeta peptide. Second, it increases the amount of the neuroprotective sAPPalpha (Table 3).

Several drugs can stimulate alpha-secretase activity (agonists of muscarinic, glutamate, and serotonin receptors, statins, estrogens, testosterone and PKC activators) and have been tested in clinical trials, but no evidence supports their use in AD yet.

Many stumbling blocks still impede the rational design of therapeutic agents that act by alpha-secretase activation and it is generally thought that potentiating alpha-secretase cleavage is more challenging than inhibiting beta- and gamma-secretase (185). Direct stimulation of ADAM 9/10/17 activity through activation of G protein–coupled receptors is a possible strategy for stimulating alpha-secretase processing of APP and indeed such alpha-secretase activators exist. However, most of these drugs are intended for other pharmacological actions and this lack of specificity represents a major limitation. An

alternative approach to enhancing alpha-secretase activity may be through activation of PKC. Indeed, the activation of ADAM proteases is controlled by the protein phosphorylation signal transduction pathway of PKC. Phorbol esters are PKC activators that have been shown to significantly reduce Abeta production both *in vitro* and *in vivo*. However, their tumor-promoting characteristics preclude their development as AD therapeutics. The design of other agents has been successful and includes benzolactam-based compounds and linoleic acid derivatives that alter Abeta processing. Bryostatin 1, a molecule that was initially being investigated because of its promising anticancer activity, has sub-nanomolar affinity for PKC. At these concentrations, it is able to promote sAPPalpha secretion in fibroblasts taken from patients with AD.

A new generation of agonists of the M1 muscarinic acetylcholine receptor was tested in pre-clinical studies and in AD patients. The M1 receptor is required for alpha-secretase cleavage, as reported in a recent study using M1 receptor deficient mice (186). The new M1 agonists are more specific and better tolerated than previous muscarinic agonists.

The crucial question remaining is whether there are side effects connected with enhanced ADAM10 activation in brain or peripheral structures. Of course, because ADAM10-based AD therapy will take place in elderly people, interference with developmental signaling pathways does not appear to be so relevant. Also, regarding prion disease, upregulation of ADAM10 might be beneficial.

However, although being a valuable alternative, ADAM10 activation must be moderate and closely monitored.

8. PERSPECTIVE

Although research is promising, many hurdles must be overcome before studies in humans can be attempted for drugs modifying Abeta generation (187). These hurdles include finding a molecule with high specificity for the targeted secretase, adequate absorption and pharmacokinetic profile, low toxicity and sufficient BBB permeability. Moreover, nearly all current drug design approaches are based firmly on the amyloid hypothesis, which postulates that by removing, preventing or reducing the amyloid deposits, AD will somehow be "cured." The weakness of this approach is that amyloid deposition may be only another symptom rather than a cause of degenerative processes and therefore its disruption it will leave the underlying disease processes intact. In some individuals, AD occurs secondary to genetic predisposition, in others, it arises as a long-term consequence of head trauma, and in others, it is completely sporadic. From a therapeutic perspective, targeting the mechanisms of FAD makes the implicit assumption that familial disease is fundamentally similar to the common sporadic late-onset form (188).

To solve these complicated issues, it is necessary to improve our knowledge of AD pathogenesis, of the secretases, of their regulating mechanisms and of the molecular details of their cell biology.

9. REFERENCES

1. S. S. Sisodia and P. H. St George-Hyslop: gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? *Nat Rev Neurosci*, 3(4), 281-90 (2002)

2. D. J. Selkoe: Alzheimer disease: mechanistic understanding predicts novel therapies. *Ann Intern Med*, 140(8), 627-38 (2004)

3. J. Hardy: Amyloid double trouble. *Nat Genet*, 38(1), 11-2 (2006)

4. D. J. Selkoe: Biochemistry and molecular biology of amyloid beta-protein and the mechanism of Alzheimer's disease. *Handb Clin Neurol*, 89, 245-60 (2008)

5. C. Haass: Take five--BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *EMBO J*, 23(3), 483-8 (2004)

6. S. Lammich, E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass and F. Fahrenholz: Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A*, 96(7), 3922-7 (1999)

7. M. Zimmermann, F. Gardoni, E. Marcello, F. Colciaghi, B. Borroni, A. Padovani, F. Cattabeni and M. Di Luca: Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines. *J Neurochem*, 90(6), 1489-99 (2004)

8. A. Kinoshita, H. Fukumoto, T. Shah, C. M. Whelan, M. C. Irizarry and B. T. Hyman: Demonstration by FRET of BACE interaction with the amyloid precursor protein at the cell surface and in early endosomes. *J Cell Sci*, 116(Pt 16), 3339-46 (2003)

9. S. Kitazume, Y. Tachida, R. Oka, K. Shirotani, T. C. Saido and Y. Hashimoto: 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(24), 13554-9 (2001)

10. R. Vassar, B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M. A. Jarosinski, A. L. Biere, E. Curran, T. Burgess, J. C. Louis, F. Collins, J. Treanor, G. Rogers and M. Citron: Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*, 286(5440), 735-41 (1999)

11. S. Sinha, J. P. Anderson, R. Barbour, G. S. Basi, R. Caccavello, D. Davis, M. Doan, H. F. Dovey, N. Frigon, J.

Hong, K. Jacobson-Croak, N. Jewett, P. Keim, J. Knops, I. Lieberburg, M. Power, H. Tan, G. Tatsuno, J. Tung, D. Schenk, P. Seubert, S. M. Suomensaari, S. Wang, D. Walker, J. Zhao, L. McConlogue and V. John: Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature*, 402(6761), 537-40 (1999)

12. I. Hussain, D. Powell, D. R. Howlett, D. G. Tew, T. D. Meek, C. Chapman, I. S. Gloger, K. E. Murphy, C. D. Southan, D. M. Ryan, T. S. Smith, D. L. Simmons, F. S. Walsh, C. Dingwall and G. Christie: Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol Cell Neurosci*, 14(6), 419-27 (1999)

13. R. Yan, M. J. Bienkowski, M. E. Shuck, H. Miao, M. C. Tory, A. M. Pauley, J. R. Brashier, N. C. Stratman, W. R. Mathews, A. E. Buhl, D. B. Carter, A. G. Tomasselli, L. A. Parodi, R. L. Heinrikson and M. E. Gurney: Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature*, 402(6761), 533-7 (1999)

14. X. Lin, G. Koelsch, S. Wu, D. Downs, A. Dashti and J. Tang: Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. *Proc Natl Acad Sci U S A*, 97(4), 1456-60 (2000)

15. S. L. Cole and R. Vassar: The role of amyloid precursor protein processing by BACE1, the beta-secretase, in Alzheimer disease pathophysiology. *J Biol Chem*, 283(44), 29621-5 (2008)

16. Y. Luo, B. Bolon, M. A. Damore, D. Fitzpatrick, H. Liu, J. Zhang, Q. Yan, R. Vassar and M. Citron: BACE1 (beta-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time. *Neurobiol Dis*, 14(1), 81-8 (2003)

17. M. Ohno, S. L. Cole, M. Yasvoina, J. Zhao, M. Citron, R. Berry, J. F. Disterhoft and R. Vassar: BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mice. *Neurobiol Dis*, 26(1), 134-45 (2007)

18. F. M. Laird, H. Cai, A. V. Savonenko, M. H. Farah, K. He, T. Melnikova, H. Wen, H. C. Chiang, G. Xu, V. E. Koliatsos, D. R. Borchelt, D. L. Price, H. K. Lee and P. C. Wong: 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(50), 11693-709 (2005)

19. O. Singer, R. A. Marr, E. Rockenstein, L. Crews, N. G. Coufal, F. H. Gage, I. M. Verma and E. Masliah: Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. *Nat Neurosci*, 8(10), 1343-9 (2005)

20. H. Fukumoto, B. S. Cheung, B. T. Hyman and M. C. Irizarry: Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease. *Arch Neurol*, 59(9), 1381-9 (2002)

21. H. Tanahashi and T. Tabira: 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(1), 9-12 (2001)

22. K. R. Mowrer and M. S. Wolfe: Promotion of BACE1 mRNA alternative splicing reduces amyloid beta-peptide production. *J Biol Chem*, 283(27), 18694-701 (2008)

23. G. G. Westmeyer, M. Willem, S. F. Lichtenthaler, G. Lurman, G. Multhaup, I. Assfalg-Machleidt, K. Reiss, P. Saftig and C. Haass: Dimerization of beta-site beta-amyloid precursor protein-cleaving enzyme. *J Biol Chem*, 279(51), 53205-12 (2004)

24. B. D. Bennett, S. Babu-Khan, R. Loeloff, J. C. Louis, E. Curran, M. Citron and R. Vassar: Expression analysis of BACE2 in brain and peripheral tissues. *J Biol Chem*, 275(27), 20647-51 (2000)

25. F. Fischer, M. Molinari, U. Bodendorf and P. Paganetti: The disulphide bonds in the catalytic domain of BACE are critical but not essential for amyloid precursor protein processing activity. *J Neurochem*, 80(6), 1079-88 (2002)

26. E. H. Koo and S. L. Squazzo: Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J Biol Chem*, 269(26), 17386-9 (1994)

27. K. Sambamurti, N. H. Greig and D. K. Lahiri: Advances in the cellular and molecular biology of the beta-amyloid protein in Alzheimer's disease. *Neuromolecular Med*, 1(1), 1-31 (2002)

28. F. S. Esch, P. S. Keim, E. C. Beattie, R. W. Blacher, A. R. Culwell, T. Oltersdorf, D. McClure and P. J. Ward: Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science*, 248(4959), 1122-4 (1990)

29. J. P. Anderson, F. S. Esch, P. S. Keim, K. Sambamurti, I. Lieberburg and N. K. Robakis: Exact cleavage site of Alzheimer amyloid precursor in neuronal PC-12 cells. *Neurosci Lett*, 128(1), 126-8 (1991)

30. P. Seubert, T. Oltersdorf, M. G. Lee, R. Barbour, C. Blomquist, D. L. Davis, K. Bryant, L. C. Fritz, D. Galasko, L. J. Thal and *et al.*: Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. *Nature*, 361(6409), 260-3 (1993)

31. P. Seubert, C. Vigo-Pelfrey, F. Esch, M. Lee, H. Dovey, D. Davis, S. Sinha, M. Schlossmacher, J. Whaley, C. Swindlehurst and *et al.*: Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature*, 359(6393), 325-7 (1992)

32. M. Haniu, P. Denis, Y. Young, E. A. Mendiaz, J. Fuller, J. O. Hui, B. D. Bennett, S. Kahn, S. Ross, T. Burgess, V. Katta, G. Rogers, R. Vassar and M. Citron: Characterization of Alzheimer's beta -secretase protein

BACE. A pepsin family member with unusual properties. *J Biol Chem*, 275(28), 21099-106 (2000)

33. C. Costantini, M. H. Ko, M. C. Jonas and L. Puglielli: A reversible form of lysine acetylation in the ER and Golgi lumen controls the molecular stabilization of BACE1. *Biochem J*, 407(3), 383-95 (2007)

34. S. Benjannet, J. A. Cromlish, K. Diallo, M. Chretien and N. G. Seidah: The metabolism of beta-amyloid converting enzyme and beta-amyloid precursor protein processing. *Biochem Biophys Res Commun*, 325(1), 235-42 (2004)

35. B. D. Bennett, P. Denis, M. Haniu, D. B. Teplow, S. Kahn, J. C. Louis, M. Citron and R. Vassar: A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's beta -secretase. *J Biol Chem*, 275(48), 37712-7 (2000)

36. A. Capell, H. Steiner, M. Willem, H. Kaiser, C. Meyer, J. Walter, S. Lammich, G. Multhaup and C. Haass: Maturation and pro-peptide cleavage of beta-secretase. *J Biol Chem*, 275(40), 30849-54 (2000)

37. D. M. Skovronsky, D. B. Moore, M. E. Milla, R. W. Doms and V. M. Lee: 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(4), 2568-75 (2000)

38. S. Rossner, C. Lange-Dohna, U. Zeitschel and J. R. Perez-Polo: Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. *J Neurochem*, 92(2), 226-34 (2005)

39. X. He, F. Li, W. P. Chang and J. Tang: GGA proteins mediate the recycling pathway of memapsin 2 (BACE). *J Biol Chem*, 280(12), 11696-703 (2005)

40. L. Pastorino, A. F. Ikin, A. C. Nairn, A. Pursnani and J. D. Buxbaum: 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*, 175-85 (2002)

41. M. J. Savage, S. P. Trusko, D. S. Howland, L. R. Pinsker, S. Mistretta, A. G. Reaume, B. D. Greenberg, R. Siman and R. W. Scott: Turnover of amyloid beta-protein in mouse brain and acute reduction of its level by phorbol ester. *J Neurosci*, 18(5), 1743-52 (1998)

42. J. Walter, R. Fluhrer, B. Hartung, M. Willem, C. Kaether, A. Capell, S. Lammich, G. Multhaup and C. Haass: Phosphorylation regulates intracellular trafficking of beta-secretase. *J Biol Chem*, 276(18), 14634-41 (2001)

43. S. Benjannet, A. Elagoz, L. Wickham, M. Mamarbachi, J. S. Munzer, A. Basak, C. Lazure, J. A. Cromlish, S. Sisodia, F. Checler, M. Chretien and N. G. Seidah: 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(14), 10879-87 (2001)

44. W. He, Y. Lu, I. Qahwash, X. Y. Hu, A. Chang and R. Yan: Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. *Nat Med*, 10(9), 959-65 (2004)

45. K. S. Murayama, F. Kametani, S. Saito, H. Kume, H. Akiyama and W. Araki: Reticulons RTN3 and RTN4-B/C interact with BACE1 and inhibit its ability to produce amyloid beta-protein. *Eur J Neurosci*, 24(5), 1237-44 (2006)

46. Q. Shi, M. Prior, W. He, X. Tang, X. Hu and R. Yan: Reduced amyloid deposition in mice overexpressing RTN3 is adversely affected by preformed dystrophic neurites. *J Neurosci*, 29(29), 9163-73 (2009)

47. G. Tesco, Y. H. Koh, E. L. Kang, A. N. Cameron, S. Das, M. Sena-Esteves, M. Hiltunen, S. H. Yang, Z. Zhong, Y. Shen, J. W. Simpkins and R. E. Tanzi: Depletion of GGA3 stabilizes BACE and enhances beta-secretase activity. *Neuron*, 54(5), 721-37 (2007)

48. T. Wahle, K. Prager, N. Raffler, C. Haass, M. Famulok and J. Walter: GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network. *Mol Cell Neurosci*, 29(3), 453-61 (2005)

49. R. Spoelgen, C. A. von Arnim, A. V. Thomas, I. D. Peltan, M. Koker, A. Deng, M. C. Irizarry, O. M. Andersen, T. E. Willnow and B. T. Hyman: 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(2), 418-28 (2006)

50. E. Rogaeva, Y. Meng, J. H. Lee, Y. Gu, T. Kawarai, F. Zou, T. Katayama, C. T. Baldwin, R. Cheng, H. Hasegawa, F. Chen, N. Shibata, K. L. Lunetta, R. Pardossi-Piquard, C. Bohm, Y. Wakutani, L. A. Cupples, K. T. Cuenco, R. C. Green, L. Pinessi, I. Rainero, S. Sorbi, A. Bruni, R. Duara, R. P. Friedland, R. Inzelberg, W. Hampe, H. Bujo, Y. Q. Song, O. M. Andersen, T. E. Willnow, N. Graff-Radford, R. C. Petersen, D. Dickson, S. D. Der, P. E. Fraser, G. Schmitt-Ulms, S. Younkin, R. Mayeux, L. A. Farrer and P. St George-Hyslop: The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet*, 39(2), 168-77 (2007)

51. F. Kamenetz, T. Tomita, H. Hsieh, G. Seabrook, D. Borchelt, T. Iwatsubo, S. Sisodia and R. Malinow: APP processing and synaptic function. *Neuron*, 37(6), 925-37 (2003)

52. H. Ma, S. Lesne, L. Kotilinek, J. V. Steidl-Nichols, M. Sherman, L. Younkin, S. Younkin, C. Forster, N. Sergeant, A. Delacourte, R. Vassar, M. Citron, P. Kofuji, L. M. Boland and K. H. Ashe: 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(19), 8167-72 (2007)

53. M. Sastre, I. Dewachter, G. E. Landreth, T. M. Willson, T. Klockgether, F. van Leuven and M. T. Heneka: Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-gamma agonists modulate immunostimulated processing of amyloid precursor protein through regulation of beta-secretase. *J Neurosci*, 23(30), 9796-804 (2003)

54. E. Tamagno, P. Bardini, A. Obbili, A. Vitali, R. Borghi, D. Zaccheo, M. A. Pronzato, O. Danni, M. A. Smith, G. Perry and M. Tabaton: Oxidative stress increases expression and activity of BACE in NT2 neurons. *Neurobiol Dis*, 10(3), 279-88 (2002)

55. X. Zhang, K. Zhou, R. Wang, J. Cui, S. A. Lipton, F. F. Liao, H. Xu and Y. W. Zhang: Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. *J Biol Chem*, 282(15), 10873-80 (2007)

56. Y. Wen, O. Onyewuchi, S. Yang, R. Liu and J. W. Simpkins: Increased beta-secretase activity and expression in rats following transient cerebral ischemia. *Brain Res*, 1009(1-2), 1-8 (2004)

57. I. Blasko, R. Beer, M. Bigl, J. Apelt, G. Franz, D. Rudzki, G. Ransmayr, A. Kampfl and R. Schliebs: Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease beta-secretase (BACE-1). *J Neural Transm*, 111(4), 523-36 (2004)

58. Y. Wen, W. H. Yu, B. Maloney, J. Bailey, J. Ma, I. Marie, T. Maurin, L. Wang, H. Figueroa, M. Herman, P. Krishnamurthy, L. Liu, E. Planel, L. F. Lau, D. K. Lahiri and K. Duff: Transcriptional regulation of beta-secretase by p25/cdk5 leads to enhanced amyloidogenic processing. *Neuron*, 57(5), 680-90 (2008)

59. J. M. Cordy, I. Hussain, C. Dingwall, N. M. Hooper and A. J. Turner: 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(20), 11735-40 (2003)

60. K. S. Vetrivel, X. Meckler, Y. Chen, P. D. Nguyen, N. G. Seidah, R. Vassar, P. C. Wong, M. Fukata, M. Z. Kounnas and G. Thinakaran: Alzheimer disease Abeta production in the absence of S-palmitoylation-dependent targeting of BACE1 to lipid rafts. *J Biol Chem*, 284(6), 3793-803 (2009)

61. R. Yan, P. Han, H. Miao, P. Greengard and H. Xu: 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(39), 36788-96 (2001)

62. K. S. Murayama, F. Kametani and W. Araki: Extracellular release of BACE1 holoproteins from human neuronal cells. *Biochem Biophys Res Commun*, 338(2), 800-7 (2005)

63. I. Hussain, J. Hawkins, A. Shikotra, D. R. Riddell, A. Faller and C. Dingwall: Characterization of the ectodomain shedding of the beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). *J Biol Chem*, 278(38), 36264-8 (2003)

64. G. Thinakaran, D. B. Teplow, R. Siman, B. Greenberg and S. S. Sisodia: Metabolism of the "Swedish" amyloid precursor protein variant in neuro2a (N2a) cells. Evidence that cleavage at the "beta-secretase" site occurs in the golgi apparatus. *J Biol Chem*, 271(16), 9390-7 (1996)

65. I. Qahwash, W. He, A. Tomasselli, R. F. Kletzien and R. Yan: Processing amyloid precursor protein at the beta-site requires proper orientation to be accessed by BACE1. *J Biol Chem*, 279(37), 39010-6 (2004)

66. S. F. Lichtenthaler, D. I. Dominguez, G. G. Westmeyer, K. Reiss, C. Haass, P. Saftig, B. De Strooper and B. Seed: The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1. *J Biol Chem*, 278(49), 48713-9 (2003)

67. Q. Li and T. C. Sudhof: Cleavage of amyloid-beta precursor protein and amyloid-beta precursor-like protein by BACE 1. *J Biol Chem*, 279(11), 10542-50 (2004)

68. L. Pastorino, A. F. Ikin, S. Lamprianou, N. Vacaresse, J. P. Revelli, K. Platt, P. Paganetti, P. M. Mathews, S. Harroch and J. D. Buxbaum: BACE (beta-secretase) modulates the processing of APLP2 *in vivo*. *Mol Cell Neurosci*, 25(4), 642-9 (2004)

69. D. Y. Kim, L. A. Ingano, B. W. Carey, W. H. Pettingell and D. M. Kovacs: Presenilin/gamma-secretase-mediated cleavage of the voltage-gated sodium channel beta2-subunit regulates cell adhesion and migration. *J Biol Chem*, 280(24), 23251-61 (2005)

70. X. Hu, C. W. Hicks, W. He, P. Wong, W. B. Macklin, B. D. Trapp and R. Yan: Bace1 modulates myelination in the central and peripheral nervous system. *Nat Neurosci*, 9(12), 1520-5 (2006)

71. M. Willem, A. N. Garratt, B. Novak, M. Citron, S. Kaufmann, A. Rittger, B. DeStrooper, P. Saftig, C. Birchmeier and C. Haass: Control of peripheral nerve myelination by the beta-secretase BACE1. *Science*, 314(5799), 664-6 (2006)

72. E. Rockenstein, M. Mante, M. Alford, A. Adame, L. Crews, M. Hashimoto, L. Esposito, L. Mucke and E. Masliah: High beta-secretase activity elicits neurodegeneration in transgenic mice despite reductions in amyloid-beta levels: implications for the treatment of Alzheimer disease. *J Biol Chem*, 280(38), 32957-67 (2005)

73. A. Padovani, L. Pastorino, B. Borroni, F. Colciaghi, L. Rozzini, R. Monastero, J. Perez, C. Pettenati, M. Mussi, G. Parrinello, E. Cottini, G. L. Lenzi, M. Trabucchi, F. Cattabeni and M. Di Luca: Amyloid precursor protein in platelets: a peripheral marker for the diagnosis of sporadic AD. *Neurology*, 57(12), 2243-8 (2001)

74. M. Freeman: Rhomboid proteases and their biological functions. *Annu Rev Genet*, 42, 191-210 (2008)

75. B. De Strooper, P. Saftig, K. Craessaerts, H. Vanderstichele, G. Guhde, W. Annaert, K. Von Figura and F. Van Leuven: Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*, 391(6665), 387-90 (1998)

76. B. De Strooper: Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex. *Neuron*, 38(1), 9-12 (2003)

77. N. Takasugi, T. Tomita, I. Hayashi, M. Tsuruoka, M. Niimura, Y. Takahashi, G. Thinakaran and T. Iwatsubo: The role of presenilin cofactors in the gamma-secretase complex. *Nature*, 422(6930), 438-41 (2003)

78. W. T. Kimberly, M. J. LaVoie, B. L. Ostaszewski, W. Ye, M. S. Wolfe and D. J. Selkoe: Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc Natl Acad Sci U S A*, 100(11), 6382-7 (2003)

79. D. Edbauer, E. Winkler, J. T. Regula, B. Pesold, H. Steiner and C. Haass: Reconstitution of gamma-secretase activity. *Nat Cell Biol*, 5(5), 486-8 (2003)

80. T. Sato, T. S. Diehl, S. Narayanan, S. Funamoto, Y. Ihara, B. De Strooper, H. Steiner, C. Haass and M. S. Wolfe: Active gamma-secretase complexes contain only one of each component. *J Biol Chem*, 282(47), 33985-93 (2007)

81. P. Osenkowski, H. Li, W. Ye, D. Li, L. Aeschbach, P. C. Fraering, M. S. Wolfe and D. J. Selkoe: Cryoelectron microscopy structure of purified gamma-secretase at 12 A resolution. *J Mol Biol*, 385(2), 642-52 (2009)

82. S. S. Hebert, L. Serneels, T. Dejaegere, K. Horre, M. Dabrowski, V. Baert, W. Annaert, D. Hartmann and B. De Strooper: Coordinated and widespread expression of gamma-secretase *in vivo*: evidence for size and molecular heterogeneity. *Neurobiol Dis*, 17(2), 260-72 (2004)

83. K. Shirotani, D. Edbauer, M. Kostka, H. Steiner and C. Haass: Immature nicastrin stabilizes APH-1 independent of PEN-2 and presenilin: identification of nicastrin mutants that selectively interact with APH-1. *J Neurochem*, 89(6), 1520-7 (2004)

84. L. Serneels, J. Van Biervliet, K. Craessaerts, T. Dejaegere, K. Horre, T. Van Houtvin, H. Esselmann, S. Paul, M. K. Schafer, O. Berezovska, B. T. Hyman, B. Sprangers, R. Sciot, L. Moons, M. Jucker, Z. Yang, P. C. May, E. Karran, J. Wiltfang, R. D'Hooge and B. De Strooper: gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science*, 324(5927), 639-42 (2009)

85. K. Ahn, C. C. Shelton, Y. Tian, X. Zhang, M. L. Gilchrist, S. S. Sisodia and Y. M. Li: Activation and intrinsic {gamma}-secretase activity of presenilin 1. *Proc Natl Acad Sci U S A*, 107(50), 21435-40 (2010)

86. M. S. Wolfe, W. Xia, B. L. Ostaszewski, T. S. Diehl, W. T. Kimberly and D. J. Selkoe: Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature*, 398(6727), 513-7 (1999)

87. B. De Strooper, W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J. S. Mumm, E. H. Schroeter, V. Schrijvers, M. S. Wolfe, W. J. Ray, A. Goate and R. Kopan: A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature*, 398(6727), 518-22 (1999)

88. G. Struhl and I. Greenwald: Presenilin is required for activity and nuclear access of Notch in Drosophila. *Nature*, 398(6727), 522-5 (1999)

89. T. E. Golde, M. S. Wolfe and D. C. Greenbaum: Signal peptide peptidases: a family of intramembrane-cleaving proteases that cleave type 2 transmembrane proteins. *Semin Cell Dev Biol*, 20(2), 225-30 (2009)

90. H. Steiner, M. Kostka, H. Romig, G. Basset, B. Pesold, J. Hardy, A. Capell, L. Meyn, M. L. Grim, R. Baumeister, K. Fechteler and C. Haass: Glycine 384 is required for presenilin-1 function and is conserved in bacterial polytopic aspartyl proteases. *Nat Cell Biol*, 2(11), 848-51 (2000)

91. C. Sato, Y. Morohashi, T. Tomita and T. Iwatsubo: Structure of the catalytic pore of gamma-secretase probed by the accessibility of substituted cysteines. *J Neurosci*, 26(46), 12081-8 (2006)

92. A. Tolia, L. Chavez-Gutierrez and B. De Strooper: Contribution of presenilin transmembrane domains 6 and 7 to a water-containing cavity in the gamma-secretase complex. J Biol Chem, 281(37), 27633-42 (2006)

93. S. Takagi, A. Tominaga, C. Sato, T. Tomita and T. Iwatsubo: Participation of transmembrane domain 1 of presenilin 1 in the catalytic pore structure of the gamma-secretase. J Neurosci, 30(47), 15943-50

94. A. Tolia, K. Horre and B. De Strooper: Transmembrane domain 9 of presenilin determines the dynamic conformation of the catalytic site of gamma-secretase. J Biol Chem, 283(28), 19793-803 (2008)

95. W. G. Annaert, C. Esselens, V. Baert, C. Boeve, G. Snellings, P. Cupers, K. Craessaerts and B. De Strooper: Interaction with telencephalin and the amyloid precursor

protein predicts a ring structure for presenilins. *Neuron*, 32(4), 579-89 (2001)

96. A. Khandelwal, D. Chandu, C. M. Roe, R. Kopan and R. S. Quatrano: Moonlighting activity of presenilin in plants is independent of gamma-secretase and evolutionarily conserved. *Proc Natl Acad Sci U S A*, 104(33), 13337-42 (2007)

97. C. Goutte, W. Hepler, K. M. Mickey and J. R. Priess: aph-2 encodes a novel extracellular protein required for GLP-1-mediated signaling. *Development*, 127(11), 2481-92 (2000)

98. G. Yu, M. Nishimura, S. Arawaka, D. Levitan, L. Zhang, A. Tandon, Y. Q. Song, E. Rogaeva, F. Chen, T. Kawarai, A. Supala, L. Levesque, H. Yu, D. S. Yang, E. Holmes, P. Milman, Y. Liang, D. M. Zhang, D. H. Xu, C. Sato, E. Rogaev, M. Smith, C. Janus, Y. Zhang, R. Aebersold, L. S. Farrer, S. Sorbi, A. Bruni, P. Fraser and P. St George-Hyslop: Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing. *Nature*, 407(6800), 48-54 (2000)

99. K. Shirotani, D. Edbauer, A. Capell, J. Schmitz, H. Steiner and C. Haass: Gamma-secretase activity is associated with a conformational change of nicastrin. *J Biol Chem*, 278(19), 16474-7 (2003)

100. C. Goutte, M. Tsunozaki, V. A. Hale and J. R. Priess: APH-1 is a multipass membrane protein essential for the Notch signaling pathway in Caenorhabditis elegans embryos. *Proc Natl Acad Sci U S A*, 99(2), 775-9 (2002)

101. H. Hasegawa, N. Sanjo, F. Chen, Y. J. Gu, C. Shier, A. Petit, T. Kawarai, T. Katayama, S. D. Schmidt, P. M. Mathews, G. Schmitt-Ulms, P. E. Fraser and P. St George-Hyslop: Both the sequence and length of the C terminus of PEN-2 are critical for intermolecular interactions and function of presenilin complexes. *J Biol Chem*, 279(45), 46455-63 (2004)

102. P. C. Fraering, M. J. LaVoie, W. Ye, B. L. Ostaszewski, W. T. Kimberly, D. J. Selkoe and M. S. Wolfe: Detergent-dependent dissociation of active gamma-secretase reveals an interaction between Pen-2 and PS1-NTF and offers a model for subunit organization within the complex. *Biochemistry*, 43(2), 323-33 (2004)

103. M. J. LaVoie, P. C. Fraering, B. L. Ostaszewski, W. Ye, W. T. Kimberly, M. S. Wolfe and D. J. Selkoe: Assembly of the gamma-secretase complex involves early formation of an intermediate subcomplex of Aph-1 and nicastrin. *J Biol Chem*, 278(39), 37213-22 (2003)

104. A. Capell, D. Beher, S. Prokop, H. Steiner, C. Kaether, M. S. Shearman and C. Haass: Gamma-secretase complex assembly within the early secretory pathway. *J Biol Chem*, 280(8), 6471-8 (2005)

105. K. Michelsen, H. Yuan and B. Schwappach: Hide and run. Arginine-based endoplasmic-reticulum-sorting motifs in the assembly of heteromultimeric membrane proteins. *EMBO Rep*, 6(8), 717-22 (2005)

106. C. Kaether, J. Scheuermann, M. Fassler, S. Zilow, K. Shirotani, C. Valkova, B. Novak, S. Kacmar, H. Steiner and C. Haass: Endoplasmic reticulum retention of the gamma-secretase complex component Pen2 by Rer1. *EMBO Rep*, 8(8), 743-8 (2007)

107. D. Spasic, T. Raemaekers, K. Dillen, I. Declerck, V. Baert, L. Serneels, J. Fullekrug and W. Annaert: Rer1p competes with APH-1 for binding to nicastrin and regulates gamma-secretase complex assembly in the early secretory pathway. *J Cell Biol*, 176(5), 629-40 (2007)

108. A. Y. Kornilova, F. Bihel, C. Das and M. S. Wolfe: The initial substrate-binding site of gamma-secretase is located on presenilin near the active site. *Proc Natl Acad Sci U S A*, 102(9), 3230-5 (2005)

109. C. Sato, S. Takagi, T. Tomita and T. Iwatsubo: The C-terminal PAL motif and transmembrane domain 9 of presenilin 1 are involved in the formation of the catalytic pore of the gamma-secretase. *J Neurosci*, 28(24), 6264-71 (2008)

110. A. J. Beel and C. R. Sanders: Substrate specificity of gamma-secretase and other intramembrane proteases. *Cell Mol Life Sci*, 65(9), 1311-34 (2008)

111. G. Struhl and A. Adachi: Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. *Mol Cell*, 6(3), 625-36 (2000)

112. K. M. Jung, S. Tan, N. Landman, K. Petrova, S. Murray, R. Lewis, P. K. Kim, D. S. Kim, S. H. Ryu, M. V. Chao and T. W. Kim: Regulated intramembrane proteolysis of the p75 neurotrophin receptor modulates its association with the TrkA receptor. *J Biol Chem*, 278(43), 42161-9 (2003)

113. S. H. Kim and S. S. Sisodia: Evidence that the "NF" motif in transmembrane domain 4 of presenilin 1 is critical for binding with PEN-2. *J Biol Chem*, 280(51), 41953-66 (2005)

114. N. Sestan, S. Artavanis-Tsakonas and P. Rakic: Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. *Science*, 286(5440), 741-6 (1999)

115. S. Hitoshi, T. Alexson, V. Tropepe, D. Donoviel, A. J. Elia, J. S. Nye, R. A. Conlon, T. W. Mak, A. Bernstein and D. van der Kooy: Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev*, 16(7), 846-58 (2002)

116. T. Dejaegere, L. Serneels, M. K. Schafer, J. Van Biervliet, K. Horre, C. Depboylu, D. Alvarez-Fischer, A. Herreman, M. Willem, C. Haass, G. U. Hoglinger, R. D'Hooge and B. De Strooper: Deficiency of Aph1B/C-gamma-secretase disturbs Nrg1 cleavage and sensorimotor

gating that can be reversed with antipsychotic treatment. *Proc Natl Acad Sci U S A*, 105(28), 9775-80 (2008)

117. K. S. Vetrivel, H. Cheng, S. H. Kim, Y. Chen, N. Y. Barnes, A. T. Parent, S. S. Sisodia and G. Thinakaran: Spatial segregation of gamma-secretase and substrates in distinct membrane domains. *J Biol Chem*, 280(27), 25892-900 (2005)

118. H. Cheng, K. S. Vetrivel, R. C. Drisdel, X. Meckler, P. Gong, J. Y. Leem, T. Li, M. Carter, Y. Chen, P. Nguyen, T. Iwatsubo, T. Tomita, P. C. Wong, W. N. Green, M. Z. Kounnas and G. Thinakaran: S-palmitoylation of gamma-secretase subunits nicastrin and APH-1. *J Biol Chem*, 284(3), 1373-84 (2009)

119. S. B. Roberts, J. A. Ripellino, K. M. Ingalls, N. K. Robakis and K. M. Felsenstein: Non-amyloidogenic cleavage of the beta-amyloid precursor protein by an integral membrane metalloendopeptidase. *J Biol Chem*, 269(4), 3111-6 (1994)

120. H. Koike, S. Tomioka, H. Sorimachi, T. C. Saido, K. Maruyama, A. Okuyama, A. Fujisawa-Sehara, S. Ohno, K. Suzuki and S. Ishiura: Membrane-anchored metalloprotease MDC9 has an alpha-secretase activity responsible for processing the amyloid precursor protein. *Biochem J*, 343 Pt 2, 371-5 (1999)

121. J. Arribas, L. Coodly, P. Vollmer, T. K. Kishimoto, S. Rose-John and J. Massague: Diverse cell surface protein ectodomains are shed by a system sensitive to metalloprotease inhibitors. *J Biol Chem*, 271(19), 11376-82 (1996)

122. S. Parvathy, I. Hussain, E. H. Karran, A. J. Turner and N. M. Hooper: Alzheimer's amyloid precursor protein alpha-secretase is inhibited by hydroxamic acid-based zinc metalloprotease inhibitors: similarities to the angiotensin converting enzyme secretase. *Biochemistry*, 37(6), 1680-5 (1998)

123. M. Racchi, D. C. Solano, M. Sironi and S. Govoni: Activity of alpha-secretase as the common final effector of protein kinase C-dependent and -independent modulation of amyloid precursor protein metabolism. *J Neurochem*, 72(6), 2464-70 (1999)

124. D. Hartmann, B. de Strooper, L. Serneels, K. Craessaerts, A. Herreman, W. Annaert, L. Umans, T. Lubke, A. Lena Illert, K. von Figura and P. Saftig: The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Hum Mol Genet*, 11(21), 2615-24 (2002)

125. K. Reiss, T. Maretzky, A. Ludwig, T. Tousseyn, B. de Strooper, D. Hartmann and P. Saftig: ADAM10 cleavage of N-cadherin and regulation of cell-cell adhesion and beta-catenin nuclear signalling. *EMBO J*, 24(4), 742-52 (2005)

126. S. M. Le Gall, P. Bobe, K. Reiss, K. Horiuchi, X. D. Niu, D. Lundell, D. R. Gibb, D. Conrad, P. Saftig and C. P. Blobel: ADAMs 10 and 17 represent differentially regulated components of a general shedding machinery for membrane proteins such as transforming growth factor alpha, L-selectin, and tumor necrosis factor alpha. *Mol Biol Cell*, 20(6), 1785-94 (2009)

127. E. Kojro, R. Postina, C. Buro, C. Meiringer, K. Gehrig-Burger and F. Fahrenholz: The neuropeptide PACAP promotes the alpha-secretase pathway for processing the Alzheimer amyloid precursor protein. *FASEB J*, 20(3), 512-4 (2006)

128. M. Ahmad, T. Takino, H. Miyamori, T. Yoshizaki, M. Furukawa and H. Sato: Cleavage of amyloid-beta precursor protein (APP) by membrane-type matrix metalloproteinases. *J Biochem*, 139(3), 517-26 (2006)

129. S. Naus, S. Reipschlager, D. Wildeboer, S. F. Lichtenthaler, S. Mitterreiter, Z. Guan, M. L. Moss and J. W. Bartsch: Identification of candidate substrates for ectodomain shedding by the metalloprotease-disintegrin ADAM8. *Biol Chem*, 387(3), 337-46 (2006)

130. A. Chantry, N. A. Gregson and P. Glynn: A novel metalloproteinase associated with brain myelin membranes. Isolation and characterization. *J Biol Chem*, 264(36), 21603-7 (1989)

131. L. Howard, X. Lu, S. Mitchell, S. Griffiths and P. Glynn: Molecular cloning of MADM: a catalytically active mammalian disintegrin-metalloprotease expressed in various cell types. *Biochem J*, 317 (Pt 1), 45-50 (1996)

132. F. Colciaghi, B. Borroni, L. Pastorino, E. Marcello, M. Zimmermann, F. Cattabeni, A. Padovani and M. Di Luca: [alpha]-Secretase ADAM10 as well as [alpha]APPs is reduced in platelets and CSF of Alzheimer disease patients. *Mol Med*, 8(2), 67-74 (2002)

133. L. B. Gatta, A. Albertini, R. Ravid and D. Finazzi: Levels of beta-secretase BACE and alpha-secretase ADAM10 mRNAs in Alzheimer hippocampus. *Neuroreport*, 13(16), 2031-3 (2002)

134. A. Anders, S. Gilbert, W. Garten, R. Postina and F. Fahrenholz: Regulation of the alpha-secretase ADAM10 by its prodomain and proprotein convertases. *FASEB J*, 15(10), 1837-9 (2001)

135. F. Grams, R. Huber, L. F. Kress, L. Moroder and W. Bode: Activation of snake venom metalloproteinases by a cysteine switch-like mechanism. *FEBS Lett*, 335(1), 76-80 (1993)

136. F. Loechel, B. J. Gilpin, E. Engvall, R. Albrechtsen and U. M. Wewer: Human ADAM 12 (meltrin alpha) is an active metalloprotease. *J Biol Chem*, 273(27), 16993-7 (1998)

137. U. P. Shinde, J. J. Liu and M. Inouye: Protein memory through altered folding mediated by intramolecular chaperones. *Nature*, 389(6650), 520-2 (1997)

138. R. J. Peters, A. K. Shiau, J. L. Sohl, D. E. Anderson, G. Tang, J. L. Silen and D. A. Agard: Pro region C-terminus:protease active site interactions are critical in catalyzing the folding of alpha-lytic protease. *Biochemistry*, 37(35), 12058-67 (1998)

139. S. S. Sisodia: Beta-amyloid precursor protein cleavage by a membrane-bound protease. *Proc Natl Acad Sci U S A*, 89(13), 6075-9 (1992)

140. M. Citron, T. Oltersdorf, C. Haass, L. McConlogue, A. Y. Hung, P. Seubert, C. Vigo-Pelfrey, I. Lieberburg and D. J. Selkoe: Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature*, 360(6405), 672-4 (1992)

141. S. L. Kuentzel, S. M. Ali, R. A. Altman, B. D. Greenberg and T. J. Raub: The Alzheimer beta-amyloid protein precursor/protease nexin-II is cleaved by secretase in a trans-Golgi secretory compartment in human neuroglioma cells. *Biochem J*, 295 (Pt 2), 367-78 (1993)

142. S. Tomita, Y. Kirino and T. Suzuki: Cleavage of Alzheimer's amyloid precursor protein (APP) by secretases occurs after O-glycosylation of APP in the protein secretory pathway. Identification of intracellular compartments in which APP cleavage occurs without using toxic agents that interfere with protein metabolism. *J Biol Chem*, 273(11), 6277-84 (1998)

143. S. R. Sahasrabudhe, A. M. Brown, J. D. Hulmes, J. S. Jacobsen, M. P. Vitek, A. J. Blume and J. L. Sonnenberg: Enzymatic generation of the amino terminus of the beta-amyloid peptide. *J Biol Chem*, 268(22), 16699-705 (1993)

144. L. Hendriks, C. M. van Duijn, P. Cras, M. Cruts, W. Van Hul, F. van Harskamp, A. Warren, M. G. McInnis, S. E. Antonarakis, J. J. Martin and *et al.*: Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. *Nat Genet*, 1(3), 218-21 (1992)

145. P. W. Janes, N. Saha, W. A. Barton, M. V. Kolev, S. H. Wimmer-Kleikamp, E. Nievergall, C. P. Blobel, J. P. Himanen, M. Lackmann and D. B. Nikolov: Adam meets Eph: an ADAM substrate recognition module acts as a molecular switch for ephrin cleavage in trans. *Cell*, 123(2), 291-304 (2005)

146. J. Pruessmeyer and A. Ludwig: The good, the bad and the ugly substrates for ADAM10 and ADAM17 in brain pathology, inflammation and cancer. *Semin Cell Dev Biol*, 20(2), 164-74 (2009)

147. K. Reiss and P. Saftig: The "a disintegrin and metalloprotease" (ADAM) family of sheddases: physiological and cellular functions. *Semin Cell Dev Biol*, 20(2), 126-37 (2009)

148. D. R. Edwards, M. M. Handsley and C. J. Pennington: The ADAM metalloproteinases. *Mol Aspects Med*, 29(5), 258-89 (2008)

149. E. Jorissen, J. Prox, C. Bernreuther, S. Weber, R. Schwanbeck, L. Serneels, A. Snellinx, K. Craessaerts, A. Thathiah, I. Tesseur, U. Bartsch, G. Weskamp, C. P. Blobel, M. Glatzel, B. De Strooper and P. Saftig: The disintegrin/metalloproteinase ADAM10 is essential for the establishment of the brain cortex. *J Neurosci*, 30(14), 4833-44

150. R. M. Nitsch, B. E. Slack, R. J. Wurtman and J. H. Growdon: Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science*, 258(5080), 304-7 (1992)

151. C. Prinzen, U. Muller, K. Endres, F. Fahrenholz and R. Postina: Genomic structure and functional characterization of the human ADAM10 promoter. *FASEB J*, 19(11), 1522-4 (2005)

152. S. Lammich, D. Buell, S. Zilow, A. K. Ludwig, B. Nuscher, S. F. Lichtenthaler, C. Prinzen, F. Fahrenholz and C. Haass: Expression of the anti-amyloidogenic secretase ADAM10 is suppressed by its 5'-untranslated region. *J Biol Chem*, 285(21), 15753-60

153. S. Lammich, S. Schobel, A. K. Zimmer, S. F. Lichtenthaler and C. Haass: Expression of the Alzheimer protease BACE1 is suppressed via its 5'-untranslated region. *EMBO Rep*, 5(6), 620-5 (2004)

154. T. O'Connor, K. R. Sadleir, E. Maus, R. A. Velliquette, J. Zhao, S. L. Cole, W. A. Eimer, B. Hitt, L. A. Bembinster, S. Lammich, S. F. Lichtenthaler, S. S. Hebert, B. De Strooper, C. Haass, D. A. Bennett and R. Vassar: Phosphorylation of the translation initiation factor eIF2alpha increases BACE1 levels and promotes amyloidogenesis. *Neuron*, 60(6), 988-1009 (2008)

155. J. D. Buxbaum, K. N. Liu, Y. Luo, J. L. Slack, K. L. Stocking, J. J. Peschon, R. S. Johnson, B. J. Castner, D. P. Cerretti and R. A. Black: Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J Biol Chem*, 273(43), 27765-7 (1998)

156. F. Checler: Processing of the beta-amyloid precursor protein and its regulation in Alzheimer's disease. *J Neurochem*, 65(4), 1431-44 (1995)

157. J. D. Buxbaum, M. Oishi, H. I. Chen, R. Pinkas-Kramarski, E. A. Jaffe, S. E. Gandy and P. Greengard: Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc Natl Acad Sci U S A*, 89(21), 10075-8 (1992)

158. R. A. Nixon: Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. *Neurobiol Aging*, 26(3), 373-82 (2005)

159. D. Xu, C. Sharma and M. E. Hemler: Tetraspanin12 regulates ADAM10-dependent cleavage

of amyloid precursor protein. *FASEB J*, 23(11), 3674-81 (2009)

160. Y. Hiraoka, M. Ohno, K. Yoshida, K. Okawa, H. Tomimoto, T. Kita and E. Nishi: Enhancement of alpha-secretase cleavage of amyloid precursor protein by a metalloendopeptidase nardilysin. *J Neurochem*, 102(5), 1595-605 (2007)

161. R. Epis, E. Marcello, F. Gardoni, C. Vastagh, M. Malinverno, C. Balducci, A. Colombo, B. Borroni, H. Vara, M. Dell'Agli, F. Cattabeni, M. Giustetto, T. Borsello, G. Forloni, A. Padovani and M. Di Luca: Blocking ADAM10 synaptic trafficking generates a model of sporadic Alzheimer's disease. *Brain*, 133(11), 3323-35

162. E. Marcello, F. Gardoni, D. Mauceri, S. Romorini, A. Jeromin, R. Epis, B. Borroni, F. Cattabeni, C. Sala, A. Padovani and M. Di Luca: Synapse-associated protein-97 mediates alpha-secretase ADAM10 trafficking and promotes its activity. *J Neurosci*, 27(7), 1682-91 (2007)

163. H. S. Hoe, M. J. Cooper, M. P. Burns, P. A. Lewis, M. van der Brug, G. Chakraborty, C. M. Cartagena, D. T. Pak, M. R. Cookson and G. W. Rebeck: The metalloprotease inhibitor TIMP-3 regulates amyloid precursor protein and apolipoprotein E receptor proteolysis. *J Neurosci*, 27(40), 10895-905 (2007)

164. E. Kojro, P. Fuger, C. Prinzen, A. M. Kanarek, D. Rat, K. Endres, F. Fahrenholz and R. Postina: Statins and the squalene synthase inhibitor zaragozic acid stimulate the non-amyloidogenic pathway of amyloid-beta protein precursor processing by suppression of cholesterol synthesis. *J Alzheimers Dis*, 20(4), 1215-31 (2010)

165. E. Kojro, G. Gimpl, S. Lammich, W. Marz and F. Fahrenholz: Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. *Proc Natl Acad Sci U S A*, 98(10), 5815-20 (2001)

166. P. H. Kuhn, H. Wang, B. Dislich, A. Colombo, U. Zeitschel, J. W. Ellwart, E. Kremmer, S. Rossner and S. F. Lichtenthaler: ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. *EMBO J*, 29(17), 3020-32

167. M. L. Kim, B. Zhang, I. P. Mills, M. E. Milla, K. R. Brunden and V. M. Lee: Effects of TNFalpha-converting enzyme inhibition on amyloid beta production and APP processing *in vitro* and *in vivo*. *J Neurosci*, 28(46), 12052-61 (2008)

168. F. Fahrenholz: Alpha-secretase as a therapeutic target. *Curr Alzheimer Res*, 4(4), 412-7 (2007)

169. F. Mangialasche, A. Solomon, B. Winblad, P. Mecocci and M. Kivipelto: Alzheimer's disease: clinical trials and drug development. *Lancet Neurol*, 9(7), 702-16

170. J. A. Schneider, Z. Arvanitakis, S. E. Leurgans and D. A. Bennett: The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol*, 66(2), 200-8 (2009)

171. L. S. Schneider and D. K. Lahiri: The perils of Alzheimer's drug development. *Curr Alzheimer Res*, 6(1), 77-8 (2009)

172. M. S. Wolfe: Secretase as a target for Alzheimer's disease. *Curr Top Med Chem*, 2(4), 371-83 (2002)

173. S. Roggo: Inhibition of BACE, a promising approach to Alzheimer's disease therapy. *Curr Top Med Chem*, 2(4), 359-70 (2002)

174. G. Evin and V. B. Kenche: BACE inhibitors as potential therapeutics for Alzheimer's disease. *Recent Pat CNS Drug Discov*, 2(3), 188-99 (2007)

175. V. John: Human beta-secretase (BACE) and BACE inhibitors: progress report. *Curr Top Med Chem*, 6(6), 569-78 (2006)

176. I. Hussain, J. Hawkins, D. Harrison, C. Hille, G. Wayne, L. Cutler, T. Buck, D. Walter, E. Demont, C. Howes, A. Naylor, P. Jeffrey, M. I. Gonzalez, C. Dingwall, A. Michel, S. Redshaw and J. B. Davis: 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(3), 802-9 (2007)

177. T. Li, H. Wen, C. Brayton, F. M. Laird, G. Ma, S. Peng, L. Placanica, T. C. Wu, B. J. Crain, D. L. Price, C. G. Eberhart and P. C. Wong: Moderate reduction of gamma-secretase attenuates amyloid burden and limits mechanism-based liabilities. *J Neurosci*, 27(40), 10849-59 (2007)

178. A. Schmechel, M. Strauss, A. Schlicksupp, R. Pipkorn, C. Haass, T. A. Bayer and G. Multhaup: Human BACE forms dimers and colocalizes with APP. *J Biol Chem*, 279(38), 39710-7 (2004)

179. Y. Zhou, Y. Su, B. Li, F. Liu, J. W. Ryder, X. Wu, P. A. Gonzalez-DeWhitt, V. Gelfanova, J. E. Hale, P. C. May, S. M. Paul and B. Ni: Nonsteroidal anti-inflammatory drugs can lower amyloidogenic Abeta42 by inhibiting Rho. *Science*, 302(5648), 1215-7 (2003)

180. T. Yang, D. Arslanova, Y. Gu, C. Augelli-Szafran and W. Xia: Quantification of gamma-secretase modulation differentiates inhibitor compound selectivity between two substrates Notch and amyloid precursor protein. *Mol Brain*, 1(1), 15 (2008)

181. F. Chen, H. Hasegawa, G. Schmitt-Ulms, T. Kawarai, C. Bohm, T. Katayama, Y. Gu, N. Sanjo, M. Glista, E. Rogaeva, Y. Wakutani, R. Pardossi-Piquard, X. Ruan, A. Tandon, F. Checler, P. Marambaud, K. Hansen, D. Westaway, P. St George-Hyslop and P. Fraser: TMP21 is a presenilin complex component that modulates gamma-secretase but not epsilon-secretase activity. *Nature*, 440(7088), 1208-12 (2006)

182. A. Thathiah, K. Spittaels, M. Hoffmann, M. Staes, A. Cohen, K. Horre, M. Vanbrabant, F. Coun, V. Baekelandt, A. Delacourte, D. F. Fischer, D. Pollet, B. De Strooper and P. Merchiers: The orphan G protein-coupled receptor 3 modulates amyloid-beta peptide generation in neurons. *Science*, 323(5916), 946-51 (2009)

183. G. He, W. Luo, P. Li, C. Remmers, W. J. Netzer, J. Hendrick, K. Bettayeb, M. Flajolet, F. Gorelick, L. P. Wennogle and P. Greengard: Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease. *Nature*, 467(7311), 95-8

184. R. Visintin, S. Prinz and A. Amon: CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science*, 278(5337), 460-3 (1997)

185. M. Hooper: What do you think is a non-disease? Diet, lifestyle, exercise, spirituality, and the search for meaning are ignored at our peril. *BMJ*, 324(7342), 912 (2002)

186. A. A. Davis, J. J. Fritz, J. Wess, J. J. Lah and A. I. Levey: Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology *in vitro* and *in vivo*. *J Neurosci*, 30(12), 4190-6

187.E. R. Siemers, J. F. Quinn, J. Kaye, M. R. Farlow, A. Porsteinsson, P. Tariot, P. Zoulnouni, J. E. Galvin, D. M. Holtzman, D. S. Knopman, J. Satterwhite, C. Gonzales, R. A. Dean and P. C. May: Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology*, 66(4), 602-4 (2006)

188. M. Citron: Alzheimer's disease: strategies for disease modification. *Nat Rev Drug Discov*, 9(5), 387-98 (2010)

Abbreviations: aa: Amino Acids, Abeta: Amyloid peptide, AChEIs: Acetylcholinesterase Inhibitors, AD: Alzheimer's Disease , ADAM: A Disintegrin And Metalloproteinase, AICD: APP Intracellular Domain, APH1: Anterior Pharynx Defective 1, APLP: APP Like Proteins, APP: Amyloid Precursor Protein, APPSwe: APP with Swedish familiar AD causing mutation, BACE: Beta Site APP Cleaving Enzyme, BBB: Brain Blood Barrier, CHO: Chinese Hamster Ovary, CNS: Central Nervous System, COX: CicloOxigenase, CTF: Carboxy terminal Fragment, EGF: Epidermal Growth Factor, ER: Endoplasmic Reticulum, INF: Interferon, GGA: Golgi-Localized Gamma-ear containing ADP, HEK: Human Embryonic Kidney 293, MMP: Metalloprotease, NCT: Nicastrin, NTF: N-Terminal fragment, NSAIDs: Non-steroidal Anti-inflammatory Drugs, PACAP: Pituitary Adenilate Cyclase Activating Polypeptide, PEN-2: Presenilin Enhancer 2, PLC: Phospholipase C, PKC: Protein Kinase C, PMA: Phorbol-12-Myristate-13-Acetate, PS: Presenilin, RNAi: RNA interference, SALA: Selective Abeta lowering agents, SH-SY5Y: Human derived Neuroblastoma, STAT: Signal Traducer and activator of transcription, TNF: Tumor Necrosis factor, TGN: Trans Golgi Network, TMD: Trans Membrane Domain, TSPAN: Tetraspannin

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