

**ALZHEIMER'S DISEASE: THE MOLECULAR CLOCKWORK
OF A TICKING TIME BOMB**

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Keywords: Alzheimer's disease / amyloid β -peptide / secretases / processing

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SUMMARY

Alzheimer's Disease is by far the most frequent age dependent neurodegenerative disorder and heavily threatens public health in our senescing society. Cure is desperately required, however conventional symptomatic attempts failed. In contrast, novel mechanism based therapeutic approaches are now providing strong hope that progression of Alzheimer's disease may be slowed or even prevented. These novel therapeutic approaches are based on the identification of a variety of key enzymes, the secretases, which allow the proteolytic generation of the disease characterizing Amyloid β -peptide.

The amyloid cascade

It is precisely 100 years ago that *Auguste D.* reported to Alois Alzheimer, a German psychiatrist in Frankfurt, with her famous words: "I lost myself". This is probably the best description of the disease symptoms, a slow progression of memory loss. As the major risk factor for AD is aging, one must expect a sharp increase of the number of patients in the near future.

Based on the abundant deposition of senile plaques and neurofibrillar tangles, a cascade of events was described, which is now known as the Amyloid Cascade (Hardy and Selkoe, 2002). Amyloid stands for Amyloid β -peptide (A β) and describes a highly hydrophobic peptide, which aggregates to form small oligomers (Glennner and Wong, 1984). These oligomers are still soluble and may cause first detrimental effects on memory (Walsh et al., 2002). If these oligomers aggregate further they start forming fibers, which eventually precipitate and accumulate in the disease defining amyloid plaques (Fig. 1). While the fibers are deposited in the brain

parenchyma additional toxic aggregation events appear to be induced within the cell. Tau, a protein, which stabilizes microtubules dissociates from the cytoskeleton and aggregates within the soma of neurons to form the intracellular tangles (Mandelkow and Mandelkow, 1998) (Fig. 1). This process may then initiate a variety of less understood toxic insults, ultimately leading to the tremendous neuronal loss observed in AD brains (Gotz et al., 2001). This process, which appears to be initiated by the oligomerization of A β (Gotz et al., 2001) is now known as the amyloid cascade (Aguzzi and Haass, 2003; Hardy and Selkoe, 2002).

Physiological generation of A β

Surprisingly and against all predictions, A β was found in the early 90s to be a physiologically normal metabolite generated even by healthy persons (Busciglio et al., 1993; Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992). This finding not only predicted a very high age dependent risk for AD due to continuous A β accumulation over time, but also immediately provided the field with a desperately required tool to investigate A β generation at the cellular and molecular level and to screen for A β lowering drugs (Haass and Selkoe, 1993). This was possible due to the finding that even simple cultured cells of almost any type produce and secrete A β under completely physiological conditions. Moreover, even non-neuronal cells could be used to study the central aspect of AD, A β generation, since enzymes and mechanisms involved are identical to those in neurons (Haass et al., 1992). Such cultured cells were subsequently used to screen thousands of compounds for amyloid lowering drugs. Indeed, as described below, numerous amyloid lowering drugs have now been identified with the help of this finding and some of them are currently investigated already in human trials. Thus, the finding of A β generation under

physiological conditions in cultured cells started a new age in modern AD research and paved the route to drug development.

A β is generated by two consecutive proteolytic cleavages

Generation of A β occurs by processing of the β -amyloid precursor protein (APP) via proteases called secretases. Three secretases are known, α -, β -, and γ -secretase (Haass and Selkoe, 1993). While β -, and γ -secretase mediate the amyloidogenic cleavage events (Fig. 1), α -secretase on the contrary prevents A β generation by cleaving APP in the middle of the A β domain. Before describing the molecular and cellular properties of the amyloidogenic secretases, I will first introduce the pathological consequences of AD associated mutations in the human genome.

Subtle changes in A β generation are sufficient to cause very aggressive forms of AD

In very rare cases, familial AD (FAD) is inherited in a familial autosomal dominant manner and can then occur at a very young age. Although these mutations are indeed not abundant at all and certainly account only for a very small minority of AD cases, they tremendously helped us to understand disease pathology and mechanisms of sporadic cases (Haass, 1996; Haass and Baumeister, 1998). Accordingly, in FAD cases mutations in three genes, APP itself and the two homologous Presenilins PS1 and PS2, cause at the end increased production of the two amino acids longer A β 42 peptide (Citron et al., 1997; Duff et al., 1996; Suzuki et al., 1994);summarized in (Haass, 1996)). A β 42 aggregates much faster, and consequently causes a much earlier onset of the disease (Hardy and Selkoe, 2002).

Moreover, since small changes in the total amount of A β are already sufficient to significantly lower the age of onset in FAD cases, one may even speculate that A β metabolism in human brains could be associated with a risk late onset AD. Indeed, two major A β degrading enzymes were identified, Neprilysin (Nep) (Iwata et al., 2000) and the Insulin degrading enzyme (IDE) (Qiu et al., 1998). In fact gene knock down of Nep and IDE are sufficient to increase A β deposition in transgenic models of AD pathology (Iwata et al., 2001; Leissring et al., 2003). Furthermore, genetic linkage of late onset AD on chromosome 10 (Bertram et al., 2000; Ertekin-Taner et al., 2000) may also point to at least one of the two enzymes, namely IDE. All these findings thus strongly suggest, that A β generation is the heart of AD pathology. If one wants to slow A β generation and its accumulation and deposition, one must therefore try to block the A β generating secretases. These enzymes are now identified and their molecular characterization is now sophisticated enough to allow the design a target oriented treatment.

β -Secretase is a membrane bound aspartyl protease

β -secretase (also called BACE-1 for β -site APP cleaving enzyme) was identified as a type 1 transmembrane protein containing an aspartyl protease activity (Lin et al., 2000; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999) reviewed in (Vassar and Citron, 2000). BACE belongs to the pepsin family of aspartyl proteases, and defines a novel subgroup of membrane-associated hydrolases. BACE-1 mediates the primary amyloidogenic cleavage of APP and generates the membrane bound 99 amino acid APP C-terminal fragment (APPC99), which is the immediate precursor for the intramembraneous γ -secretase cleavage (Fig. 1).

BACE-1 is clearly the only protease with a well-defined β -secretase activity. This was unambiguously shown by the homozygous knockout of the BACE-1 gene, which does not allow any A β generation anymore (Cai et al., 2001; Luo et al., 2001). Furthermore, increased BACE-1 expression in transgenic mouse models for AD pathology displayed an enhanced and earlier A β deposition, which again is consistent with the notion that BACE-1 is “*the*” β -secretase (Bodendorf et al., 2002; Willem et al., 2004).

A close homologue of BACE-1, BACE-2, was identified as well (Vassar, 2001); however, BACE-2 exhibits an α -secretase-like activity, which cleaves APP in the middle of the A β -domain at amino acid 19 and 20 (Farzan et al., 2000; Fluhner et al., 2002). Thus, BACE-2 does not contribute to the amyloidogenic processing of APP, which is consistent with the complete lack of A β generation in a BACE-1 knockout.

BACE-1 is generated as a *preproenzyme*. Upon removal of the signal peptide, the prodomain is cleaved by a furin-like protease (Bennett et al., 2000; Capell et al., 2000). BACE-1 is then targeted through the secretory pathway (Capell et al., 2000) to the plasma membrane and clusters within lipid rafts (Ehehalt et al., 2003). APP processing by BACE-1 also occurs preferentially within lipid rafts (Ehehalt et al., 2003). Consistent with the finding of BACE-1 in lipid rafts, BACE-1 is sorted apically in polarized cells (Capell et al., 2002), the side where rafts are thought to accumulate. At the plasma membrane BACE-1 (like APP) can be internalized to endosomes (Huse et al., 2000; Walter et al., 2001). Thus BACE-1 and APP follow very similar trafficking routes and meet within endosomes, which may be the preferential site of BACE-1 activity due to its acidic pH optimum (Vassar et al., 1999).

Little is known about the physiological substrates of BACE-1. As described above, BACE-1 has only limited access to basolaterally sorted APP in polarized cells, thus suggesting that APP is not the main substrate. This is supported by the finding that APP does not bear the optimal cleavage site for BACE-1 (Liu et al., 2002). In fact one of the FAD associated mutations in APP (the so called Swedish mutation) strongly enhances BACE-1 cleavage of APP simply by creating an "optimized" cleavage site (Cai et al., 1993; Citron et al., 1992; Haass et al., 1995). Besides APP, additional candidate BACE-1 substrates were identified recently; these include P-selectin glycoprotein ligand-1 (Lichtenthaler et al., 2003) and the sialyl-transferase ST6Gal I (Kitazume et al., 2001). Nevertheless, the biological function of BACE-1 remains unclear and may be difficult to address since no obvious phenotype is observed upon removal of BACE-1 from the mouse genome. However, very recently changes in the life span of BACE-1/BACE-2 double knock out mice associated with electrophysiological changes in the steady state inactivation of voltage-gated sodium channels were observed, which may help to elucidate the biological function (Dominguez et al., 2005). In addition recent observations from my laboratory demonstrate a striking behavioral phenotype upon knock down of BACE-1 in zebrafish (Schmidt and Haass, unpublished observation), whose morphological analysis may soon lead to an understanding of BACE-1 function.

Most interestingly, evidence exists that BACE-1 expression is significantly enhanced in brains derived from patients with sporadic AD (Yang et al., 2003). The reasons for the enhanced BACE-1 expression are currently unclear. However, in this regard it is tempting to speculate that AD associated stressors such as oxidative stress, radicals, unfolded proteins, head trauma and others may induce BACE-1 transcription, and/or expression/activity during aging. In that regard it is also very

interesting to note that translation of BACE-1 is strongly repressed by its 5' untranslated region (UTR) (Lammich et al., 2004). One may thus speculate that increased BACE-1 expression in the absence of changes in the mRNA levels may be due to a regulative mechanisms associated with the 5' UTR. This is currently a topic of intense investigation.

γ -Secretase, a complex of four proteins

Convergent evolutionary development of intramembrane proteolysis

BACE-1 generates the immediate precursor for γ -secretase (Haass and Selkoe, 1993), which then liberates A β into biological fluids via a very unusual proteolytic cleavage, since the physiological production of A β described above predicts that the final cleavage of the amyloidogenic precursor must occur within the membrane without any previous damage. In fact, since intramembrane proteolysis was thought to be biochemically impossible scientists failed to search for a long time for normal A β generation. Thus, this is a very striking example how dangerous such dogmas can be for scientific progress. However, recent evidence now strongly suggests the convergent evolution of a number of polytopic proteases, which are all able to cleave proteins inside the hydrophobic environment of a lipid bilayer. In fact, now intramembrane proteolysis is known to be mediated by a class of novel polytopic proteases, which have their active centers located within hydrophobic transmembrane domains (Haass and Steiner, 2002; Steiner and Haass, 2000). Members of these proteases include the site-two-protease (S2P) (Brown et al., 2000), rhomboids (Lee et al., 2001; Urban et al., 2001), γ -secretase (Steiner et al., 2000; Wolfe et al., 1999), and signal peptide peptidase (SPP) (Weihofen et al., 2002; Weihofen and Martoglio, 2003). While S2P and rhomboids belong to the class of metallo- and serine proteases

respectively, γ -secretase and SPP are aspartyl proteases. Intramembrane cleaving cysteine proteases have so far not been identified.

Presenilins

The two homologous presenilins (the name suggests that they cause pre-senile dementia), PS1 and PS2 (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995), are of exceptional importance for the γ -secretase cleavage. This became obvious many years ago when the genetic linkage of PSs to numerous familial AD cases was discovered (Sherrington et al., 1995). More than 100 autosomal dominant PS point mutations have now been identified, which all cause aggressive early onset AD by the same mechanism. In some severe cases the mutations can cause dementia even in the 20s. These mutations influence the γ -secretase cleavage event by simply shifting it two amino acids to the C-terminus (Citron et al., 1997; Duff et al., 1996); reviewed in (Steiner and Haass, 2000). The subsequent increase of the A β 42 to A β 40 ratio results in the enhanced oligomerization of the highly amyloidogenic A β 42. This already suggests that PSs must be directly involved in the γ -secretase cleavage event or even be the γ -secretase itself, although this took quite some time to be appreciated by scientists in the field. A direct role of PS in A β generation was supported by the pivotal observation of De Strooper and colleagues, who found that a PS1 knockout severely reduced A β generation (De Strooper et al., 1998). Moreover, upon inactivation of the second PS gene (PS2) no A β generation was observed at all (Herreman et al., 2000; Zhang et al., 2000). It should be noted that contradictory reports suggesting some A β generation in the absence of PS1 and PS2 turned out to be not correct.

Direct evidence for PS presenting the catalytic core of γ -secretase came from the finding that all PSs contain two functionally important and highly conserved aspartate residues within TMD 6 and 7 (Wolfe et al., 1999) (Fig. 1). This was of great interest, since biochemical evidence suggested that γ -secretase belongs to the aspartyl protease family. Do these aspartates build up the catalytic center of the γ -secretase complex? When either of the two aspartates was mutagenized, A β generation was indeed abolished (Wolfe et al., 1999). Moreover, PSs are cleaved by endoproteolysis within the large cytoplasmic loop (Fig. 1) (Thinakaran et al., 1996). This cleavage results in two fragments (the N-terminal (NTF) and the C-terminal (CTF) fragment), which remain bound to each other (Capell et al., 1998). Upon mutagenesis of either one of the two critical aspartates, this cleavage event does not occur anymore and PS accumulated as a holoprotein (Wolfe et al., 1999). This phenomenon would be consistent with the holoprotein being a zymogene, which is activated by autoproteolysis (Wolfe et al., 1999). Very similar findings were found for PS2 (Kimberly et al., 2000; Steiner et al., 1999) as well and thus fully confirmed the initial observation by Wolfe and colleagues. Another piece of evidence that PSs provide the catalytic core for the aspartyl protease activity of the γ -secretase complex is that γ -secretase inhibitors can be directly cross-linked to PS (Esler et al., 2000; Li et al., 2000). However, in contrast to BACE-1 and all other conventional aspartyl proteases, PSs lack the D(T/S)G(T/S) aspartyl protease active site: instead, they contain a GxGD motif around the critical aspartate in TM7 (Haass and Steiner, 2002; Steiner et al., 2000). Most interestingly, this motif is fully conserved between presenilins, the type-4 prepilin peptidases (LaPointe and Taylor, 2000; Steiner et al., 2000), and the signal peptide peptidases and now defines the novel group of GxGD-type aspartyl proteases (see below) (Haass and Steiner, 2002; Weihofen and Martoglio, 2003). This supports

the hypothesis described above, namely the convergent evolution of a novel active site for a common problem: intramembrane proteolysis.

Obviously, PSs did not evolve to produce the deleterious A β peptide. In fact, A β generation seems to be a rather unfortunate byproduct of an otherwise highly important biological function of PSs. A rather paradoxical function of PS became evident, when PSs were eliminated in mice. The knock out of PS, a gene whose function is directly related to AD via A β generation, causes an early embryonic phenotype, which closely resembles that of a Notch knock out (De Strooper et al., 1999). This finding strongly suggested that Notch may be a pivotal substrate for γ -secretase activity in vivo and also predicted an intramembrane cleavage, which may be associated with a function in nuclear signaling. Indeed, the function of the intramembrane cleavage of Notch is now well understood. Intramembrane cleavage of Notch is required to liberate the Notch intracellular domain (ICD) for nuclear signaling and subsequent regulation of target gene transcription (summarized by (Selkoe and Kopan, 2003).

A number of additional substrates of presenilins have also been identified besides APP and Notch (De Strooper, 2003). These include the APP homologues APLP-1 and -2, ErbB-4, E-cadherin, N-cadherin, LRP, Nectin-1- α , the Notch ligands Delta and Jagged, and CD44. Interestingly, intramembrane cleavage can also negatively regulate nuclear signaling. Robakis and colleagues (Marambaud et al., 2003) demonstrated that the ICD generated from N-cadherin functions as a potent repressor of CBP/CREB-mediated transcription. Moreover, generation of the ICD was reduced by FAD mutations. Interestingly, there is also evidence that the APP intracellular domain interacts with the nuclear adaptor protein Fe65 and with the histone acetyltransferase Tip60 to form a transcriptionally active complex (Cao and

Südhof, 2001). Similar to the effects of FAD mutants on ICD generation of N-cadherin, some FAD mutations affect the generation of the APP and the Notch intracellular domain (Moehlmann et al., 2002). This suggests a loss of function (in signaling) in addition to the pathological gain of function related to A β 42 generation. Recently it was also shown that Nephrilysin expression appears to be under the control of AICD (Pardossi-Piquard et al., 2005), although some controversies appear to emerge (Hass and Yankner, 2005). Thus it still remains to be unequivocally proven that AICD and many other ICDs generated by γ -secretase are indeed involved in nuclear signaling. It seems to be equally plausible that γ -secretase is predominantly involved in the removal of protein fragments left over in the membrane after shedding of their extracellular domains. Furthermore, from recent data observed by Leyssen et al. (Leyssen et al., 2005) one may conclude that γ -secretase cleavage of APP C-terminal fragments may also be required to terminate signaling via a membrane bound fragment. This becomes apparent during APP induced neurite arborization upon brain damage. In this case even APP CTFs can mediate signaling for neurite outgrowth, but surprisingly this event is independent of γ -secretase activity.

A presenilin dependent dual cleavage mechanism

At least two intramembraneous cleavage events are required to liberate ICDs (Fig. 2) and their corresponding A β or A β -like peptide (Okochi et al., 2002; Sastre et al., 2001). One cleavage event occurs within the middle of the TM domains (at amino acids 40 or 42). Other cleavages can occur close to the cytoplasmic border of the TM domain and are required to liberate ICD. It is possible that hydrophobic TMDs prevent efficient liberation of ICDs generated by the cleavage in the middle of the membrane, and thus require a second cleavage event to remove this “sticky” domain.

Both cleavage events appear to occur almost simultaneously and are fully PS dependent (Sastre et al., 2001). However, very recent results implicate that there may indeed be sequential cleavages. In this work it is proposed that a total of three cleavages are required to finally liberate A β 40 or A β 42. These cleavages begin at the cytoplasmic border (the ϵ -cleavage) and are followed by a novel ζ -cut roughly in the middle of ϵ - and γ -cleavages (Qi-Takahara et al., 2005; Zhao et al., 2005; Zhao et al., 2004) (Fig. 2). Only, when these initial ϵ - and ζ -cleavages take place, A β seems to be finally released by the γ -secretase cleavage. Although this model predicts that γ -secretase is strictly dependent on ζ/ϵ -cleavage, other data demonstrate A β generation in the absence of ϵ -cleavage (Moehlmann et al., 2002). Thus clearly more work is required, to investigate the role of the individual cuts in ICD liberation.

Nct, APH-1 and PEN-2

PSs are not sufficient for γ -secretase activity, but rather require additional cofactors to form a multi-protein high molecular weight complex (De Strooper, 2003). Biochemical purification of this complex led to the identification of nicastrin (Nct) (Yu et al., 2000). The remaining components of the complex were isolated by genetic screens for enhancers of a PS-dependent Notch deficient phenotype in *Caenorhabditis elegans*. This led to the identification of two additional components: APH-1 (anterior pharynx-defective phenotype) and PEN-2 (PS-enhancer) (De Strooper, 2003; Francis et al., 2002; Goutte et al., 2002). When all four components were expressed together in *Saccharomyces cerevisiae*, an organism, which lacks any endogenous γ -secretase activity, fully active γ -secretase was reconstituted (Edbauer et al., 2003).

Although this quartet of proteins is clearly sufficient to reconstitute full γ -secretase complex activity, additional regulatory factors, which are not intrinsic γ -

secretase complex components, are expected. In fact, recently CD 147 a putative inhibitor of γ -secretase was identified (Zhou et al., 2005).

PS expression and γ -secretase activity are regulated in a coordinated manner. Because overexpression of PS does not lead to the expression of higher levels of PS fragments (Haass and Steiner, 2002), nor to enhanced γ -secretase activity, a limiting factor regulating γ -secretase activity was postulated. Maybe there is no defined limiting factor: instead, the least abundant γ -secretase complex component, be it PS, PEN-2, APH-1, or Nct, may become limiting. If there is a disequilibrium of any of these components, excess amounts are destabilized or, in the case of Nct, fail to mature (De Strooper, 2003).

Although the composition and the biological function of the γ -secretase complex seem to be largely solved, Nct appears to be the only component whose function is now clarified. Obviously, the lack of knowledge about the function of PEN-2 and APH-1 may be due to the difficulties in analyzing these components individually in the absence of the other partners. This is different for Nct, which by itself can be expressed and was recently shown to be the receptor for γ -secretase substrates (Shah et al., 2005). Interestingly, during evolution an amino peptidase-like domain was preserved in Nct, which contains the site for substrate recognition. Since the complete (Shirovani et al., 2003) luminal domain of Nct bound to the membrane (Morais et al., 2003) is required for γ -secretase activity within the complex, it was tempting to speculate that this globular, tightly folded ectodomain is also responsible for measuring the length of the membrane-retained stub-like substrates. Such a function must be postulated for one of the γ -secretase complex components, because substrates are only recognized and proteolyzed if their ectodomain was previously truncated (Struhl and Adachi, 2000).

ER retention and γ -secretase complex assembly

Cellular assembly of the γ -secretase complex was investigated indirectly by knocking down individual components. In the absence of PEN-2, PS is stabilized as an uncleaved holoprotein probably with the help of APH-1 and Nct (Luo et al., 2003; Takasugi et al., 2003). Thus APH-1 may be a stabilizer of the PS holoprotein, whereas PEN-2 is apparently required to initiate endoproteolysis of PS (Hu and Fortini, 2003; Luo et al., 2003; Takasugi et al., 2003). These findings may give some insights into the assembly of the γ -secretase complex. Since Nct is fairly stable in the ER it is likely that it may be the scaffold for a second binding partner. APH-1 would then be recruited to Nct, as evidenced by the identification of a putative Nct/APH-1 precomplex (LaVoie et al., 2003; Morais et al., 2003). Binding of PS to the APH-1/Nct complex would then result in the formation of a trimeric complex (Takasugi et al., 2003). Finally, PEN-2 joins the complex, thus allowing PS endoproteolysis and the eventual activation of the γ -secretase activity (Hu and Fortini, 2003; Takasugi et al., 2003). However, this is all still quite speculative, since it has so far proved impossible to monitor the assembly of the complex *in vivo*. Interestingly, recent findings support this model and suggest complex assembly within the ER (Capell et al., 2005; Kim et al., 2004). Unincorporated PS is retained within the ER by an ER retention signal (Kaether et al., 2004). This signal appears to be hidden upon binding other complex components. Under these conditions, PS assembled into a native γ -secretase complex is released from the ER. The complex is then targeted to its sites of biological activity at the plasma membrane and endosomes (Kaether et al., 2002; Kaether and Haass, manuscript submitted).

SPP and SPPLs, the cousins of γ -secretase?

How unique is γ -secretase? We already discussed above that the C-terminal critical aspartate of PSs is located within a conserved GxGD motif (Steiner et al., 2000), whereas the N-terminal aspartate is embedded within a YD sequence segment. The GxGD signature motif is highly conserved in SPP, an unrelated polytopic aspartyl protease (Martoglio and Golde, 2003; Weihofen et al., 2002; Weihofen and Martoglio, 2003; Wolfe and Kopan, 2004) as well as in the type-four prepilin peptidases (TFPP) (LaPointe and Taylor, 2000; Steiner et al., 2000). Moreover, mutagenesis of the corresponding aspartate residue blocks proteolytic activity of SPP (Weihofen et al., 2002), PS1 (Wolfe et al., 1999), PS2 (Kimberly et al., 2000; Steiner et al., 1999), and TFPP (LaPointe and Taylor, 2000). This strongly suggests that the aspartate within the GxGD motif comprises the C-terminal active site of these proteases. Furthermore, SPP can also be targeted with highly specific γ -secretase inhibitors demonstrating the similarity of the catalytic centers of SPP and γ -secretase (Kornilova et al., 2003; Nyborg et al., 2004; Weihofen et al., 2003). Further similarities are observed between PSs, SPP, and TFPPs, which also include a PxL motif within the C-terminal domain. Although these are striking similarities of SPP and γ -secretase, SPP appears to act independent of other supportive factors such as Nct, APH-1 or PEN-2. It rather seems to interact with itself to form functionally active homo dimers.

SPP is required for the removal of signal peptides (Weihofen and Martoglio, 2003). In addition, SPP is also involved in immune surveillance and processing of the Hepatitis C viral core protein (Weihofen et al., 2002) suggesting a more general role of SPP in the liberation of bioactive peptides (Martoglio and Golde, 2003; Weihofen et al., 2002; Weihofen and Martoglio, 2003; Wolfe and Kopan, 2004). Like γ -

secretase this function appears to require a previous proteolytic cut, which removes parts of the upstream protein sequences. However, in contrast to γ -secretase substrates, which only cut type-1 oriented proteins, SPP cleaves substrates in type-2 orientation.

Besides SPP a family of homologous proteins was identified by database searches (Grigorenko et al., 2002; Martoglio and Golde, 2003; Ponting et al., 2002; Weihofen et al., 2002; Weihofen and Martoglio, 2003). These proteins were named SPPL (SPP-like) 2(a,b,c), and 3 (in yeast an additional SPPL, SPPL4, exists) (Weihofen et al., 2002) or PSHs 1-5 (Ponting et al., 2002). It now appears that all members of the SPP family are GxGD-type proteases. Moreover, at least SPPL2a and SPPL2b are not located within the ER, where one would expect signal peptide clearing proteases (Krawitz et al., 2005). Thus, these proteases may have completely novel and unexpected functions in signaling.

Taken together SPP and SPPLs seem to be relative close relatives of γ -secretase, although some rather striking differences occur in terms of co-factor requirement and substrate orientation. Maybe these differences can be used in a comparative study to elucidate the function of APH-1 and Pen-2 for γ -secretase mediated proteolysis.

Amyloid lowering drugs

Having identified all components required for γ -secretase function, it should be rather straightforward to interfere with A β production with the help of selective inhibitors. Several high affinity inhibitors are indeed available and significantly reduce the A β burden in animal models. However, some concern remains. γ -Secretase is required for Notch signaling (and most likely for several other signaling

pathways as well). Accordingly, deleterious site effects have been observed *in vivo* (Geling et al., 2002; Hadland et al., 2001). However, as for any other novel drug one needs to define a therapeutic window, which allows some γ -secretase activity for signaling but reduces A β generation significantly enough to slow aggregation and deposition. In addition some non-steroidal anti-inflammatory drugs (NASIDS) selectively lower A β 42 generation (Weggen et al., 2001). This results in a consecutive increase of a truncated 38 amino acid A β (which appears not to be amyloidogenic) and this switch of cleavage specificity does not affect Notch signaling via its ICD. Mechanistically, this seems to occur via allosteric changes of PS within the γ -secretase complex (Lleo et al., 2004). These alternative γ -secretase modulators are currently under intense investigation and human trials are on the way.

BACE-1 is another obvious target for A β lowering drugs. In contrast to γ -secretase, BACE-1 can be fully ablated in mice without causing an obvious deleterious phenotype. Thus, interfering with BACE-1 may not result in unwanted site effects although as described above caution is still required, since we have not yet identified the physiological function of BACE-1. Based on the three-dimensional structure of BACE-1 (Hong et al., 2000), peptidomimetic and nonpeptidomimetic inhibitors have been generated (Hong et al., 2000; Vassar, 2001). These need to be developed further to improve their affinity with the rather large active site cleft of BACE-1 before they can be used in human trials. But highly sensitive inhibitors will certainly be available in the near future.

Thus, due to the enormous increase in our knowledge on secretase mediated A β generation a large selection of inhibitors have now been generated and tested *in vivo*. I strongly believe that it will only be a matter of time until the first drugs based on these inhibitors can be used to reduce the amyloid burden in humans. Such a

striking success would have been impossible without intensive research on A β production and secretase biology. When I entered the field in 1990 we did not even think about treatment, in fact I believed that it will take many decades until we have collected enough knowledge to allow the design of a mechanism based therapy. To my opinion AD research is therefore extremely successful and can serve as a spectacular example for the benefits of modern biomedical research.

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Figure Legend:

Figure 1

Generation of A β from APP via proteolytic processing by β -, and γ -secretase.

(1.) APP is cleaved by BACE-1 to generate C99, the immediate precursor for A β generation. (2.) C99 docks to the γ -secretase complex by binding to Nct. Nct binds to C99 via its free N-terminus (asterisks). (3) C99 is transferred to the catalytic center of the γ -secretase provided by the two critical aspartate residues in transmembrane domains 6 and 7 (4.). (5.) Upon cleavage the intracellular domain (ICD) is liberated into the cytoplasm and may (at least on the case of the Notch ICD) signal to the nucleus (6.). (7.) A β is secreted into biological fluids and rapidly forms oligomeric aggregates (8.). (9.) Upon further fibrillization, A β becomes deposited into the disease defining amyloid plaques. These events initiate the amyloid cascade resulting in additional intracellular aggregates of the tau protein, which then form tangles (the black structures surrounding the amyloid plaque).

Fig. 2: Sequential intramembrane cleavage of C99 at the ϵ -, ζ -, $\square\square\square$ γ -cleavage sites.