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Amyloid-beta precursor protein processing in neurodegeneration

Valérie Wilquet and Bart De Strooper

The amyloid-β precursor protein is proteolytically cleaved by secretases, resulting in a series of fragments, including the amyloid-β peptide of Alzheimer's disease. The amyloid precursor protein, when membrane anchored, could operate as a receptor. After cleavage, the soluble ectodomain exerts a trophic function in the subventricular zone. The amyloid-β peptide itself has a depressant role in synaptic transmission, with both physiological and pathological implications.
During the past two years, much time has been invested in determining the molecular pathways that regulate the processing and the signal transduction of the amyloid precursor protein. However, the absence of consistent and informative phenotypes in different loss of function animal models make elucidating the molecular actions of the amyloid-β precursor protein an ongoing challenge.

Addresses
Laboratory for Neuronal Cell Biology and Gene Transfer, K.U. Leuven and VIB, Department of Human Genetics, Herestraat 49, 3000 Leuven, Belgium
e-mail: bart.destrooper@med.kuleuven.ac.be

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Abbreviations

Aβ	amyloid-β peptide
AD	Alzheimer's disease
AICD	APP intracellular domain
APP	amyloid-β precursor protein
APPs	secreted ectodomain of APP
CTF	carboxy terminal fragment
LRP	LDL receptor-related protein

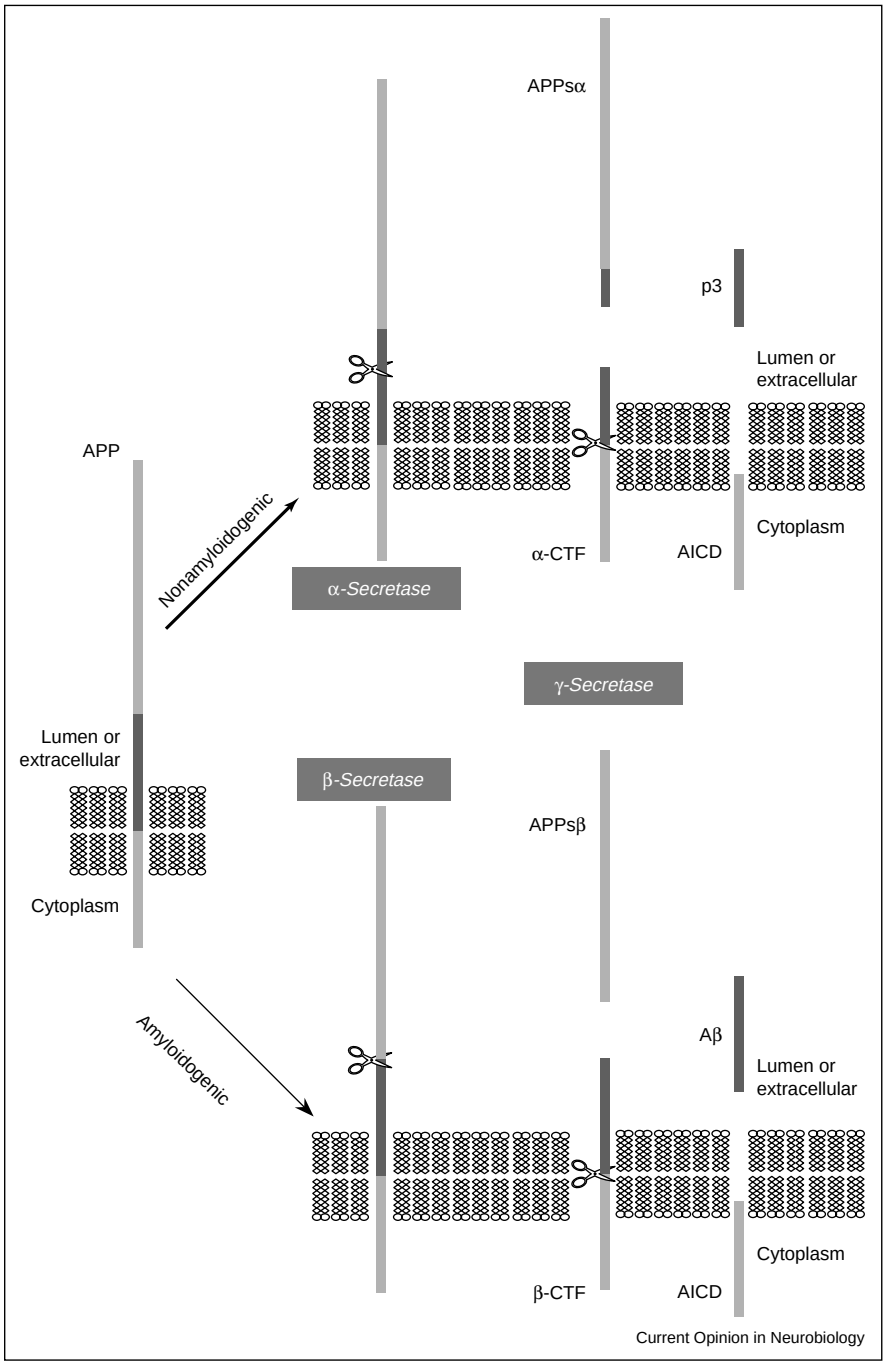
Introduction

Changes in the generation or the degradation of the amyloid-β peptide (Aβ) are, according to the amyloid hypothesis, the triggering molecular events in the pathogenic cascade of Alzheimer's disease (AD) [1]. Aβ is a proteolytic fragment cleaved from the amyloid-β precursor protein (APP) by two proteases, β- and γ-secretase. A third secretase, α-secretase, cleaves the Aβ sequence itself and is therefore usually considered as nonamyloidogenic (Figure 1). α-Secretase (a member of the ADAM family of metalloproteases) and β-secretase (a membrane-bound aspartyl protease also called BACE) cleave the ectodomain of APP, resulting in the shedding of APPsα and APPsβ. γ-Secretase finally cleaves the transmembrane domain of the APP carboxy terminal fragments (α-CTF and β-CTF), releasing the p3 and the Aβ peptide, respectively, into the extracellular milieu and the APP intracellular domain (AICD) into the cytoplasm (Figure 1). The strongest evidence that abnormal proteolytic processing and increased Aβ generation are central to the disease process comes from studies of very rare inherited forms of AD [1]. Missense mutations in APP and in the presenilins, the core proteins of the γ-secretase complex, all affect Aβ generation, aggregation or degradation [2•]. Moreover, all mutations identified in APP are clustered around the three secretase cleavage sites (see Figure 2). Although the cause(s) of sporadic AD is (are) unknown, it is assumed that external factors could affect Aβ production or degradation at several levels. Obviously, a better understanding of the proteolytic processing and the biological significance of APP processing events are major issues in AD research. Previous reviews have discussed APP structure, posttranslational modifications and APP-interacting proteins [3,4]. We focus here on more recent insights into the function of the proteolytic fragments of APP and their relevance for our understanding of AD pathogenesis.

The biological function of the amyloid-β precursor protein and its proteolytic fragments

APP can be considered as a membrane-anchored receptor. Only recently, however, a candidate ligand for APP_{695} (the predominant isoform in the brain; 695 refers to the number of amino acids encoded by the transcript) was identified. F-spondin, a secreted neuronal glycoprotein thought to function in axonal path finding and neuronal regeneration, binds to APP (and its closely related family members APLP-1 and -2). The interaction with F-spondin apparently inhibits β-secretase and AICD-dependent transactivation in cultured cells [5•]. Thus, the binding of ligands can in principle regulate APP cleavage. It remains unclear where exactly in the cell APP would operate as a receptor. Interaction with F-spondin is thought to occur at the cell surface. However, APP could also be a receptor in intracellular axonal transport vesicles, transporting cargo to the synaptic terminal through its interaction with kinesin and the microtubule cytoskeleton [6]. An interesting interaction also exists between APP and the large low-density lipoprotein (LDL) receptor-related protein (LRP) via their cytoplasmic domains, both of which bind the adaptor protein Fe65 [7]. This particular interaction increases APP proteolytic processing. Whether the reverse is true, that is, LRP processing being enhanced

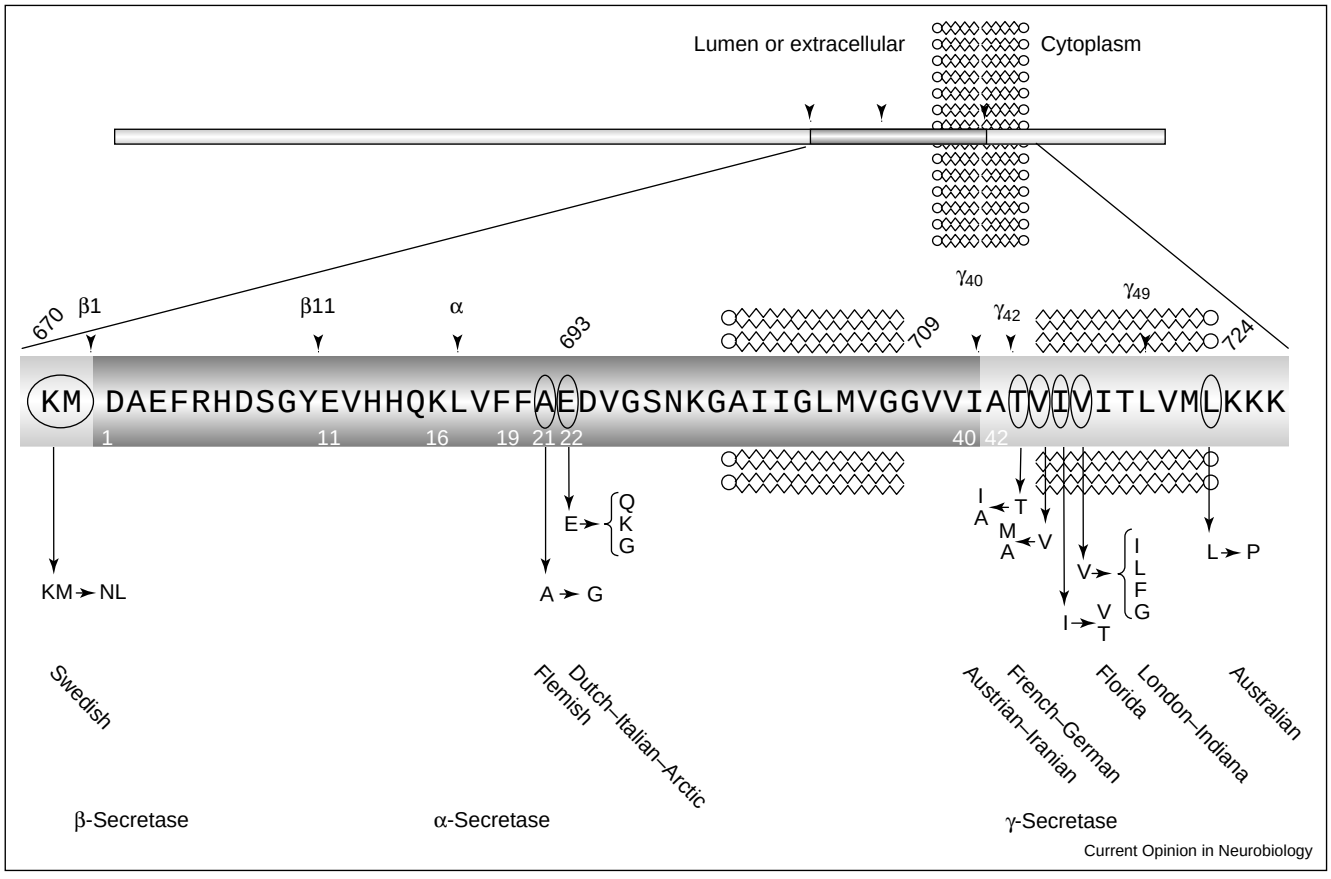
Figure 1



Proteolytic processing of amyloid precursor protein (APP) by the secretases. APP is a type-I transmembrane glycoprotein. The majority of APP is processed in the nonamyloidogenic pathway (thick arrow); APP is first cleaved by α-secretase within the amyloid-β peptide (Aβ) domain (darker shaded region), leading to APPsα secretion and precluding Aβ generation. Membrane-anchored α-carboxy terminal fragment (CTF) is then cleaved by γ-secretase within the membrane, releasing the p3 peptide and APP intracellular domain (AICD). Alternatively, amyloidogenesis (thin arrow) takes place when APP is first cleaved by β-secretase, producing APPsβ. Aβ and AICD are generated upon cleavage by γ-secretase of the β-CTF fragment retained in the membrane. Scissors indicate the cleavage sites of α-, β- and γ–secretase.

by interaction with APP, is not yet known. However, both LRP and APP become γ-secretase substrates once their ectodomain is removed, and the resulting cytoplasmic domains are potentially involved in gene regulation. Kinoshita *et al.* [8] have suggested that the LRP intracellular domain negatively regulates gene transcription by

Figure 2



The mutations causing familial Alzheimer's disease (AD) in amyloid precursor protein (APP) surround the cleavage sites of the three secretases. The amino acid sequence of the region encompassing amyloid-β peptide (Aβ) is given; amino acids mutated in familial AD are circled, and the corresponding amino acid changes (KM->NL: KM mutated in NL) are indicated. The country from where the families originate is also mentioned in the schematic (this is sometimes used in the literature as an alternative name for the mutations). Amino acid numbering of APP is written in black, and of Aβ in white. Arrowheads indicate the cleavage sites of α-, β- and γ-secretase (for an overview see http://molgen-www.uia.ac.be/ADMutations/).

the AICD/Fe65/Tip60 complex (see below). Whether this mechanism also regulates an APP receptor-associated activity *in vivo* remains currently unanswered.

Many years ago, it was proposed that the secreted ectodomain of APP, APPs, exerts growth-promoting activities on fibroblasts [9]. Abundant $APPs_{695}$-binding sites were recently found *in vivo* in the subventricular zone, a neurogenic area of the adult brain. $APPs_{695}$, together with epidermal growth factor (EGF), increased the proliferation of adult progenitor cells in the subventricular zone but not in the dentate gyrus of the hippocampus [10•]. Although this work substantiates considerably the concept that APPs can act as a growth factor, it now becomes crucial to identify the receptor and the signaling pathways mediating this function.

In parallel with APPs, Aβ and p3 peptides are secreted into the extracellular environment of neurons in the brain. For p3, little is known about its fate and whether it could contribute to amyloidogenesis (the p3 peptide is strongly hydrophobic). By contrast, the potential deleterious neurotoxic effect of the Aβ peptide has received enormous attention. Many mechanisms [11–13] have been proposed but it remains unclear the extent to which these really contribute to the neurodegeneration in AD brains. Most recently, attention has shifted again to oligomeric forms of Aβ [14]. These peptides interfere with long-term hippocampal potentiation [13], and could potentially precipitate incipient synaptic dysfunction in AD. An intriguing question in this regard is whether the Aβ peptide, which is continuously secreted under normal physiological conditions, could have a physiological role by itself. *In vitro* data [15] suggest that a decrease in Aβ levels compromises the viability of neuronal cells but apparently not of non-neuronal cells. Kamenetz *et al.* investigated the effect of Aβ on neuronal transmission in hippocampal slices [16••]. Interestingly, modulation of APP processing by

neuronal stimulation resulted in increased Aβ production and depressed synaptic transmission. Whether this effect was mediated by oligomeric forms of the Aβ peptide as proposed by Walsh *et al.* [13] was not investigated, but the observations suggest that Aβ could participate in a negative feedback loop involved in synaptic homeostasis. How Aβ or Aβ fibrils interact with the synaptic machinery remains elusive.

Much excitement has also surrounded the intracellular γ-secretase proteolytic fragment AICD, postulated to function in nuclear signaling [17], because of the similitude between Notch and APP processing. Definitive proof of this hypothesis is still lacking, although AICD fused to the DNA binding domain of the Gal4 transcription factor is able to drive, in combination with the adaptor protein Fe65 protein and the histone acetyltransferase Tip60, the expression of an upstream activating sequence (UAS)-dependent reporter [18]. Intriguingly, it appeared recently that AICD exerts its main role in this complex while still membrane bound [19] by recruiting and activating Fe65. Upon γ-cleavage, AICD and activated Fe65 are released but only the latter is involved directly in transactivating gene transcription. A similar model but with membrane bound APP in a passive anchoring role for Fe65 was proposed by Minopoli *et al.* [20]. A potential weakness when evaluating AICD function is the use of heterologous reporter assays, making it somewhat difficult to extrapolate the findings towards the role of AICD in regulating the expression of endogenous promoters. For example, Baek *et al.* [21] demonstrated that the transcription of the endogenous tetraspanin KAI1 gene is activated by the AICD/Fe65/Tip60 complex by dismissing a N-CoR co-repressor complex from its promoter. Whether AICD plays a similar indirect role in this action as in the afore-mentioned Gal4 reporter assay remains uncertain.

Regulation of the trafficking and processing of the amyloid-β precursor protein

Both from a fundamental and from a more clinical perspective, the relationship between APP trafficking and proteolytic processing is of interest. The balance between nonamyloidogenic α- and amyloidogenic β-secretase processing is partially regulated by this subcellular transport. α-Secretase typically cleaves in the transport route to and at the cell surface. β-Secretase apparently meets APP at the cell surface, and both are internalized together into early endosomes. The bulk of β-secretase cleavage of APP takes place in the endosomes [22,23], in accordance with the acid pH optimum for the β-secretase. Somewhat surprisingly, however, in dynK44A-expressing HeLA cells with blocked endocytic pathways, large amounts of Aβ peptide are still generated at the cell surface [24]. Whether this also occurs under normal conditions is less clear, because it is likely that in these cells, when endocytosis is blocked, β-secretase accumulates at the cell surface and that the increased concentration of β-secretase in this compartment compensates for the less than optimal pH conditions. A recent hypothesis implies that lipid rafts are involved in the regulation of the β-secretase cleavage step [25•]. Lipid rafts are sphingolipid- and cholesterol-rich microdomains in the cell membrane [26]. BACE is a lipid raft constituent [27], and some evidence suggests that APP can also partially localize in these rafts [25•]. Rafts are very small entities, and the likelihood of APP and BACE being together in the same raft is not very high. However, antibody crosslinking of rafts [25•] or inserting a glycosyl-phosphatidyl-inositol anchor on BACE (enhancing its localization in rafts) [28] increases APPsβ and Aβ production. This effect was abolished upon cholesterol depletion, which disrupts raft domains. The proposed model [25•] implies that rafts fuse upon endocytosis. The model could explain why statins, or depletion of cholesterol in general, can decrease Aβ production in cell culture and in guinea pigs [29]. However, it should be noted that the methods used for depleting cholesterol and disrupting rafts in cell culture experiments were extremely crude and it remains to be seen to what extent aspects of the current model can be confirmed in more physiological settings. Finally, β-secretase cleavage of APP is regulated by proteoglycans [30•].

Trafficking and processing of APP can also be regulated by the many adaptor proteins known to bind to the APP cytosolic tail (for a review see [3,4]). However, little is known about the physiological or pathological relevance of most of these interactions. The most extensively studied APP-binding partners are members of the X11 and the Fe65 families of 'adaptor' proteins that possess phosphotyrosine-binding domains (among others). Although Fe65 is involved in gene transcription regulation, as discussed above, it seems also to promote Aβ secretion [4], and in transfected cells, the Fe65–APP interaction can regulate cell motility [31]. As the proteins co-localize *in vivo* in synapses and in actin-rich mobile structures within growth cones [31], a role is proposed in growth cone motility and synaptic plasticity.

An aspect of APP trafficking that has received little attention until now is how the α- or β-secretase generated APP CTFs finally become associated with the γ-secretase complex. This multiprotein complex comprises at least presenilin, nicastrin, Aph-1 and Pen-2 ([32••,33••,34••], reviewed in [35]) and, besides APP, cleaves a whole series of other integral membrane proteins, most notoriously Notch. Although inhibiting γ-secretase is technically feasible (several small compounds have been identified that inhibit its activity), the concomitant inhibition of Notch signaling potentially results in severe side effects in the immune system and other organs [36,37•]. Further analysis of the role of the different γ-secretase subunits

might reveal subtle differences in the cleavage of Notch and APP. There is much controversy in this regard, with some groups [38,39] confirming and others [40,41] invalidating competitive inhibition between APP and Notch substrates. Part of the explanation could come from the fact that the γ-secretase complex apparently binds its substrates at an exosite remote from the catalytic cleft [42,43]. Recently, competition between APP and Notch for the cleavage site, but not for the binding site, was observed [43]. Targeting these binding sites could thus result in the development of more specific drugs. Apparently, *in vivo*, additional complex regulatory mechanisms operate that make APP and Notch processing cell-type specific. Efficient γ-secretase processing of APP, for example, is restricted to neurons in the eye of the developing *Drosophila* [44•].

Some investigators believe that presenilin–γ–secretase, in addition to its proteolytic function, also plays a role in the subcellular trafficking of APP [45–47] and of other proteins [42,48]. In the absence of presenilin or nicastrin, APP CTFs generated by α- and β-secretases accumulate at the plasma membrane [47,49], where γ-secretase is thought to be active [24,46]. Nevertheless, although some authors argue that presenilin and/or nicastrin affect APP trafficking along the secretory pathway [45], others think that it affects APP reinternalization in the endocytic pathway [46,47].

Further advances in understanding the role of APP and the amyloid-β peptide in the pathogenic cascade of Alzheimer's disease

In the past few years, a further refinement of available transgenic mouse models for AD has been realized [50,51,52••]. The most sophisticated models combine tau mutants with APP clinical mutant overexpression or with injection of Aβ into the brain, and this is required for the manifestation of both plaques and tangles in the animal model. Interestingly in these mice, tangle formation is enhanced by Aβ, demonstrating an interaction between Aβ and tangle formation *in vivo*, and at least suggesting a molecular link between the two typical AD lesions. In a triple-transgenic mouse model that also overexpresses a mutant presenilin1 allele [52••], the following sequence of events is observed: long-term potentiation deficits are already present in six-month-old mice, then Aβ deposits and, finally, tau pathology. Moreover, both plaques and tangles are restricted to the hippocampus, amygdala and cerebral cortex, the most relevant brain regions in AD pathology.

Because all currently available mouse models for AD are generated by transgenic overexpression of human APP in the brains of mice, for example, the Tg2576+ transgenic line [53], it is impossible to know whether deficits in memory are due to overexpression of mutant human APP *per se* or to the excessive production of APP-processing products, or indeed are simply a consequence of Aβ-peptide generation. Therefore, $Tg2576^{+}$ mice were crossed with β-secretase-deficient ($BACE1^{-/-}$) mice. These mice do not generate Aβ peptide, although in principle all other fragments remain available. Because the memory deficits, the hippocampal cholinergic dysfunction and the excessive Aβ levels of the $Tg2576^{+}$ mice could be rescued by the BACE1 deficiency, Ohno *et al.* conclude that Aβ itself is likely to be the causative species of the $Tg2576^{+}$ phenotype [54•]. Finally, mouse models are now used to identify and validate new potential proteins in the amyloid cascade. Much attention has been directed towards amyloid peptide clearance mechanisms. Obviously, it is conceptually attractive to postulate that part of sporadic AD is caused by loss of such clearance mechanisms (e.g. associated with aging). Several proteases have been identified that are able to degrade Aβ in cell culture or in tissue extracts (reviewed in [55]), and it is now important to explore the role of these proteases further *in vivo*. Insulin-degrading enzyme inactivation in mice or rats leads to a decrease in Aβ degradation in brain fractions and an increase in cerebral Aβ [56,57]. Neuron-specific transgenic overexpression of insulin-degrading enzyme or neprilysin in the background of APP transgenic mice resulted in reduced cerebral Aβ levels and prevented plaque formation and its associated cytopathology [58•].

Conclusions

Many *in vitro* studies have implied that cell-bound APP has a receptor function, and that its proteolytic fragments are ligands involved in a variety of cellular activities. However, the next major problem to tackle is extrapolating the many intriguing findings in cell culture to relevant *in vivo* observations in (genetic knock out) model organisms. We foresee in the years to come more in depth analysis of genetic knock out models (for instance the combined APP–APLP1–APLP-2 knock out mice and work in *Caenorhabditis elegans* and other genetic model organisms) that will hopefully lead to the confirmation of a clear phenotype. In addition, the identification of receptors or ligands for APP and genetic dissection of their role in the context of the intact animal will be important approaches to gain an understanding of the physiological function of this protein. Even if this work turns out to be not directly crucial for our understanding of the pathogenesis of Alzheimer's disease, the understanding of the function of APP remains an important scientific aim that is worthwhile pursuing.

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