

BACE, APP PROCESSING, AND SIGNAL TRANSDUCTION IN ALZHEIMER'S DISEASE

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1.1 INTRODUCTION

Alzheimer's disease (AD) is a remarkably, and to date inexplicably, common disease, affecting over five million Americans at a national cost of approximately \$150 billion annually – a cost that does not begin to address the impact of the disease on families, individuals, and society. With the graying of America, the prediction is for approximately 13 million cases by 2050 and, given the late appearance of symptoms in the pathogenic process, many more pre-Alzheimer's cases, including both mild cognitive impairment (MCI) and pre-MCI conditions. Thus, AD is unfolding as one of the most important global health concerns.

Since the first description of the disease just over 100 years ago, extensive clinical, pathological, genetic, and biochemical data have been accumulated, implicating the amyloid-beta (A β) peptides, especially A β 1-42, as key mediators in the pathogenesis of this disorder, the so-called amyloid cascade hypothesis. However, the physiological role of these peptides remains unknown, as does the mechanism(s) of their neurodegenerative effect.

The A β peptides are derived proteolytically from the β -amyloid precursor protein (APP) by β -site APP cleaving enzyme (BACE) (or β -secretase) cleavage of the extracellular domain, followed by γ -secretase cleavage of the transmembrane domain. However, APP is also cleaved at other sites, for example, at the α -site by α -secretase (with ADAM10 being the most likely candidate) and at the cytosolic caspase site by caspases (with caspase-8 and caspase-6 being the most likely candidates, given their P4 preference along with co-immunoprecipitation and kinetic data). With four major cleavage sites, theoretically 14 peptides could be produced, and it is becoming more apparent that other APP-derived peptides beyond A β also

play critical roles in elements of the Alzheimer's phenotype [1–3]. Therefore, A β may turn out to be one part of a much larger pathogenetic scenario, and thus, BACE may ultimately be one target of a cocktail of drugs that modulates APP processing at more than one site, as well as affecting targets other than APP.

In neurodegenerative diseases such as AD, neurons in various nuclei are lost in disease-specific distributions. However, the neuronal loss is a relatively late event, typically following synaptic dysfunction, synaptic loss, neurite retraction, and the appearance of other abnormalities such as axonal transport defects. This progression argues that cell death programs may play at best only a secondary role in the neurodegenerative process. However, emerging evidence from several laboratories has suggested an alternative possibility: that although cell death itself occurs late in the degenerative process, the pathways involved in cell death *signaling* do indeed play critical roles in neurodegeneration, both in sub-apoptotic events such as synapse loss and in the ultimate neuronal loss itself [3–6].

Although initial comparisons of the intrinsic suicide program in genetically tractable organisms such as the nematode *Caenorhabditis elegans* failed to disclose obvious relationships to genes associated with familial AD – for example, presenilin-1 and the β -APP do not bear an obvious relationship to any of the major *C. elegans* cell death genes (*ced-3*, *ced-4*, or *ced-9*) – more recent studies suggest a fundamental relationship between developmental and degenerative processes [3, 4, 7–10]. For example, Nikolaev et al. recently found that in a culture model of trophic factor withdrawal in developing neurons, one pathway involved in neurite retraction is mediated by a cleavage product of sAPP β [3], the latter released after BACE cleavage of APP. A detailed understanding of the interrelationship between fundamental cell death programs and neurodegenerative processes is still evolving, and it promises to offer novel approaches to the treatment of these diseases. In this review, we will discuss BACE cleavage of APP and how it might be involved in certain aspects of Alzheimer's pathology.

1.2 BACE CLEAVAGE OF APP AS A MOLECULAR SWITCHING MECHANISM

Neurons, as well as other cells, depend for their survival on stimulation that is mediated by various receptors and sensors, and programmed cell death may be induced in response to the withdrawal of trophic factors, hormonal support, electrical activity, extracellular matrix support, or other trophic stimuli [11]. For years, it was generally assumed that cells dying as a result of the withdrawal of required stimuli did so because of the loss of a positive survival signal, for example, mediated by receptor tyrosine kinases [12]. While such positive survival signals are clearly critical, data obtained over the past 15 years argue for a complementary effect that is pro-apoptotic, activated by trophic stimulus withdrawal, and mediated by specific receptors dubbed “dependence receptors” [13, 14]. Over a dozen such receptors have now been identified, and examples include DCC (deleted in colorectal cancer), Unc5H2 (uncoordinated gene 5 homologue 2), neogenin, rearranged during transfection (RET), Ptc, and APP [14–24]. These receptors interact in their intracytoplasmic

domains with caspases, including apical caspases such as caspase-9, and may therefore serve as sites of induced proximity and activation of these caspases. Caspase activation leads in turn to receptor cleavage, producing pro-apoptotic fragments [15, 22]; however, caspase cleavage site mutation of dependence receptors suppresses the cell death signals mediated by the receptors [13, 22]. A striking example of this effect was obtained in studies of neural tube development: withdrawal of Sonic hedgehog from the developing chick spinal cord led to apoptosis mediated by its receptor, Patched, preventing spinal cord development; however, transfection of a caspase-uncleavable mutant of Patched blocked apoptosis and restored significant development, even in the absence of Sonic hedgehog [25].

Thus, cellular dependence on specific signals for survival is mediated, at least in part, by specific dependence receptors that induce apoptosis in the absence of the required stimulus – when unoccupied by a trophic ligand, or when bound by a competing, anti-trophic ligand – but block apoptosis following binding to their respective ligands [11, 14, 23]. Expression of these dependence receptors thus creates cellular states of dependence on the associated trophic ligands. These states of dependence are not absolute, since they can be blocked downstream in some cases by the expression of anti-apoptotic genes such as bcl-2 or p35 [11, 20, 26]; however, they result in a shift of the apoptat [27, 28] toward an increased likelihood of triggering apoptosis. In the aggregate, these receptors may serve as part of a molecular integration system for trophic signals, analogous to the electrical integration system composed of the dendritic arbors within the nervous system.

Although cellular dependence on trophic signals was originally described in the developing nervous system, neurodegeneration may also utilize the same pathways, since APP exhibits several features characteristic of dependence receptors: an intracytoplasmic caspase cleavage site (Asp664) [18, 29], co-immunoprecipitation with an apical caspase (caspase-8), caspase activation, derivative pro-apoptotic peptides (including the A β peptide; see below), and suppression of apoptosis induction by mutation of the caspase cleavage site [18, 30].

These findings raise several questions: first, does BACE cleavage of APP play a role in APP's putative dependence-related pro-apoptotic function? Second, does the caspase cleavage of APP occur in human brain and, if so, is this increased in patients with AD and coordinated with BACE cleavage? Third, if this cleavage is prevented, is the Alzheimer's phenotype affected? These questions are addressed below.

1.3 AD: AN IMBALANCE IN CELLULAR DEPENDENCE?

Although cellular dependence on trophic signals was originally described in molecules and pathways critical for the developing nervous system, degeneration in neurons of the aged organism may also utilize the same pathways. For example, APP, a molecule holding a central position in AD pathogenesis because of its intimate relationship to A β peptides, exhibits several features characteristic of dependence receptors: a caspase cleavage site (Asp664) [1, 29], interaction with an apical

caspase (caspase-8), derivative pro-apoptotic peptides released after caspase activation, and suppression of apoptosis induction by mutation of the caspase cleavage site [1, 30, 31]. Although APP demonstrates many of the characteristics of a dependence receptor, what is unclear is whether there is a physiological ligand that maintains the balance of APP in favor of survival rather than neuronal death, as has been seen with other trophic factor receptors.

In this context, BACE may hold a surprising central position. This is because cleavage of APP by BACE generates not only the C-terminal fragment of APP that is the direct precursor of A β , but this cleavage also releases sAPP β , which can interact with DR6 to effect neuronal damage (see below). What is unclear is whether BACE cleavage of APP plays a role in APP's putative dependence-related proapoptotic function. And second, whether caspase cleavage of APP occurs in human brain and, if so, is this increased in patients with AD and coordinated with BACE cleavage? And lastly, if this cleavage is prevented, is the Alzheimer's phenotype affected? These questions are addressed below.

Extensive genetic and biochemical data have implicated the A β peptide as a central mediator of AD, but the mechanism(s) of action remains controversial: the ability of A β to generate a sulfuranyl radical involving methionine 35 has been implicated, and so have its direct effects on postsynaptic structures, the metal-binding property of A β , as well as its aggregating property, and its ability to form pore-like structures in membranes, just to list a few of the mechanisms proposed [32]. These proposed mechanisms share a focus on the chemical and physical properties of the A β peptide. However, cellular signaling is emerging as a complementary mechanism by which A β exerts its critical effects, and multiple candidates have surfaced as key downstream mediators, including APP itself, the insulin receptor, and tau, among others [30, 33, 34]. These cellular signals may also mediate neuronal dependence on trophic support, as described below.

1.4 BACE CLEAVAGE, CASPASE CLEAVAGE, AND NEURONAL TROPHIC DEPENDENCE

Neopeptide antibodies directed against residues 657–664 of human APP disclosed the presence of caspase-cleaved APP fragments in human brain, especially in the hippocampal region [8], with an approximately fourfold increase in Alzheimer's patients over age-matched controls. However, in brains without Alzheimer's pathology, there was an inverse relationship between age and immunohistochemical detection of APPneo, with a different distribution from AD brains: in the Alzheimer brains, the staining was primarily in neuronal somata and peri-neuronally, whereas in the non-Alzheimer brains, the staining was observed predominantly in the processes. These findings suggest that the caspase cleavage of APP occurs physiologically and is reduced with age, but that this process remains more active and perhaps aberrant in cellular distribution in association with AD.

To test whether preventing the caspase cleavage of APP has any consequences on the Alzheimer's phenotype, a transgenic mouse model of age-associated amyloid

pathology was generated in which APP containing the Swedish and Indiana familial AD mutations were combined with a mutation of the caspase site (D664A) was expressed under the control of the neuronal-specific platelet-derived growth factor-B (PDGF-B) promoter. Although the caspase mutation (D664A) had no effect on amyloid production or plaque formation, these animals did not exhibit any overt synapse loss, early p21-activated kinase (PAK) phosphorylation, dentate gyrus atrophy, electrophysiological abnormalities (including reductions in excitatory postsynaptic potentials [EPSPs] and long-term potentiation [LTP]), neophobia, or memory deficits that frequently characterize these APP overexpressing mice [4, 6, 35]. These findings indicate that key features of the Alzheimer's associated phenotype in a standard transgenic mouse model depend on the presence of the caspase cleavage site within APP. This finding, when combined with the extensive previous work showing that the Alzheimer's phenotype is critically dependent on A β , suggests that the APP caspase site may lie downstream from the A β accumulation and that cleavage of this site is one pathway that contributes to neuronal injury [30, 33]. This possibility has received support from studies showing that A β interacts directly with APP in the A β region itself, leading to APP multimerization, caspase cleavage at Asp664, and cell death signaling [30, 33].

1.5 BACE CLEAVAGE OF APP, DEPENDENCE RECEPTORS, AND ALZHEIMER PATHOLOGY

The above model of caspase cleavage of APP focuses primarily on the cytosolic region of APP where the caspase site is situated. Experimental evidence suggested that A β -induced multimerization and subsequent caspase cleavage of APP can take place with either APP or the BACE-cleaved C99 APP fragment. However, there did not appear to be any preference between these two substrates. How, then, might BACE cleavage of APP relate to the caspase cleavage of APP? Recent work from the Tessier-Lavigne lab [3] provides surprising new insight into this question: following trophic factor withdrawal from developing neurons in culture, BACE was apparently activated, resulting in the shedding of sAPP β . Following further processing near the amino-terminus of sAPP β by an unidentified protease, the resulting amino-terminal peptide of the APP ectodomain was able to interact with death receptor 6 (DR6). This binding led to caspase-6 activation and subsequent neurite retraction, which is of interest given previous studies showing that APP is cleavable by caspase-6, and that its caspase site (VEVD) is indeed most compatible with a caspase-6 site [1]. This is a surprising observation because the large sAPP ectodomain (mainly sAPP α) has traditionally been thought to play a neurotrophic rather than toxic role. Nonetheless, increased BACE cleavage of APP should lead to the production of more A β peptide, and this in turn will result in more A β -C99 interaction and potentially more caspase cleavage of APP. Thus, BACE cleavage of APP results in two downstream pathways that damage neurons: through the sAPP β fragment interacting with DR6 receptor (via N-APP production) and through A β -induced caspase cleavage of APP C99 fragment.

binding to and facilitating APP oligomerization, recruiting and activating caspase-8 (and possibly caspase-6), engendering the processing of APP at Asp664, and inducing neurite retraction, then, ultimately, neuronal cell death [4, 30, 33]. Whether the D664A mutation of APP exerts effects beyond the prevention of caspase cleavage (e.g., an alteration of the intracytoplasmic structure of APP) is not yet known. However, regardless of the mechanism, the results suggest that APP signal transduction may be important in mediating AD [39], at least in the transgenic mouse model, possibly downstream from A β oligomerization and binding of APP. The results also suggest that BACE cleavage and caspase cleavage of APP may work in concert to lead to neurite retraction, and potentially other aspects of the AD phenotype.

The results obtained in the transgenic mouse model of AD also suggest an alternative to the classic models of AD. As noted above, chemical and physical properties of A β have been cited as the proximate cause of AD pathophysiology. However, these theories do not explain why A β is produced ubiquitously and constitutively, nor do they offer a physiological function for the A β peptide, or account for the improvement in AD model mice that occurs with a reduction in tau protein [34].

An alternative model, presented in Figs. 1.1–1.3, proposes that APP is indeed a dependence receptor, and that it functions normally as a molecular switch in *synaptic element interdependence* (Fig. 1.4): in this model, both the presynaptic element and the postsynaptic element are dependent on trophic support, including soluble factors such as netrin, substrate adherence molecules such as laminin, neurotransmitters, and neuronal activity, as well as other factors. In the presence of adequate trophic support, APP is cleaved at the α - and γ -secretase sites, generating three peptides – sAPP α , p3, and APP intracytoplasmic domain (AICD) – that support cell

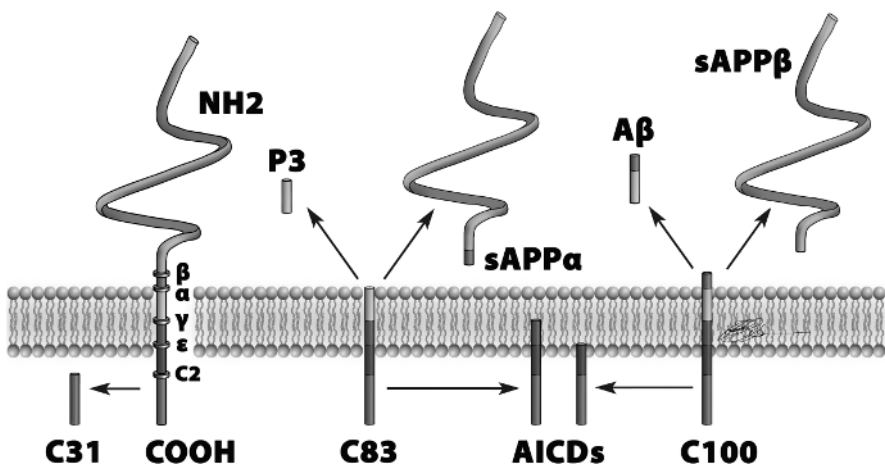


Figure 1.2 Alternative cleavage patterns of APP generate distinct extracellular, transmembrane, and intracytoplasmic fragments. (See color insert.)

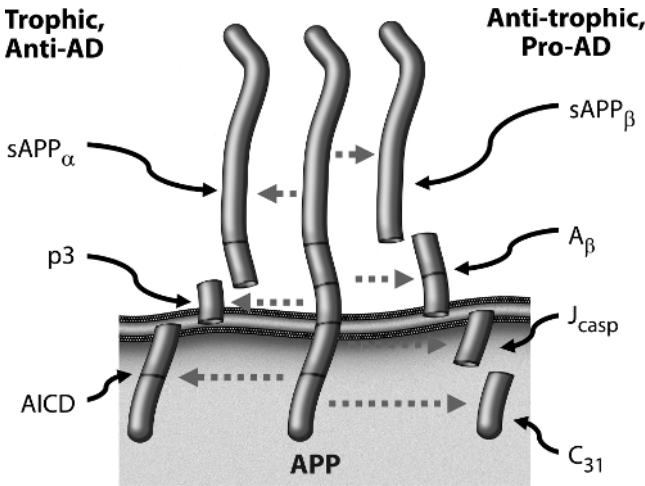


Figure 1.3 Alternative cleavage of APP to produce four peptides that mediate synaptic loss, neurite retraction, and ultimately, programmed cell death (“the four horsemen”); or three peptides that mediate synaptic maintenance and inhibit programmed cell death (“the wholly trinity”). Among the factors that mediate the decision between these two pathways are included trophic effects such as netrin-1 and anti-trophic effects such as A β peptide.

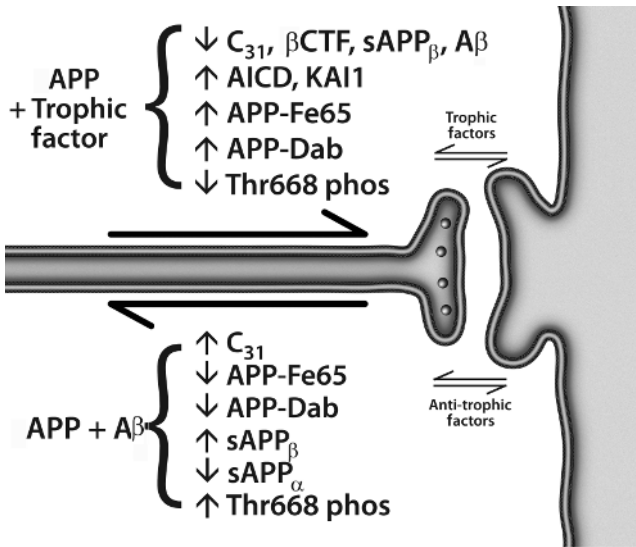


Figure 1.4 Synaptic element interdependence model of synaptic maintenance, reorganization, and Alzheimer’s disease. The presynaptic and postsynaptic elements are interdependent, and provide both trophic influences (e.g., neurotrophins, netrin-1, laminin, collagen, and synaptic activity itself) and anti-trophic influences (e.g., amyloid- β peptide). Trophic support leads to the processing of APP into three peptides that support synaptic maintenance, whereas the withdrawal of trophic support leads to alternative processing, to four peptides that mediate synaptic inhibition, synaptic loss, neurite retraction and, ultimately, programmed cell death. In this model, the A β peptide functions as an anti-trophin and, since it leads to APP processing that produces additional A β peptide, it is “prionic,” that is, A β begets additional A β .

survival and synaptic maintenance. However, a reduction in trophic support (e.g., due to head trauma) alters the processing of APP, thereby activating BACE cleavage of APP to alter the ratio of α/β cleavages, and leading to the production of four peptides – sAPP β , A β , Jcasp, and C31 (Fig. 1.3) – that mediate a reduction in synaptic transmission, synaptic loss, neurite retraction and, ultimately, programmed cell death [1, 3, 4, 9, 35]. In this model, neuronal and synaptic injury in AD is suggested to result from an imbalance in physiological signaling pathways that mediate synaptic maintenance versus synaptic reorganization, mediated at least in part by APP, functioning in synaptic element interdependence (Fig. 1.4), as part of a plasticity module that includes other receptors such as the common neurotrophin receptor, p75^{NTR} and the axon guidance receptor DCC, among others [40].

1.6 KEY MUTATIONS PROXIMAL OF APP PROCESSING TO A β

Mutations in APP that are associated with familial AD typically affect A β processing and lead to an increase in the ratio of A β 1-42 to A β 1-40. These mutations may be located in proximity to the β -cleavage site or the γ -cleavage site. Mutations near the α -secretase cleavage site affect the primary structure of the A β peptide, and are often associated with cerebral hemorrhagic syndromes, but in at least some cases, such as the Arctic mutation (see Table 1.1), also lead to AD.

TABLE 1.1 APP Mutations Around the Key Cleavage Sites Involved in APP Processing

Nearest cleavage site	Mutation	Type	References
Beta-site	Lys670 → Asn	Swedish	Mullan et al., 1992 [41]
	Met671 → Leu	Swedish	
Alpha-site	Ala692 → Gly	Flemish	Kumar-Singh et al., 2002 [42]
	Glu693 → Gln	Dutch	Van Broeckhoven et al., 1990 [43]
Gamma-site	Glu693 → Gly	Arctic	Nilsbeth et al., 2001 [44]
	Asp694 → Asn	Iowa	Grabowski et al., 2001 [45]
	Ala713 → Thr	Austrian	Carter et al. 1992 [46]
	Thr714 → Ile/Ala	Austrian/Iranian	Kumar-Singh et al., 2000 [47]; Pasalar et al., 2002 [48]
	Val715 → Met	French	Ancolio et al., 1999 [49]
	Ile716 → Val	Florida	Eckman et al., 1997 [50]
Epsilon-site	Val717 → Phe/Gly/Ile	Indiana/ /London	Murell et al., 1991 [51]; Chartier-Harlin et al., 1991 [52]; Goate et al., 1991 [53]
	Leu723 → Pro	Australian	Kwok et al., 2000 [54]
	Lys724 → Asn	Belgium	Theuns et al., 2006 [55]

1.7 FINAL REMARKS

BACE processing of APP represents an important therapeutic target in AD. However, such processing may be part of a larger set of pathogenetic events in this disease, featuring imbalanced signal transduction mediated by APP and potentially other receptors.

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