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The Functions of the Amyloid Precursor Protein Gene and Its Derivative Peptides: I Molecular Biology and Metabolic Processing

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ABSTRACT

The amyloid precursor protein gene (*APP*) *and its derivative peptides have important functions in the central nervous system. APP and Aβ fulfil criteria as neuractive peptides*: *presence, release and identity of action. Aβ is a peptide of* 1 - 43 *amino acids in length, derived from APP and the major component of the core of neuritic plaques found in Alzheimer's disease. Analysis of the cDNA of Aβ revealed its origins from the larger precursor protein. There are at least four types of mRNA generated by alternative splicing of exons* 7 *and* 8. *Exon* 7 *encodes a* 57 *amino acid sequence found in the extracellular domain with major homology to the Kunitz-type of serine protease inhibitors. APP is cleaved by three secretases known as α, β and γ secretase which act on APP at different sites producing various fragments of differing amino acid length. The γ secretase is a macromolecular enzyme complex composed of presenilin* 1, 2 *and other molecular constitutents essential for its function.*

Keywords**:** *Amyloid Precursor Protein Gene, Biochemistry*

1. Introduction

The amyloid precursor gene (APP) has important functions in the central nervous system. In Alzheimer's disease (AD) APP is the precursor of amyloid peptides (Aβ) which forms the amyloidogenic cores of neuritic plaques, hallmarks of the disease. Understanding of the normal function of APP and its pharmacology will provide insights into the pathophysiology of AD and its treatment.

2. Molecular Biology of APP

AD is characterized by neuronal loss, senile plaques, and neuro-fibrillary tangles [1]. The major component of senile plaques is amyloid [2,3]. Amyloid is an insoluble extracellular protein that forms β-pleated sheets. This protein had become known as Aβ peptide and is 4 - 4.3 kDa in molecular weight and is composed of 40 - 43 amino acids.

Analysis of the cDNA revealed that it originated from a larger precursor molecule of about 700 amino acids termed the Amyloid Precursor Protein (APP) [4,5]. APP gene transcripts were identified in human brain [6]. APP is also found in the peripheral nervous system and skeletal muscle [7]. When APP was identified in virtually all cell types, and in all mammals and vertebrates tested, the term APP became widely accepted [8-10].

Examination of the hydrophobicity plot indicated that APP was a single-chain surface membrane-spanning peptide with a large extracellular and smaller cytoplasmic domain suggestive of a cell-surface receptor [11], the Aβ region being both in the extracellular and transmembrane domains.

Analysis of other cDNA clones revealed at least four types of mRNAs generated by alternative splicing of exons 7 and 8 [12-16].

The different forms of APP are designated by the number of amino acids: APP 695, APP 714, APP 751 and APP 770. Other isoforms have been discovered which result from differential splicing of exon 15 and are found in lymphocytes, macrophages and microglial cells —designated appican or L-APP [17,18]. The splicing of exon 15 determines the chondroitin sulfate attachment site of APP [19].

Trophic factors like NGF may also influence the splicing of APP and may be important in apoptosis and the mechanisms of neurodegeneration [20]. Splicing of APP may be modulated by acetylcholine nicotinic re-

ceptor modulation [21]. APP 770 is mostly found in tissues outside the CNS and contains both exons 7 and 8. These splice variants are regulated during development [22-24].

Exon 7 encodes a 56 amino acid sequence found in the extracellular domain that produces a region with major homology to the Kunitz-type of serine protease inhibitors (KPI) [25-28].

Hence:

APP 751 and 770 = KPI +

APP 695 and 714 = KPI –

This KPI+ domain was found to be identical to protease nexin II secreted by activated astrocytes [27,29]. Protease nexin II has powerful antichymotrypsin activity *in vitro* [30]. (**Figure 1**).

The 5′ upstream regulatory region of APP has been cloned and sequenced in rats and humans [31,32]. Rat APP has 97% homology to human APP, making it an excellent experimental animal to investigate the functions of APP [15,33]. There is significant homology in the APP promoter between monkeys and humans [34]. The regulatory region is high in G and C content, and has consensus DNA sites for AP-1, AP-2, AP-4, GCF and SP-1 proteins; it does not have a TATA or CAAT box [32-36]. These features establish APP as a house-keeping gene. Such genes are functionally required in all cell types irrespective of the specialized role of these cells. Other examples of housekeeping genes are vimentin and nerve growth factor (NGF) receptor genes. *In vitro* studies suggest that DNA methylation may affect APP gene expression by interaction with the GC-rich sequence [37] The promoter region of APP also contains recognition sequences for the heat-shock control element binding protein upstream of the RNA start sites. The heat-shock consensus sequence (CTGGACTTTTCTAG) is located at position –317 bp in the regulatory elements of APP [38].

When the promoter region was fused with chloramphenicol acetyltranferase (CAT) reporter gene and the function genes transfected into cell lines, deletional analysis revealed a negative regulatory element between –153 and –100 base pairs (bp) which contains two nuclear protein binding sites, one of which is probably SP1 [39].

An analysis of the APP promoter revealed that sequences –203 to +104 were sufficient for promoter function and regulatory elements were found from –128 to –63; furthermore the same proximal elements were recognised by nuclear factors from both neuronal and non-neuronal cells [40].

In the rat promoter region a position 375 bp upstream of the start codon drives APP expression and is regulated by two sites at –260 to –248 and –223 to –192 [41]. Levels of APP mRNA may be modulated by cystolic protein—RNA interactions involving AU-rich regions and a 29 base element in the 3' nontranslated region [42,43]. Analysis of the rat promoter showed that the CAA and GAG regions interact with SP1, nuclear proteins, and human upstream stimulatory factor (USF) protein; furthermore the SP1 and USF sites regulate transcription of the CAA element [44,45]. The transcriptional regulatory factor NF-kappa-B/Rel recognises sequences in the 5' regulatory region which specifically interact with p50-containing members of this family (p50/p50; p50/p65; p50/$_c$rel). The nuclear complex binding APP-kappa-B sites is an integral component of neurons and lymphocytes [46]. Interleukin-1β and glutamate may activate the NF-kappa-B/Rel binding site [47].

In a mouse embryonal carcinoma line it was shown that alternative splicing of APP pre-mRNA was influenced by the immediate early gene c-*jun* in that the encoding exons 7 and 8 were preserved maintaining greater relative amounts of APP 770 in comparison to APP695 [48]; this change impairing the ability of these cells to differentiate into neurons. A protein homologous to the ubiquitin activating enzyme E1 has been found to bind to the carboxyl terminal of APP and may be important in regulating its function [49].

In a study using Cos-1 and P19 cells it was shown that exogenous APP mRNA may regulate its own expression. Cells over-expressing sAPPβ 770 and sAPPβ 695 had reduced levels of sAPPβ 770 and sAPPβ 695 mRNA [50]. There is no evidence that aluminium influences the expression of APP [51]. Phosphorylation may influence the expression of APP through kinase activity [52]. The transcriptional regulation of APP may be through the action of cyclic AMP and may be mediated by two sites on APP promoter not involving AP-2 sites [53-55].

APP mRNA may be regulated by nucleolin and heterogeneous nuclear ribonucleoprotein (hnRNP) through post-transcriptional interaction with the three untranslated regions of APP mRNA [56].

Further studies have shown that APP is a member of a family of structurally related proteins in mammals and other species. APLP 1 and 2 do not have the Aβ region [17,57-59]. APLP 2 may have a role in axogenesis in the olfactory epithelium [60]. APLP1 is found in the cerebral cortex postsynaptic density of rats and humans and supports a role in synaptic function [61]. Mouse cortical neurons lacking APP reveal normal neurite growth suggesting that APLP 1 and 2 might supplement this function of APP [62]. The protein and mRNAs for APLP1 and 2 are found in a similar distribution in human brain as APP providing further evidence for similarity of func-

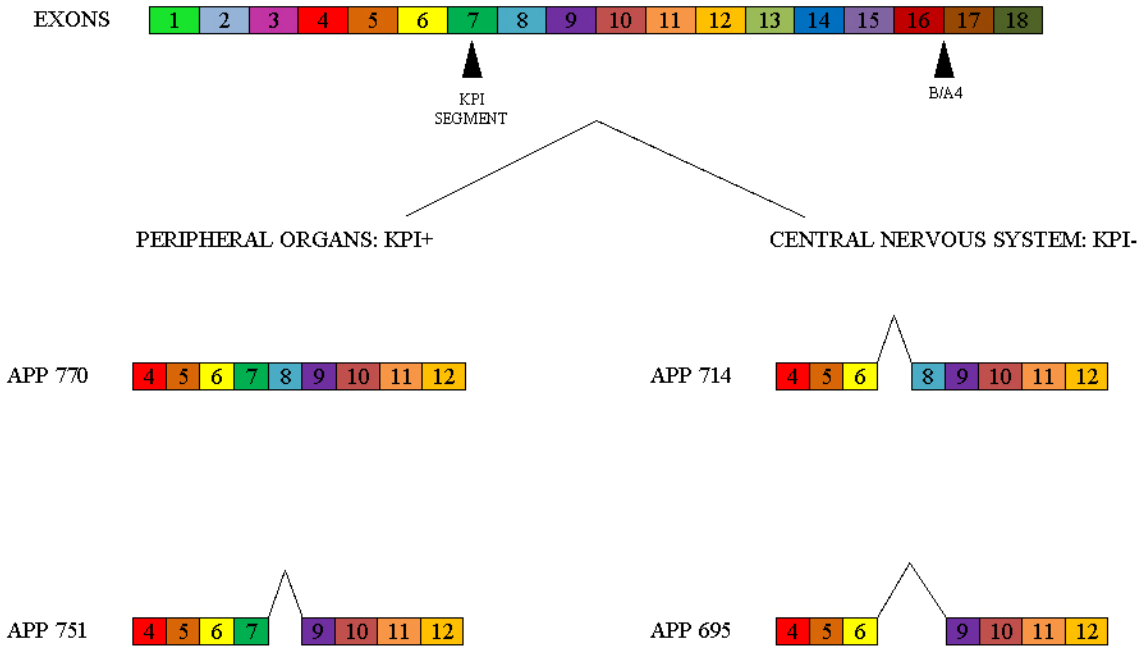

Figure 1. Exon structure of APP transcripts. These are generated by alternative splicing of exons 7 and 8. Exon 7 codes for the KPI segment and exon 8 for the MRC OX-2 antigen. Transcripts containing exon 7 are found in peripheral organs, whilst exon 7 is absent in the CNS transcripts. Alternative splicing of exon 15 from all of the above transcripts generates mRNA isoforms, found by RT-PCR, in most tissues except neurons [17]. The β/A4 encoding sequence is shared between exons 16 and 17. The transmembrane segment is encoded by exon 17.

tion [63]. APLP2 can provoke neurite outgrowth in chick sympathetic neurons as does sAPPβ 695 and 751 again suggesting redundancy of function [64]. The function of APLP molecules may be to supplement the functions APP in times of homeostatic stress. That is, a duplication of function.

3. The Metabolic Processing of APP

APP is proteolytically cleaved by a number of membrane bound putative enzymes known as secretases [65-68]. Protein phosphorylation is also important in the secretory processing of APP through the action of protein kinase C [69]. The β secretase has been isolated, cloned, and sequenced [70].

The action of α secretase produces an N-terminal fragment known as sAPPα and a C-terminal fragment which remains in the membrane. The α-secretase may act in the presence of different amino acid sequences in the juxta-membranous region of APP [71].

The β-secretase acts at the N-terminal end of the Aβ domain and produces sAPPβ which contains the undisrupted Aβ region. The activity of the β-secretase may depend on the primary structure of APP [72]. The action of γ-secretase cleaves the C-terminal end of Aβ from the C-terminal fragment of sAPPβ. Cleavage of the N-terminal domain of Aβ is essential for its secretion [73].

APP is found in neuronal cell membranes [74]. APP is metabolized in the Golgi apparatus and progresses to the liposomes for degradation and to the plasma membrane for secretion [75-77]. The activation of protein kinase C regulates the production of sAPPβ and decreases Aβ in Golgi secretory vesicles [78]. The β-secretase cleavage may also occur in the Golgi apparatus [79]. The regions which determine secretion are in the extracellular domain [80]. Secretory vesicle budding and trafficking is not essential for γ-secretase action which can occur in the trans-Golgi network [81].

β-secretase activity resides in the late endosomal compartment and a γ-cleavage in early endosomes generating Aβ peptides [82]. The endoplasmic reticulum and secretory vesicles generate Aβ(1 - 42), the trans-Golgi network Aβ(1 - 40) suggesting that intracellular organelles produce Aβ of differing amino acid lengths [83].

Recently sumoylation, the covalent process which modifies cellular proteins with small ubiquitin-like modifier (SUMO) proteins and enhances their stability or modulates subcellular compartmentalization, has been shown to regulate secretase cleavage of APP. Augmentation of SUMO3 was shown to reduce Aβ by production by enhancing the function of the α-secretase [84]. SUMO2 was shown to inhibit β secretase leading to the possibility that agents acting on SUMO2 might decrease Aβ pro-

duction in AD.

A major advance was the discovery that presenilin 1 (PS1) and APP interact in the endoplasmic reticulum and Golgi where PS1 is essential for γ-secretase activity on C-terminal fragments of APP and the generation Aβ [85]. Mutations in PS1 increase the production of Aβ 1 - 42 in cultured cells and knock-out mice not containing PS1 gene do not produce Aβ [86,87]. PS1 is required for the proper γ-secretase processing of APP C-terminal fragments, however the role of PS1 in the physiological function of APP is unknown and its role in sporadic AD is not understood [88,89]. The effect of PS1 is independent of endoplasmic trafficking of APP [90]. The relationship of PS1 to the processing of APP processing is similar to the metabolism of cholesterol which is regulated by proteolysis of a membrane-bound transcription factor through the action of a metalloprotease—the sterol regulatory element binding protein (SREBP). M19 cells deficient in the S2P gene, which encodes a metalloprotease essential for the proteolysis of SREBP, does not produce Aβ indicating there may be homology with this metalloprotease and the putative γ-secretase [91]. These findings strongly support the notion that presenilin proteins are important in the proteolytic processing of APP.

Presenilin (PS) proteins have homology to *Notch* genes. Notch genes are involved in intracellular signalling and development and may have important roles in the physiological regulation of differentiation within the haemopoietic system. These functional properties may limit the development of compounds which antagonize the actions of PS proteins. Mutagenesis experiments of two transmembrane aspartates in PS1 and PS2 abrogate γ-secretase activity and the production of Aβ suggesting that aspartate sites are critical in the proteolytic cleavage of APP [92]. PS1 mRNA is found in the same neurons as APP [93] and PS1 is identified in endoplasmic reticulum, Golgi apparatus and N-terminal fragments are found in synaptic organelles [94]. The γ-secretase is a multimolecular complex composed of PS1, 2, nicastrin, APH-1 and PEN-2 [95]. (**Figure 2**)

There may be a stoichometric interaction between APP and PS as both of these proteins form complexes with each other in living cells [96,97]. There may also be an interaction between these proteins at the cell surface which may be important in cell-cell adhesion and signalling since this protein complex activates tyrosine kinase [98].

The metabolic handling of APP by γ-secretase is probably distinct from endoplasmic reticulum processing [99]. The C-terminal fragment is important in trafficking and secretion of APP [100]. The action of cAMP-dependent protein kinase A influences the processing of

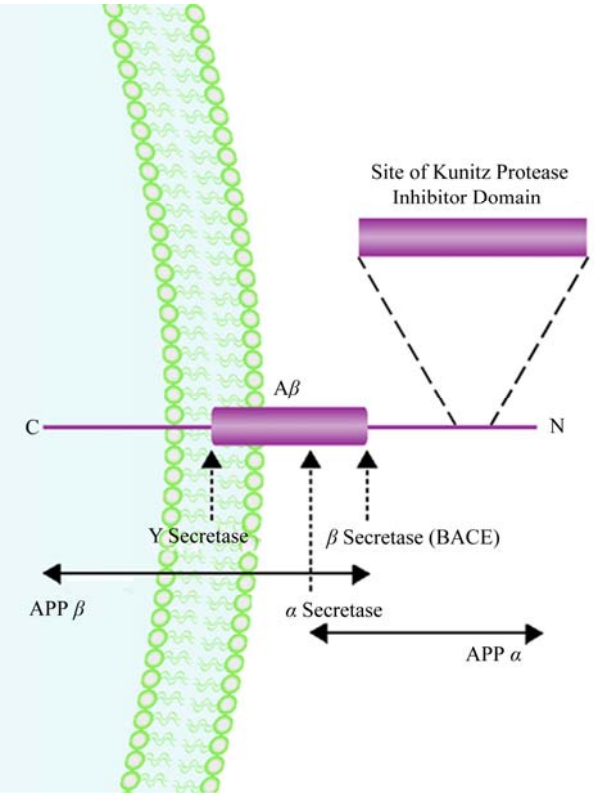

Figure 2. The structure of the amyloid precursor protein and its enzymatic cleavage. The largest transcript has 770 amino acids, the smallest 677, after alternative splicing of exons 7, 8, and 15. Two alternative spliced exons of 56 and 19 amino acids are inserted at residue 289, the first of these exons (exon 7) codes for the serine protease inhibitor domain of the Kunitz type (KPI) located in the extracellular domain. The amyloidogenic fragment Aβ has 28 amino acids in the extracellular domain with the next 11-15 in the transmembrane segment. The single transmembrane region is from positions 700 - 723. Alpha sectretase cleavage after position 687 yields soluble APP alpha (sAPPα) which is found in body fluids; the 83 amino acid fragment remains in the membrane. Cleavage before position 671 generates a 12 kDa fragment soluble APP beta (sAPPβ) which contains the undisrupted sequence of Aβ and serves as an intermediate in the production of free Aβ through the action of beta-secretase (BACE). The site of action of the gamma-secretase at the C-terminal end of the Aβ membrane is shown. There is a 17 amino acid signal peptide at the N-terminal.

APP towards sAPPα, decreasing both sAPPβ and reducing Aβ [101].

The heparin binding consensus sequence VHHQKL also contains the α-secretase cleavage site [102,103]. Levels of iron may modulate α-secretase activity through inhibitory action of an iron response element [104]. A protease has been associated with acetylcholinesterase which releases APP from the cell membrane and may

regulate APP secretion in amyloid plaques [105]. If energy metabolism is inhibited in the cell the secretase handling of APP is modulated such that more Aβ is produced [106]. A detergent-insoluble glycolipid-enriched membrane domain (DIG) contains Aβ, PS1 and APP suggesting that it is within the intramembranous compartment that the cleavage of APP occurs [107].

APP might regulate its own metabolism such that KPI+ isoforms may decrease α-secretase activity and increase the production of amyloidogenic Aβ(1 - 42) [108]. This finding has therapeutic implications for AD and requires further analysis at a gene expression level as this may lead to a new approach to treatment [109]. In human platelets APP is cleaved near the transmembrane region and involves Ca^{2+} dependent cysteine proteases [110,111]. The expression of human APP in yeast reveals that α, β and γ-secretases are conserved in yeast and their action might be linked to glycosyl-phosphatidylinositol on the cell surface [112,113].

Other enzyme systems may be involved in the metabolism of APP and are of uncertain functional significance; these include a non-lysosomal multicatalytic proteinase (ingensin) [114]. Cathepsin B also has γ-secretase activity [115]. Metallopeptidases, similar to neuropeptidases, have been identified which cleave APP at a lys-leu-region [116,117]. Experiments with phosphoramidon, a proteinase inhibitor, further support a role for metalloproteases in the production of Aβ [118]. Cathepsin D and S lysosomal enzymes have activities supportive of a role as γ-secretases [119,120]. Other cathepsins E and B may also be involved in the amyloidogenic processing of APP [121].

A gelatinase in HeLa cells possesses β-secretase like activity [122]. Zyme, a serine protease inhibitor in perivascular and microglial cells may also be involved in the metabolism of APP [123]. Tumour necrosis factor must also affect the α-secretase cleavage of APP [124]. The proteolytic processing of APP by caspases may occur in the cytoplasmic domain and be activated in the presence of PS2 mutations leading to an increase in C-terminal fragments and Aβ leading to neuronal death [125].

Other proteins bind to APP which may be important in its metabolism include thrombin [126], the chaperone protein BiP/GRP78 [127], X11α [128,129], PAT1 a microtubule-interacting protein which recognizes the basolateral sorting signal peptide of APP [130], UV-DDB (ultra-violet damaged DNA binding protein) binds to the APP YENPTY sequence in its cytoplasmic domain and may be involved in internalization and secretion of APP [131], and a novel XII like neuron specific protein with PDZ domains which interacts with the NPXY motif of APP and regulates phosphatidyl-inosityl binding to APP [132].

The protease inhibitor KPI+ domain of APP has been shown to inhibit the processing of a proenkephalin processing enzyme prohormone thiol protease (PTP) and APP KPI+ colocalizes with PTP and met-enkephalin in secretory vesicles [133]. PTP may therefore be the first physiological substrate of the protease inhibitor isoforms of APP.

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