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 constituents 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-
Protease nexin II has powerful antichymotrypsin activity in vitro.

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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.

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].

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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-
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A major advance was the discovery that presenilin 1 (PS1) and APP interact in the endoplasmic reticulum and Golgi where PS1 is essential for \( \gamma \)-secretase activity on C-terminal fragments of APP and the generation A\( \beta \) [85]. Mutations in PS1 increase the production of A\( \beta \) 1 - 42 in cultured cells and knock-out mice not containing PS1 gene do not produce A\( \beta \) [86, 87]. PS1 is required for the proper \( \gamma \)-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 A\( \beta \) indicating there may be homology with this metalloprotease and the putative \( \gamma \)-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 signaling 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 \( \gamma \)-secretase activity and the production of A\( \beta \) 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 \( \gamma \)-secretase is a multimolecular complex composed of PS1, 2, nicastrin, APH-1 and PEN-2 [95]. (Figure 2)

There may be a stoichiometric 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 signaling since this protein complex activates tyrosine kinase [98].

The metabolic handling of APP by \( \gamma \)-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 APP towards sAPP\( \alpha \), decreasing both sAPP\( \beta \) and reducing A\( \beta \) [101].

The heparin binding consensus sequence VHHQKL also contains the \( \alpha \)-secretase cleavage site [102, 103]. Levels of iron may modulate \( \alpha \)-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

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**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 A\( \beta \) 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 secretase cleavage after position 687 yields soluble APP alpha (sAPP\( \alpha \)) 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 (sAPP\( \beta \)) which contains the undisrupted sequence of A\( \beta \) and serves as an intermediate in the production of free A\( \beta \) through the action of beta-secretase (BACE). The site of action of the gamma-secretase at the C-terminal end of the A\( \beta \) membrane is shown. There is a 17 amino acid signal peptide at the N-terminal.
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 iscleaved near the transmembrane region and involves Ca2+ dependent cysteine proteases [110,111]. The expression of human APP in yeast reveals that human platelets APP is cleaved near the transmembrane region and involves Ca2+ 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 neuropetidases, 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 can 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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