Journal of Neurochemistry

JNC

JOURNAL OF NEUROCHEMISTRY | 2014 | 129 | 756–769 doi: 10.1111/jnc.12675

REVIEW

Insights into the physiological function of the β-amyloid precursor protein: beyond Alzheimer's disease

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Abstract

The β-amyloid precursor protein (APP) has been extensively studied for its role as the precursor of the β-amyloid protein (Aβ) of Alzheimer's disease. However, the normal function of APP remains largely unknown. This article reviews studies on the structure, expression and post-translational processing of APP, as well as studies on the effects of APP *in vitro* and *in vivo*. We conclude that the published data provide strong evidence that APP has a trophic function. APP is likely to be involved in neural stem cell development, neuronal survival, neurite outgrowth and neurorepair. However, the mechanisms by which APP exerts its actions remain to be elucidated. The available evidence suggests that APP interacts both intracellularly and extracellularly to regulate various signal transduction mechanisms.

Keywords: Alzheimer's disease, amyloid precursor protein, growth factor, heparin, neurotrophic, receptor.

J. Neurochem. (2014) **129**, 756–769.

The β-amyloid precursor protein (APP) is a type I transmembrane glycoprotein that is expressed in a wide variety of mammalian and non-mammalian cells (Muller-Hill and Beyreuther 1989). APP is the precursor of the β-amyloid protein (Aβ), which is the major protein component of amyloid plaques in the Alzheimer's disease (AD) brain (Masters *et al.* 1985). Aβ was identified by Glenner and Wong (1984) and the first complete cDNA sequence encoding human APP was cloned in 1987 (Kang *et al.* 1987). The regulation of APP expression, the mechanisms of APP trafficking, post-translational modification and proteolytic cleavage of APP are now well understood. The production of Aβ from APP, which is generally considered to be a key event in the pathogenesis of AD, has also been well studied. However, despite more than two and a half decades of APP research, the normal function of the protein remains unclear. Circumstantial evidence points towards a number of potential biological roles for APP, but a clearly defined mechanism of action has been elusive. The aim of this article is to examine the putative functions of APP in relation to the expression, post-translational processing and structure of APP.

Expression of APP

APP belongs to a family of evolutionarily and structurally related proteins. The human APP cDNA sequence was first cloned from a brain tissue library (Kang *et al.* 1987). Subsequently, a number of homologous APP family members were identified in a variety of mammalian and non-mammalian organisms (Muller and Zheng 2012). The APP family in mammals consists of three members: APP, the APP-like protein-1 (APLP1) and the APP-like protein-2 (APLP2) (Wasco *et al.* 1992, 1993). In humans, the APP gene is located on chromosome 21 (21q21.3), contains 18 exons and extends over a distance of approximately 240 kilobases (Yoshikai *et al.* 1990) (Fig. 1).

Received January 5, 2014; revised manuscript received February 2, 2014; accepted February 3, 2014.

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Abbreviations used: Aβ, β-amyloid; AD, Alzheimer's disease; ADAM, A disintegrin and metalloprotease; AICD, APP intracellular domain; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APLP, APP-like protein; APP, β-amyloid precursor protein; BACE, β-site APP-cleaving enzyme 1; CuBD, copper/metal-binding domain; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HBD, heparin-binding domain; KO, knock-out; KPI, Kunitz protease inhibitor; LRP, low-density lipoprotein-receptor related family proteins; LTP, long-term potentiation; NGF, nerve growth factor; NSPCs, neural stem or progenitor cells; RIP, regulated intramembrane proteolysis; sAPP, secreted β-amyloid precursor protein; Tip60, Tat-interactive protein 60; TrkA, tyrosine receptor kinase A.

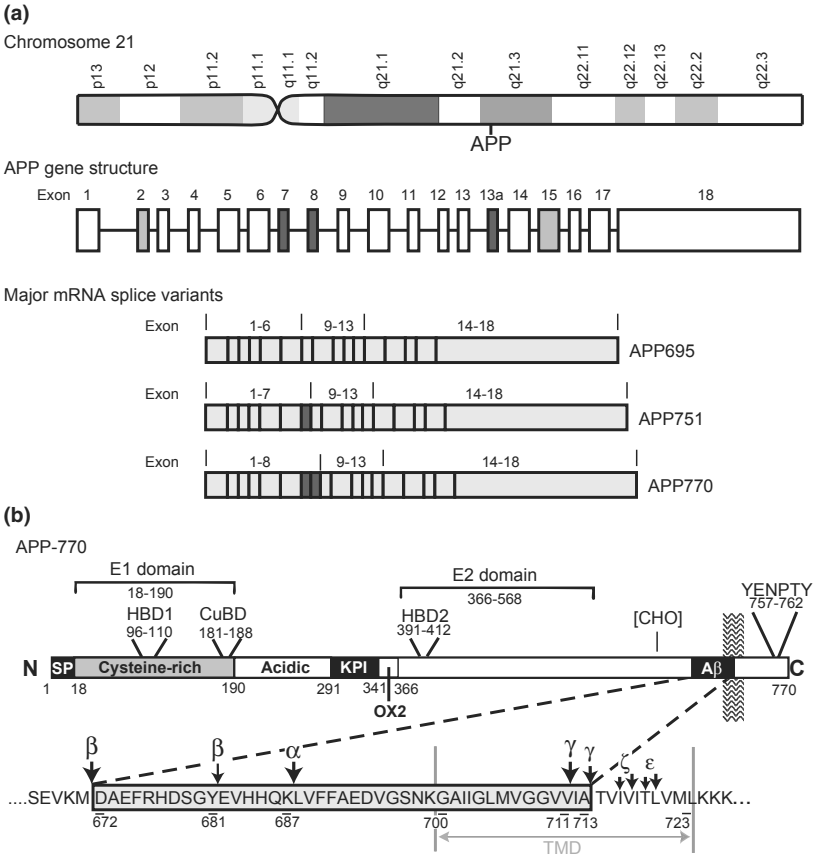

Fig. 1 Structure of the amyloid precursor protein (APP) gene, mRNA and protein. (a) The APP gene is located on chromosome 21q21.3. The gene has 18 exons. Differential mRNA splicing of Exons 7,8 (dark grey) can lead to the expression of 695, 751 and 770 amino-acid isoforms. Exons 2 and 15 (light grey) are spliced out in APP639 and L-APP, respectively. (b) Protein structure. APP has an N-terminal signal peptide (SP). The E1 domain has a heparin-binding domain (HBD1), and a copper-binding domain (CuBD); the E2 domain contains a second heparin-binding domain (HBD2). APP751 and APP770 contain a Kunitz protease inhibitor (KPI) and an Ox-2 antigen domain. Between the E2 and Aβ region are two potential N-linked glycosylation sites (CHO). In this region, there is also a potential chondroitin sulphate attachment site that is formed when exon 15 is spliced out. The amino-acid sequence of the Aβ region is shown along with the secretase cleavage sites. The intracellular C-terminal domain contains a YENPTY sorting motif. TMD, transmembrane domain.

The APP promoter sequence indicates that the APP gene belongs to the class of housekeeping genes. The promoter lacks typical TATA and CAAT boxes, but contains consensus sequences for the binding of a number of transcription factors including SP-1, AP-1 and AP-4 sites, a heat shock control element and two Alu-type repetitive sequences (Salbaum *et al.* 1988; Izumi *et al.* 1992; Quitschke and Goldgaber 1992). The presence of SP-1, AP-1 and AP-4 sites in the APP promoter, which regulate the expression of proteins associated with cell proliferation and mitosis, as well as cell differentiation, suggests that APP has a function related to cell growth or maturation. Consistent with this idea, the expression of APP or APP-like proteins is increased during development and in association with neurite outgrowth and synaptogenesis (Clarris *et al.* 1995).

During transcription, differential splicing of APP mRNA can result in a number of APP splice variants (Fig. 1). The major expressed isoforms of APP have 770, 751 or 695 amino acid residues. The APP751 and APP695 isoforms are produced as a result of splicing out of exons 7 and/or 8 (Fig. 1a) (Kang *et al.* 1987; Tanzi *et al.* 1988; Weidemann *et al.* 1989). Some less common splice variants have also been reported, such as L-APP, which lacks exon 15 (Pangalos *et al.* 1996) and APP639, which lacks exons 2, 7 and 8 (Tang *et al.* 2003).

APP mRNA is expressed in a wide variety of tissues including the nervous system (brain, spinal cord, retina), immune system (thymus, spleen), muscle (smooth, cardiac and skeletal), kidney, lung, pancreas, prostate gland and thyroid gland (Liu *et al.* 2008). However, the mRNA splice variants of APP are expressed in different amounts in different cells. APP695 is the predominant neuronal isoform (Kang *et al.* 1987), but non-neuronal cells express mostly APP770 and APP751 (Rohan de Silva *et al.* 1997). L-APP is expressed in leucocytes, microglia and astrocytes (Konig *et al.* 1992). APP639 is expressed widely in foetal tissue, but only in the liver of adults (Tang *et al.* 2003). The widespread expression, distribution and sequence homology of the APP gene family members suggest that APP plays an important role that is common to many different tissues and organisms.

Gene knock-out (KO) studies can be a powerful method for investigating protein function. APP-KO mice are viable and fertile, indicating that the APP gene alone does not play an essential role in development (Zheng *et al.* 1995). Similarly, KO of the *Drosophila* APP homologue (APPL) does not result in a lethal phenotype (Luo *et al.* 1992). However, APP-KO does result in a number of subtle phenotypic abnormalities. APP-KO mice are slightly smaller, with a reduced weight of 15–20% and reduced brain weight (Zheng *et al.* 1995; Magara *et al.* 1999) and APPL-KO in

Drosophila results in a behavioural defect (Luo *et al.* 1992). Importantly, several studies suggest that APP may have a function that is related to the function of other APP family members. Like APP-KO mice, APLP1-KO mice and APLP2-KO mice are both viable and fertile (Heber *et al.* 2000). Double KO of APLP1 and APP does not produce a lethal phenotype; however, both APP/APLP2 double KO mice and APLP1/APLP2 double KO mice have a postnatal lethal phenotype (Heber *et al.* 2000). Furthermore, knockout of APL-1, which is the only APP gene in *C. elegans*, results in a lethal phenotype (Hornsten *et al.* 2007). Therefore these studies suggest that APP has a function that is likely to be related or overlapping with that of APLP2 in mammals.

Post-translational modification, trafficking and processing of APP

After it has been expressed, the newly translated APP polypeptide can undergo a number of post-translational modifications including glycosylation, sulphation, phosphorylation and palmitoylation (Selkoe 2001; Bhattacharyya *et al.* 2013). After modification in the Golgi apparatus, APP is trafficked to the cell surface (Koo *et al.* 1996) before being internalised by clathrin-mediated endocytosis and incorporated into the endosomal-lysosomal system (Yamazaki *et al.* 1996). Most APP is trafficked from the endosome to the lysosome, where it is degraded (Haass *et al.* 1992). However, a portion can be returned to the cell surface (Yamazaki *et al.* 1996).

APP can be post-translationally processed by enzymes termed secretases, which can cleave the protein to produce a number of smaller fragments. The proteolytic processing of APP will not be discussed in detail, as this topic has been well reviewed elsewhere (Haass *et al.* 2012). APP can initially be cleaved by two proteases, α-secretase or β-secretase (Fig. 1b), to produce the secreted ectodomains sAPPα and sAPPβ. Following APP cleavage by α- or β-secretase, the membrane-associated C-terminal fragments (C83 and C99, respectively) can be cleaved by γ-secretase to yield p3 or Aβ, respectively, and a short C-terminal peptide known as the APP intracellular domain (AICD).

A number of enzymes can act as α-secretases. All of them are members of the A disintegrin and metalloprotease (ADAM) family (Buxbaum *et al.* 1998; Koike *et al.* 1999; Lammich *et al.* 1999). The β-secretase has been identified as a type 1 transmembrane aspartyl protease termed the β-site APP-cleaving enzyme 1 (BACE 1) (Hussain *et al.* 1999; Sinha *et al.* 1999; Vassar *et al.* 1999; Yan *et al.* 1999; Lin *et al.* 2000). BACE1 also cleaves APP at position 11 of the Aβ sequence, although the significance of this cleavage is unclear (Fig. 1b) (Liu *et al.* 2002). γ-Secretase is a transmembrane complex consisting minimally of four protein subunits, presenilin 1 or 2, nicastrin, anterior pharynx-defective phenotype and presenilin enhancer 2 (De Strooper *et al.* 1998; Yu *et al.* 2000; Francis *et al.* 2002; Kimberly *et al.* 2003). γ-Secretase cleavage is a type of regulated intramembrane proteolysis (RIP), as cleavage occurs in the middle of the transmembrane domain (Lichtenthaler *et al.* 2011). RIP of APP is thought to occur as a series of cleavages, starting from the C terminal end of the substrate and moving towards the N-terminal region of the transmembrane domain. These cleavage sites have been termed the γ- ε- and ζ- sites (Fig. 1b) (Lichtenthaler *et al.* 2011).

Although the proteolytic processing of APP by β-secretase can lead to the pathological production of Aβ, β-cleavage is a normal process. Generally, the cleavage of transmembrane proteins by an ADAM or BACE (ectodomain shedding) is commonly involved in the activation of a number of functional pathways. Ectodomain shedding by ADAMs is essential for the release of many cytokines and growth factor ligands, such as epidermal growth factor (EGF) (Blobel 2005). Additionally, ADAMs are involved in ectodomain shedding of growth-factor receptors, such as human epidermal growth factor receptor 2 (Liu *et al.* 2006) and Notch (Bozkulak and Weinmaster 2009). Ectodomain shedding by BACE is also likely to be required for the proper function of a number of proteins (Klaver *et al.* 2010). For example, neuregulin is cleaved by BACE1 and ADAM17 to release an ectodomain fragment, which acts in a paracrine manner to stimulate myelination (Fleck *et al.* 2013). Therefore, cleavage by ADAMs or BACE can potentially facilitate cellular signalling in a variety of ways, either by release of growth factors or by ligand-dependent activation of cellular receptors.

RIP by γ-secretase is also a process involved in the normal function of many proteins. RIP can serve two general functions. First, it can remove the membrane-associated fragment that is produced by ectodomain shedding. Second, it can catalyse the production of intracellular signalling domains (Lichtenthaler *et al.* 2011). γ-Secretase has over 80 currently known substrates (Haapasalo and Kovacs 2011). Apart from APP, the most well known γ-secretase substrate is the developmental protein Notch, which is activated by γ-secretase cleavage (De Strooper *et al.* 1999; Struhl and Greenwald 1999). Therefore, it is also possible that γ-secretase cleavage may also be involved in the function of APP.

Structure of APP

Structurally, APP has features of an integral type I transmembrane glycoprotein (Fig. 1b). The structure of APP suggests that it may act as a cell-surface receptor (Kang *et al.* 1987) or as a growth factor (Rossjohn *et al.* 1999). The encoded protein contains a large ectodomain, which includes a cysteine-rich globular domain (E1), an acidic domain, a helix-rich domain (E2) and part of the Aβ sequence, which extends into the transmembrane domain (Fig. 1b). The relatively short cytoplasmic domain contains

the C-terminus, which has some phosphorylation sites and a YENPTY sorting motif (Fig. 1b). This section will discuss the structure and putative interactions of these domains.

E1 domain and acidic region

The cysteine-rich E1 domain of APP shares little amino-acid sequence similarity to non-APP family members. Cysteine-rich globular domains are found in a number of transmembrane domain proteins including scavenger receptors and hepsin, a cell-surface serine protease (Wu and Parry 2007). The E1 domain is divided into two distinct regions, the heparin-binding domain (HBD) and the copper/metal binding domain (Fig. 2). The HBD is formed of a single α-helix and an anti-parallel β-sheet, with a loop rich in basic residues (95-110) that binds to heparin (Small *et al.* 1994; Rossjohn *et al.* 1999) Immediately adjacent to the HBD is a hydrophobic pocket, which could form either a protein-binding site or a dimerisation site (Rossjohn *et al.* 1999). It has been proposed that this region may dimerise in the presence of heparin (Gralle *et al.* 2006; Dahms *et al.* 2010). The size of the putative binding domain at the N-terminus suggests that APP may act as a receptor for a ligand or act as a growth factor (Rossjohn *et al.* 1999), or may bind to an extracellular matrix component (e.g. proteoglycan) (Small *et al.* 1994). Adjacent to the HBD is the copper/metal binding domain, which contains a single α-helix and a short β-sheet (Fig. 2). This region can bind several metal ions (Bush *et al.* 1993). The role of this domain is unclear, but it has been suggested that copper (II) binding and reduction may be a principal function (Multhaup *et al.* 1996). On the C-terminal side of the E1 domain is an acidic region of unknown significance that is rich in glutamic acid and aspartic acid residues. This region also contains a stretch of seven threonine residues (Kang *et al.* 1987).

KPI and Ox-2 antigen domains

Longer isoforms of APP (APP770 and APP751) may contain a Kunitz-type protease inhibitor (KPI) domain and an Ox-2 antigen domain. APP isoforms containing the KPI domain are more commonly expressed in non-neuronal cells (Rohan de Silva *et al.* 1997), suggesting that they may play a role in glial functions such as in wound repair. Clues to the function of these isoforms comes from studies on blood coagulation. KPI-containing forms of APP (APP751 and APP770) are highly expressed in platelets where they can influence wound repair by regulating blood clotting serine proteases (Van Nostrand *et al.* 1991b). As serine proteases are also implicated in neuronal cell growth (Wang and Reiser 2003), it is possible that KPI-containing APP isoforms regulate cell growth by inhibiting one or more of these proteases.

The role of the Ox-2 domain in APP770 is less clear. The Ox-2 antigen is a lymphoid and neuronal cell-surface glycoprotein, which has homology to Thy-1 and immunoglobulin light chains (Clark *et al.* 1985). In APP, the Ox-2 domain is an insert of 19 amino-acid residues that is similar to a region of the Ox-2 antigen. As immunoglobulin loop domains are commonly found in cell-surface receptors and are involved in cell-surface binding and recognition, it seems likely that the Ox-2 domain in APP has a similar function.

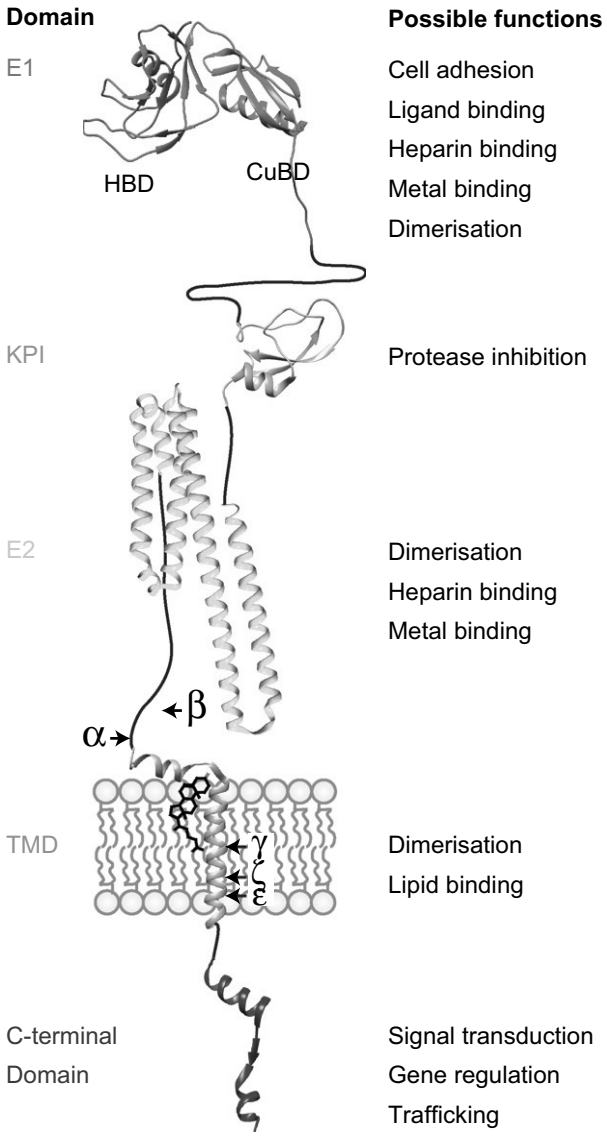

Fig. 2 Hypothetical 3-dimensional structure of amyloid precursor protein (APP) based on the following protein data bank files: E1 domain (3KTM), Kunitz protease inhibitor (KPI) domain (1ZJD), E2 domain (3UMK), transmembrane domain (TMD) (2LLM) and intracellular domain (3DXC). A cholesterol molecule is shown in the proposed lipid-binding site in the transmembrane domain. The acidic region and the region between the E2 and Aβ domains are predicated to have little secondary structure.

E2 domain

The E2 domain is a α-helix rich region (Fig. 2) that can readily dimerize (Xue *et al.* 2011) and may therefore be

involved in APP self-association. The E2 domain has a heparin-binding site (Multhaup 1994; Clarris *et al.* 1997) as well as a number of putative metal-binding sites that may hold the E2 domain in a rigid conformation (Dahms *et al.* 2012). The metal-binding site in the E2 domain has been suggested to possess a ferroxidase activity, which may function in cellular iron export through an interaction with ferroportin (Duce *et al.* 2010). A role in metal homeostasis is supported by the finding that levels of holo-APP can be regulated by iron (Rogers *et al.* 2002), although more work is needed in this area to support this idea.

Chondroitin sulphate attachment domain

An unusual spliced variant of APP (L-APP) is formed by splicing out exon 15. This creates a consensus sequence for the attachment of chondroitin sulphate (Pangalos *et al.* 1995). The proteoglycan form of APP (called 'appican'), which is formed as a consequence of this splicing event, has been found mostly in glia rather than in neurons. Appican contains a chondroitin sulphate E in the repeating disaccharide region and a 4-O-sulphated galactose in the linkage region (Tsuchida *et al.* 2001). Although the function of appican is unclear, it may be involved in adhesion events (Wu *et al.* 1997), as chondroitin sulphates are known to inhibit cell attachment and neurite outgrowth (Cui *et al.* 2013). In addition, appican has been found to bind to certain heparin-binding growth factors such as midkine and pleiotrophin, suggesting that the protein may play some role in the regulation of cell growth (Umehara *et al.* 2004).

Aβ, transmembrane domain and intracellular domain

The Aβ region on the C-terminal side of the E2 domain lies partly within the ectodomain and partly within the transmembrane domain. A GxxxG sequence motif within the transmembrane domain has been implicated in homodimerisation (Munter *et al.* 2007) and in cholesterol binding (Barrett *et al.* 2012; Fig. 2).

From a functional standpoint, the C-terminal cytoplasmic domain of APP is arguably the most interesting region. The structure and possible interactions of this region have been reviewed in detail elsewhere (Kerr and Small 2005; Schettini *et al.* 2010). The intracellular domain of APP is highly conserved among APP family members and contains a YENPTY sorting motif located between residues 757 and 762 of the APP770 isoform. This motif is involved in the facilitation of clathrin-mediated endocytosis and is present in many tyrosine receptor kinases, non-receptor tyrosine kinases, low-density lipoprotein-receptor related family proteins and integrins (Bonifacino and Traub 2003; Lemmon and Schlessinger 2010). Consistent with this role, many studies have demonstrated that the YENPTY motif in APP is involved in the regulation of its trafficking and endocytosis (Lai *et al.* 1995; Perez *et al.* 1999; Ring *et al.* 2007).

Putative functions of APP

Despite the large number of published studies on APP, there is still no clear consensus on the protein's function. This section aims to summarise the major ideas relating to the function of APP. Coverage of more specific aspects of APP function can be found in other recent reviews (Aydin *et al.* 2012; Chasseigneaux and Allinquant 2012; Muller and Zheng 2012).

Trophic actions of APP

APP has been reported to influence cell proliferation, differentiation, neurite outgrowth, cell adhesion and synaptogenesis. A number of studies suggest that the extracellular domain can stimulate cellular growth. *In vitro*, sAPPα has been reported to alter the growth of fibroblasts, keratinocytes, B109 cells, FRTL-5 cells, PC12 cells and neurons (Saitoh *et al.* 1989; Araki *et al.* 1991; Milward *et al.* 1992; Jin *et al.* 1994; Ninomiya *et al.* 1994; Pietrzik *et al.* 1998; Hoffmann *et al.* 2000; Young-Pearse *et al.* 2008). Additionally, there are some reports that infusion of sAPPα after traumatic brain injury can improve neuronal survival and recovery (Thornton *et al.* 2006). Genetic knock-in of sAPPα into APP/APLP2 double KO mice (APPsα-DM mice) rescues the lethal phenotype of the double KO (Weyer *et al.* 2011), supporting a role for sAPPα in growth. Similarly, knock-in of the extracellular domain fragment of APL-1 from *C. elegans* rescues the lethal phenotype of the APL-1 KO (Hornsten *et al.* 2007). Collectively, these studies provide good evidence that APP has a trophic function, and that the extracellular region of APP is involved in this function.

Effects on neural stem cell proliferation and differentiation

As APP is co-ordinately expressed in neuroblasts and neurons at the time of cell proliferation and differentiation (Fukuchi *et al.* 1992; Masliah *et al.* 1992; Salbaum and Ruddle 1994; Clarris *et al.* 1995; Reinhard *et al.* 2005), this has led to the idea that APP may play a role in the regulation of stem-cell proliferation or differentiation. Indeed, APP is processed in a manner that is very similar to the protein Notch, which regulates neural stem cell differentiation (Ables *et al.* 2011). Therefore, it is possible that APP may have a similar or related developmental function to that of Notch (Kimberly *et al.* 2001).

There is strong evidence that APP is able to stimulate the proliferation of neural stem or progenitor cells (NSPCs). For example, sAPPα and sAPPβ can promote the proliferation of NSPCs (Hayashi *et al.* 1994; Ohsawa *et al.* 1999; Demars *et al.* 2011; Baratchi *et al.* 2012). Hayashi *et al.* (1994) examined the effect of secreted APP770 on NSPC proliferation and found secreted APP770 had a stronger effect on NSPC proliferation than secreted APP695. A more recent study reported that inhibition of α-secretase reduced

NSPC proliferation and that sAPPα was able to rescue this effect (Demars *et al.* 2011). In another study, sAPPα infused into the ventricles of mice was found to bind to epidermal growth factor receptor (EGFR) expressing stem cells in the subventricular zone (Caille *et al.* 2004). Both the secretion of EGF and the proliferation of the EGFR-expressing cells were increased by sAPPα infusion (Caille *et al.* 2004).

To examine the specific contribution of APP to stem cell proliferation, our group examined the ability of NSPCs derived from APP transgenic mice to proliferate. We found that the expression of APP positively correlated with the proliferation of NSPCs (Hu *et al.* 2013). However, surprisingly, the APP-induced increase in NSPC proliferation was not due to the secretion of sAPPα, but rather to the secretion of cystatin C (Hu *et al.* 2013). Therefore, APP could potentially influence NSPC proliferation through two different mechanisms, i.e. either via the production of sAPPα or via cystatin C release.

Studies on APP transgenic mice also suggest the possible involvement of APP in NSPC proliferation. Some studies have reported increased NSPC proliferation in APP mice, but have also suggested the effect was due to Aβ (Verret *et al.* 2007; Kolecki *et al.* 2008; Sotthibundhu *et al.* 2009). J20 mice, which overexpress human APP with the Swedish and Indiana familial AD mutations, have a 2-fold increase in the number of proliferating stem cells in the dentate gyrus and subventricular zone at an age of 3 months (Jin *et al.* 2004; Lopez-Toledano and Shelanski 2007). In contrast, a number of studies have reported decreased NSPC proliferation in APP mice (Haughey *et al.* 2002; Dong *et al.* 2004; Donovan *et al.* 2006; Naumann *et al.* 2010) or no effect of APP on NSPC proliferation *in vivo* (Yetman and Jankowsky 2013). As Aβ starts to accumulate in APP mice, the proliferation of neural stem cells decreases (Lopez-Toledano and Shelanski 2007), suggesting that the build-up of Aβ may reduce stem cell proliferation.

APP may also play a role in regulating the differentiation of NSPCs. A study using human embryonic stem cells found that APP overexpression or addition of sAPPα enhanced neuronal differentiation (Freude *et al.* 2011). We also found that APP-overexpressing NSPCs derived from Tg2576 mice possessed a greater potential to differentiate into neurons, whereas cells derived from APP KO mice exhibited decreased neuronal differentiation (Hu *et al.* 2013). Another recent study has suggested that sAPPα/β may cause an increase in glial cell differentiation (Baratchi *et al.* 2012). APP expression is probably not mandatory for the initiation of neuronal differentiation, as embryonic stem cells derived from APP triple KO mice still form neuronal precursors (Bergmans *et al.* 2010). However, the differentiation of neuronal precursors appears to be delayed *in vivo* when APP/APLP1 and APLP2 expression is reduced (Shariati *et al.* 2013).

Effects on neurite outgrowth, synaptogenesis and synaptic plasticity

APP can promote neurite outgrowth in cell culture (Small *et al.* 1994; Allinquant *et al.* 1995). Furthermore, APP expression is upregulated rapidly in axons in response to axonal injury, possibly as part of a repair mechanism (Gentleman *et al.* 1993). One possible mechanism by which APP promotes neurite outgrowth is by regulating cell-substrate adhesion. APP is reported to bind to laminin, collagen type I and heparan sulphate (Kibbey *et al.* 1993; Beher *et al.* 1996; Clarris *et al.* 1997), all of which can influence neurite outgrowth. APP may also promote cell-cell adhesion (Soba *et al.* 2005). For example, in the presence of heparin, APP can form trans-dimers that could form cell-to-cell contacts (Gralle *et al.* 2006; Dahms *et al.* 2010). This trans-dimerisation mode of action has also been proposed as a mechanism for the stabilisation of synapses by APP (Wang *et al.* 2009). APP may also modulate the activity of other proteins involved in cell adhesion. APP reportedly interacts with several cell-adhesion molecules including integrins, fasciclin II, contactin 4, neuroglia cell adhesion molecule, and transient axonal glycoprotein-1 (Yamazaki *et al.* 1997; Ashley *et al.* 2005; Ma *et al.* 2008; Osterfield *et al.* 2008). These studies suggest a number of mechanisms by which APP may influence adhesion, although the precise mechanisms still remain obscure.

APP may also be involved in the regulation of synaptogenesis. During development, APP is expressed in both pre- and postsynaptic sites and its level is dramatically increased during the critical period of synaptogenesis (Loffler and Huber 1992; Clarris *et al.* 1995; Wang *et al.* 2009). Clarris *et al.* (1995) found that APP expression was increased in mitral cells of the olfactory bulb at precisely the stage when neurites from olfactory receptor neurons were coming in contact with the mitral cell dendrites. In neurons, a pool of APP is also preferentially found in the post-synapse, suggesting a synaptic role for this protein (Shigematsu *et al.* 1992).

APP KO mice display a number of neurological deficiencies that may be explained by an effect on synaptogenesis, such as a deficit in grip strength and locomotor activity (Zheng *et al.* 1995; Ring *et al.* 2007). APP-KO mice also have a number of deficits that are associated with altered synaptic function, such as hypersensitivity to kainate-induced seizures, alterations in dendritic spine density, and reduced performance in tests of spatial memory (Steinbach *et al.* 1998; Dawson *et al.* 1999). APP/APLP2 double KO mice have impaired neuromuscular junction formation, as demonstrated by a reduced number of synaptic vesicles, excessive terminal sprouting, incorrect apposition of pre- and post-synaptic proteins and impaired synaptic transmission (Wang *et al.* 2005). These synaptic deficits may be responsible for the lethality of the APP/APLP2 double KO (Wang *et al.* 2005).

A role for APP in regulating synaptic plasticity, learning and memory has also been proposed. APP may alter expression of the GluR2 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, which plays an important role in regulating synaptic calcium permeability (Lee *et al.* 2010). APP may also affect synaptic calcium by altering cell-surface expression of the NMDA receptor (Cousins *et al.* 2009; Hoe *et al.* 2009). As sAPPα is secreted during long-term potentiation (LTP) (Fazeli *et al.* 1994), it may play a role in the regulation of LTP. However, whether cognitive deficits in APP mice (Chapman *et al.* 1999) are due to APP-induced disruption of LTP (Weyer *et al.* 2011) or whether they are due to effects of Aβ, as seems likely (Janus *et al.* 2000; Morgan *et al.* 2000), remains to be established. Indeed, some studies suggest that APP can directly increase LTP (Ishida *et al.* 1997; Seabrook *et al.* 1999; Taylor *et al.* 2008).

Function of APP in blood coagulation

The predominant forms of APP in blood contain the KPI domain (Bush *et al.* 1990; Gardella *et al.* 1990; Van Nostrand *et al.* 1991a). These isoforms have been suggested to have a role in the regulation of blood coagulation. In platelets, APP, sAPP and Aβ accumulate in α-granules, which are vesicles that store a variety of clotting factors (Van Nostrand *et al.* 1991b; Blair and Flaumenhaft 2009). Upon platelet stimulation, APP, sAPP and Aβ are released, along with a number of another components of the coagulation cascade (Bush *et al.* 1990; Gardella *et al.* 1990; Smith *et al.* 1990; Van Nostrand *et al.* 1990; Smith 1997). The KPI domain of APP is a potent inhibitor of the coagulation factors XIa, IXa and Xa (Smith *et al.* 1990; Schmaier *et al.* 1993; Scandura *et al.* 1997). Factor XIa, for example, is strongly inhibited (K_i = 400 pM) (Smith *et al.* 1990; Scandura *et al.* 1997). Notwithstanding the role of the KPI domain, other regions of APP may also participate. For example, inhibition of factor XIa by APP is enhanced in presence of heparin, suggesting an involvement of the heparin-binding regions of APP (Smith *et al.* 1990). The E1 N-terminal heparin-binding domain of APP is also reported to inhibit the activation of factor XII and to inhibit platelet activation, independently of the KPI domain (Niwano *et al.* 1995; Henry *et al.* 1998).

KPI-containing forms of APP can inhibit blood coagulation *in vitro*, consistent with a role of APP as an inhibitor of coagulation (Schmaier *et al.* 1993; Annich *et al.* 1999). Genetic overexpression of APP in mice decreases cerebral thrombosis and also increases the severity of haemorrhage in animal models, whereas KO of APP has the opposite effect (Xu *et al.* 2005, 2007). The anti-coagulant function of APP is also conserved among APP family members (Xu *et al.* 2009). Therefore in the circulatory system, other APP family members may also have functions in the clotting cascade.

What is the mechanism of APP signalling?

Despite the many reports of effects of APP on cell proliferation, neurite outgrowth and synaptogenesis, the mechanisms that underlie these effects have not been fully elucidated. The original description of the APP gene noted that the structure of APP resembles a cell-surface receptor (Kang *et al.* 1987), however a receptor function for APP has not been unequivocally established. A major missing piece of information is the identity of a physiological ligand that activates the APP 'receptor'. F-spondin has been suggested to be a potential APP ligand (Ho and Sudhof 2004). However, the strongest support for the idea that APP functions as a receptor comes from studies that suggest APP can activate intracellular signal transduction mechanisms.

The C-terminal domain (residues 732-751) has been suggested to be a binding site for G-proteins (Nishimoto *et al.* 1993). The binding of an extracellular antibody to the N-terminal domain of APP may result in signal transduction by activating the guanosine 5′-triphosphate-binding protein $G\alpha_O$ (Okamoto *et al.* 1995; Murayama *et al.* 1996). The significance of G-protein coupled APP signalling has yet to be fully elucidated, but studies of the insect APP homologue APPL suggest that an APP-G-protein interaction could be involved in the control of neuronal migration (Ramaker *et al.* 2013).

Other mechanisms of signal transduction have also been proposed. APP has been suggested to activate gene transcription in a similar manner to Notch, which signals through the γ-secretase-mediated release of the Notch intracellular domain. This domain translocates to the nucleus and activates gene transcription. The AICD fragment of APP has also been reported to translocate to the nucleus (Cupers *et al.* 2001; Gao and Pimplikar 2001). Normally AICD is prone to degradation (Kimberly *et al.* 2001). However, AICD can be bound by Fe65, which binds to the YENPTY motif through its phosphotyrosine-binding domain (Fiore *et al.* 1995). Fe65 binding may help to stabilise AICD (Kimberly *et al.* 2001). After translocating to the nucleus, the Fe65-bound AICD has been reported to form a transcriptionally active complex in combination with Tat-interactive protein 60 (Tip60), which is a histone acetyltransferase (Cao and Sudhof 2001; Gao and Pimplikar 2001). A number of target genes have been reported for AICD. These genes include KAI1 (Baek *et al.* 2002), APP, BACE, Tip60 (von Rotz *et al.* 2004), glycogen synthase kinase-3β (Kim *et al.* 2003), EGFR (Zhang *et al.* 2007), p53 (Checler *et al.* 2007), neprilysin (Belyaev *et al.* 2009) and low-density lipoprotein-receptor related family proteins (Liu *et al.* 2007).

Despite the evidence that the AICD may be involved in the regulation of gene transcription, some studies suggest that the role of AICD may not be quite so straightforward. For example, γ-secretase-induced AICD release is not necessary

for Tip60 activation (Hass and Yankner 2005). Fe65 has also been reported to signal gene transcription independently of APP (Yang *et al.* 2006). Additionally, many of the downstream gene targets of the proposed AICD complex have been questioned (Chen and Selkoe 2007; Repetto *et al.* 2007; Waldron *et al.* 2008; Aydin *et al.* 2011) and the mechanism is still unclear (Chen and Selkoe 2007; Waldron *et al.* 2008).

APP may also exert its physiological effects via the secreted fragments sAPPα or sAPPβ. At present, it is not clear whether secreted APP can activate a specific signal transduction pathway via, for example, a growth factor receptor. Binding studies suggest that there is a high-affinity receptor for secreted APP, which interacts with the E1 domain (Reinhard *et al.* 2013). However, this receptor has not yet been identified. Some putative APP receptors include β1-integrin, lipoprotein receptor related protein-1, class A scavenger receptor, death receptor 6, p75 neurotrophin receptor and APP itself (Kounnas *et al.* 1995; Santiago-Garcia *et al.* 2001; Young-Pearse *et al.* 2008; Gralle *et al.* 2009; Nikolaev *et al.* 2009). However, APP may interact with many other extracellular proteins as well (Bai *et al.* 2008).

To complicate matters, APP's trophic effects may be mediated via other growth factors. For example, sAPP is able to potentiate the action of nerve growth factor (NGF) (Milward *et al.* 1992; Wallace *et al.* 1997; Akar and Wallace 1998). APP can also increase the secretion and expression of cystatin C, which positively modulates the growth of NSPCs. (Hu *et al.* 2013). APP has been suggested to regulate NGF/tyrosine receptor kinase A signalling, through an intracellular interaction involving the C-terminal YENPTY phosphorylation site (Matrone *et al.* 2011). Along similar lines, NGF, EGF, and fibroblast growth factor-2 have all been reported to increase the expression of APP (Ohyagi and Tabira 1993; Villa *et al.* 2001) and NGF, EGF and insulin have been reported to increase the secretion of sAPP (Slack *et al.* 1995; Solano *et al.* 2000; Ruiz-Leon and Pascual 2001; Caille *et al.* 2004). The interplay between these growth factor pathways and APP not only suggest that APP is linked to cellular growth, but also presents a challenge for establishing the direct signalling mechanisms undertaken by APP.

It has also been suggested that the production of Aβ from APP may represent a normal physiological function. However, this suggestion has been controversial. Aβ neither possesses a defined primary structure, nor is it produced as a major pathway of APP processing. Nevertheless, a number of functions for Aβ have been proposed. For example, Aβ has been suggested to be involved in cholesterol transport (Yao and Papadopoulos 2002) and Aβ peptides can increase cell adhesion and neurite outgrowth (Koo *et al.* 1993). Kamenetz *et al.* (2003) found that synaptic activity regulated Aβ production and that Aβ, in turn, selectively suppressed excitatory neurotransmission, suggesting that synaptic activity may be regulated by a negative feedback loop involving Aβ secretion. In contrast, a more recent study by Abramov *et al.* (2009) has suggested that Aβ is a positive endogenous regulator of release probability at hippocampal synapses. The identification of Aβ's normal physiological function (if it has one) is extremely important. As many therapeutic strategies for the treatment of AD aim to prevent Aβ production or increase Aβ clearance from the brain, it is important to ensure that these strategies do not disrupt a normal physiological function.

Summary and conclusions

There is strong evidence that APP plays an important role in cell growth and proliferation. There is also evidence that APP may act as a trophic factor to influence events such as neurite outgrowth and synaptogenesis. As APP is expressed at early stages of nervous system development, APP clearly plays a key role in the growth and maturation of many cells. However, the expression of APP in the mature brain and the up-regulation of APP following traumatic brain injury argue for an important tissue-repair function as well.

Although the role of APP as a growth-regulatory molecule can now be stated with some confidence, the precise mechanism by which APP regulates cell growth is still unclear. The extracellular domain of APP may interact with a cell-surface receptor or a component of the extracellular matrix. The intracellular domain is also undoubtedly important and may interact with a number of cytoplasmic adaptor molecules to facilitate signal transduction or control APP trafficking. However, further research is needed to understand APP's mechanism of action. In particular, future research needs to focus on mechanisms of APP action in which the function of APP is most clearly established. By understanding the mechanism of APP action in well-defined roles (e.g. NSPC proliferation), it may be possible to generalise the findings to understand the mechanisms of APP in relation to other less well-defined roles.

Acknowledgements and conflict of interest disclosure

This work was funded through grants to DHS from the National Health and Medical Research Council of Australia.

All experiments were conducted in compliance with the ARRIVE guidelines. The authors have no conflict of interest to declare.

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