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## Physiological Functions of APP Family Proteins

Ulrike C. Müller and Hui Zheng

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# Physiological Functions of APP Family Proteins

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Biochemical and genetic evidence establishes a central role of the amyloid precursor protein (APP) in Alzheimer disease (AD) pathogenesis. Biochemically, deposition of the  $\beta$ -amyloid (A $\beta$ ) peptides produced from proteolytic processing of APP forms the defining pathological hallmark of AD; genetically, both point mutations and duplications of wild-type APP are linked to a subset of early onset of familial AD (FAD) and cerebral amyloid angiopathy. As such, the biological functions of APP and its processing products have been the subject of intense investigation, and the past 20+ years of research have met with both excitement and challenges. This article will review the current understanding of the physiological functions of APP in the context of APP family members.

Synaptic dysfunction, cognitive decline, and plaque deposition of the  $\beta$ -amyloid peptide A $\beta$ , derived from the  $\beta$ -amyloid precursor protein APP, are hallmark features of Alzheimer disease (AD). Since the molecular cloning of APP, more than 20 years ago (Goldgaber et al. 1987; Kang et al. 1987; Tanzi et al. 1987), a large body of biochemical and genetic evidence has accumulated that identified A $\beta$  as a central trigger for AD pathogenesis. Despite this, the physiological role of APP and the question of whether a loss of its functions contributes to AD are still unclear. The secretases involved in APP processing and A $\beta$  generation have been cloned (see De Strooper et al. 2011; Haass et al. 2011) and have since become major

therapeutic targets. Understanding the physiological function of APP is also of immediate relevance for AD pathogenesis. As A $\beta$  is generated as part of normal APP processing (Haass et al. 1992), deregulation of A $\beta$  production (either during pathogenesis or as a consequence of secretase inhibitors) is expected to simultaneously affect other APP processing products and may thus compromise physiologically important signaling pathways. Two major obstacles complicate the analysis of functions of APP in vivo: (1) APP is subject to complex proteolytical processing that generates several polypeptides each of which likely performs specific functions, and (2) APP is part of a gene family with partially overlapping functions.

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## CELL BIOLOGY AND EXPRESSION

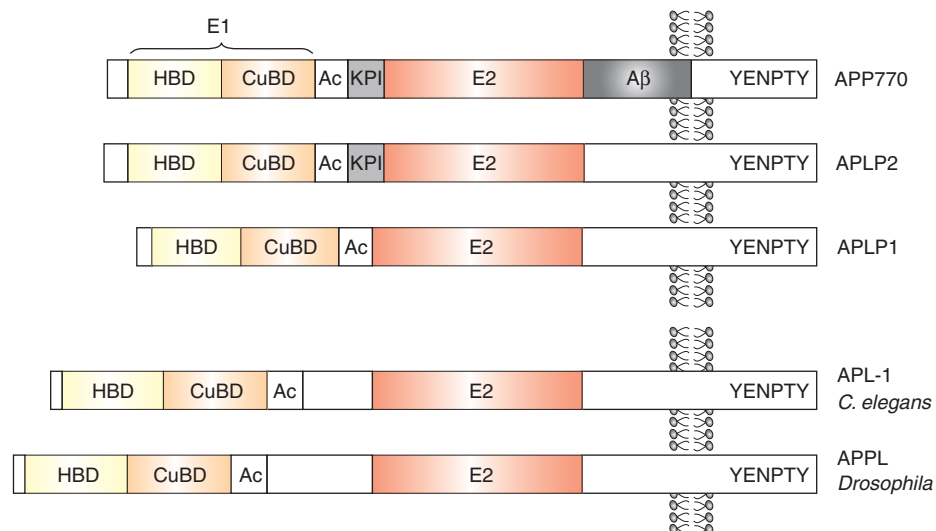
### APP Processing

APP is an integral type I transmembrane protein with a single transmembrane domain, a large extracellular ectodomain, and a short cytoplasmic tail (Fig. 1). Processing is initiated either by cleavage of APP by  $\alpha$ -secretase within the A $\beta$  region, or by cleavage by  $\beta$ -secretase (BACE) at the amino terminus of A $\beta$ , leading to the secretion of large soluble ectodomains, termed APPs $\alpha$  and APPs $\beta$ , respectively. Subsequent processing of the carboxy-terminal fragments (CTF $\beta$  or CTF $\alpha$ ) by  $\gamma$ -secretase results in the production of A $\beta$ , p3, and the APP intracellular domain (AICD). More recently, a novel amino-terminal fragment (N-APP<sub>286</sub>) derived from APPs $\beta$  was identified as a ligand for death receptor 6 (DR6), a member of the TNFR gene family (Nikolaev et al. 2009). Whereas in fibroblasts and nonneuronal cell lines (e.g., HEK293 cells)  $\alpha$ -secretase processing is the dominant pathway, primary neuronal cultures express high levels of BACE and thus generate considerable amounts of APPs $\beta$  and A $\beta$  (Simons et al. 1996; Kuhn

et al. 2010). In adult mouse brain, secreted total APPs constitutes at least 50% of all APP isoforms and in vivo studies using cycloheximide injections revealed a half-life of 4–5 h for both APPs $\alpha$  and APPs $\beta$ , whereas APP-FL is turned over much more rapidly (half-life of ca. 1 h) (Morales-Corraliza et al. 2009).

### APP Gene Family and Structure

APP is a member of an evolutionary conserved gene family including APL-1 in *Caenorhabditis elegans* (Daigle and Li 1993), APPL in *Drosophila* (Rosen et al. 1989; Luo et al. 1990), appa and appb in zebrafish (Musa et al. 2001), and in mammals besides APP the two amyloid precursor-like proteins, APLP1 and APLP2 (Wasco et al. 1992, 1993; Slunt et al. 1994). APP family proteins share conserved regions within the ectodomain, in particular the E1 and E2 domains and the intracellular tail that shows the largest sequence identity (Fig. 1). Interestingly, the extracellular juxtamembrane regions are highly divergent with the A $\beta$  sequence being unique for APP. The E1 domain can be further subdivided into a heparin-binding/growth



**Figure 1.** Schematic overview of domain structure of APP family proteins. All APP family members share conserved E1 and E2 extracellular domains, an acidic domain (Ac) and the YENPTY motif in the carboxyl terminus. Note that A $\beta$  is unique for APP. HBD, Heparin binding domain; CuBD, Copper binding domain; KPI, Kunitz-type protease inhibitor domain.



factor-like domain and a metal (copper and zinc) binding domain. The E1 domain is followed by an acidic region and a Kunitz-type protease inhibitor (KPI) domain (that is subject to alternative splicing in both APP and APLP2). The E2 region contains a second heparin binding domain and a RERMS motif implicated in trophic functions (Ninomiya et al. 1993; Roch et al. 1994). APP family proteins are posttranslationally modified including N- and O-glycosylation, sialylation, and CS GAG modification of the ectodomain and are phosphorylated at multiple sites within the intracellular carboxy-terminal domain (reviewed in Suzuki and Nakaya 2008; Jacobsen and Iverfeldt 2009). Crystal structures of several subdomains (reviewed in Reinhard et al. 2005; Gralle and Ferreira 2007), including the recently determined complete E1 structure (Dahms et al. 2010) and AICD bound to the adaptor protein Fe65 are available (Radzimanowski et al. 2010). Membrane bound APP/APLP holoproteins resemble cell surface receptors and have been shown to bind to extracellular matrix components (see below), but also interact with cell surface proteins including Alcadin (Araki et al. 2003), F-spondin (Ho and Südhof 2004), Reelin (Hoe et al. 2009b), LRP1 (Pietrzik et al. 2004), sorL1/LR11 (Schmidt et al. 2007), Nogo-66 receptor (Park et al. 2006), Notch2 (Chen et al. 2006), and Netrin (Lourenco et al. 2009). Although several of these interactions regulate APP processing, the physiological relevance of these interactions is poorly understood. Interaction screens have led to the identification of multiple intracellular binding partners (reviewed in Jacobsen and Iverfeldt 2009). Notably, the YENPTY motif that is conserved from *C. elegans* to mammalian APP/APLPs, confers clathrin mediated endocytosis, modulates A $\beta$  generation (Perez et al. 1999; Ring et al. 2007), and binds several kinases, as well as adaptor proteins including mDab1, JIP, Shc, Grb2, Numb, X11/mint family, and Fe65 family proteins. Although in vitro studies have shown that these interactions may not only modulate APP processing but may also mediate cell signaling, the in vivo relevance is only starting to be revealed.

Although APLP1 and APLP2 lack the A $\beta$  region they are similarly processed. Both APLPs undergo ectodomain shedding and soluble APLPs have been detected in conditioned medium of transfected cell lines or human cerebrospinal fluid (Slunt et al. 1994; Webster et al. 1995; Paliga et al. 1997). Likewise, p3/A $\beta$ -like fragments (Eggert et al. 2004; Minogue et al. 2009), as well as APLP1 and APLP2 intracellular fragments (termed ALIDs) are generated in a  $\gamma$ -secretase dependent manner (Scheinfeld et al. 2002; Walsh et al. 2003). Whereas there has been robust evidence indicating that APLP2 is processed by  $\alpha$ - and  $\beta$ -secretase (Eggert et al. 2004; Pastorino et al. 2004; Endres et al. 2005), APLP1 shedding appeared to be independent of BACE activity as it was not affected by BACE inhibitors (Eggert et al. 2004; Minogue et al. 2009). A recent study using BACE-KO and overexpressing mice showed, however, that BACE deficiency substantially reduces brain APLP1s levels and that ICDs of APP family members are released in the absence of BACE (Frigerio et al. 2010).

### Expression, Subcellular Localization, and Axonal Transport

APP and APLP2 are expressed ubiquitously, with particularly high expression in neurons, in largely overlapping patterns during embryonic development and in adult tissue (Slunt et al. 1994; Lorent et al. 1995; Thinakaran et al. 1995). In contrast, APLP1 is found primarily in the nervous system (Lorent et al. 1995). Regarding their subcellular localization, APP/APLPs are found both in somata and dendrites as well as in axons (Yamazaki et al. 1995; Back et al. 2007; Hoe et al. 2009a). APP/APLP expression is up-regulated during neuronal maturation and differentiation, undergoes rapid anterograde transport, and is targeted in vesicles distinct from synaptophysin transport vesicles to synaptic sites (Koo et al. 1990; Sisodia et al. 1993; Kaether et al. 2000; Szodorai et al. 2009). The initial hypothesis that APP anchors these vesicles via its carboxyl terminus to kinesin (Kamal et al. 2001), has been broadly questioned (Tienari et al. 1996; Lazarov et al. 2005;

Back et al. 2007). Using time-lapse microscopy, Szodorai recently showed unaltered velocity of APP $\Delta$ CT-GFP transport and a requirement for Rab3A GTPase activity for vesicle assembly (Szodorai et al. 2009).

## IN VITRO AND EX VIVO STUDIES OF APP

### Cell and Synaptic Adhesion

Investigations of conserved domains support an adhesion property for all members of the APP family. The extracellular sequence of APP has been found to interact with various extracellular matrix components, such as heparin (Clarris et al. 1997; Mok et al. 1997), collagen type I (Behr et al. 1996), and laminin (Kibbey et al. 1993), indicating a role of APP in cell-matrix adhesion. Structural and functional studies also implicate a role of the APP extracellular domains in facilitating cell–cell adhesion through transcellular interactions. Of interest, X-ray analysis revealed that the E2 domain of APP could form antiparallel dimers (Wang and Ha 2004). Both Dahms et al. (2010) and Gralle et al. (2006) reported that heparin binding to the extracellular E1 or E2 domain induces APP/APP dimerization. Cell culture studies revealed that APP family members form homo- or heterotypic *cis*-dimers, mainly via the E1 domain and the GxxxG motif in the transmembrane domain (Kaden et al. 2008), and that *cis*-dimerization modulates  $\gamma$ -secretase cleavage (Richter et al. 2010). *Trans*-dimerization of APP family members can promote cell–cell adhesion (Soba et al. 2005). Using a primary neuron/HEK293 mixed culture assay, Wang et al. (2009) reported that transcellular APP/APP interaction induces presynaptic specializations in cocultured neurons. These studies identify APP proteins as a novel class of synaptic adhesion molecules (SAM) with shared biochemical properties as neuexins (NX)/neuroligins (NL), SynCAMs, and leucine-rich repeat transmembrane neuronal proteins (LRRTM) (Scheiffele et al. 2000; Biederer et al. 2002; Graf et al. 2004; Sara et al. 2005; Fogel et al. 2007; Linhoff et al. 2009). Like NX/NL and SynCAM-mediated synaptic adhesion in which

extracellular sequences engage transsynaptic interactions and the intracellular domains recruit pre- or postsynaptic complexes (reviewed in Dalva et al. 2007), both the extracellular and intracellular domains of APP are required to mediate the synaptogenic activity. Consistent with Soba et al. (2005), the E1 domain plays a more active role in synaptic adhesion. Interestingly, the highly conserved GYENPTY sequence of the APP intracellular domain could form a tripartite complex with Munc 18 interacting protein (Mint/X11) and calcium/calmodulin-dependent serine protein kinase (CASK) similar to that of neuexin and SynCAM (Hata et al. 1996; Biederer and Südhof 2000; Biederer et al. 2002), and the SynCAM carboxy-terminal sequence could functionally replace the corresponding APP domain in the coculture assay (Wang et al. 2009), suggesting that the Mint/CASK complexes may be the common mediators for the different classes of synaptic adhesion proteins. Thus, the precise role of APP-mediated synaptic adhesion in central synapses, whether it involves interaction with other SAMs, and the relationship between APP-mediated synaptogenesis and synaptic dysfunction occurring in AD are interesting questions that warrant further investigation.

Besides a direct role of APP/APP interaction in cell and synaptic adhesion, APP has been shown to colocalize with integrins on the surface of axons and at the sites of adhesion (Storey et al. 1996; Yamazaki et al. 1997; Young-Pearse et al. 2008). It has also been reported to interact with other cell adhesion molecules including NCAM (Ashley et al. 2005), NgCAM (Osterfield et al. 2008), and TAG 1 (Ma et al. 2008). As such, APP may play a modulatory role through interacting with these cell adhesion molecules.

### Neural and Synapto-Trophic Functions

A large body of evidence supports a trophic function of APP in neurons and synapses. Consistent with its expression pattern, deletion or reduction of APP is associated with impaired neuronal viability in vitro and reduced synaptic activity in vivo (Allinquant et al. 1995; Perez



et al. 1997; Hérard et al. 2006). Hippocampal neurons deficient for APP (or APLPs) show initially reduced neurite outgrowth, whereas, after prolonged culture axons are elongated and neurite branching is reduced (Perez et al. 1997; Young-Pearse et al. 2008). However, it should be noted that studies using neuronal cultures derived from various APP/APLP1/APLP2 knockout combinations or obtained by differentiation of embryonic stem cells lacking APP family members failed to detect a requirement of APP proteins in either neuronal differentiation or survival (Heber et al. 2000; Bergmans et al. 2010).

The trophic activity of APP can be mediated by the full-length protein and likely involves the APP adhesion properties discussed above. In particular, binding of APP to extracellular proteoglycans has been suggested to play a role in inducing neurite outgrowth, and a peptide homologous to the APP heparin-binding domain blocked this effect (Small et al. 1994, 1999). Qiu et al. found that when APP-transfected CHO cells were used as a substrate for the growth of primary rat hippocampal neurons, increased surface APP expression stimulated short-term neuronal adhesion and longer-term neurite outgrowth (Qiu et al. 1995). Nevertheless, ample literature points to a potent role of the  $\alpha$ -secretase processed soluble fragment (APPs $\alpha$ ) in the growth promoting and neurotrophic activities. One of the earliest indications came from the observation that secreted APPs, through the "RERMS" motif in the E2 domain, promoted fibroblast proliferation (Saitoh et al. 1989; Ninomiya et al. 1993a; Jin et al. 1994). Moderate overexpression of APP in transgenic mice, infusion of APPs $\alpha$  or the RERMS pentapeptide into the ventricle, or an indirect increase of APPs $\alpha$  levels because of overexpression of  $\alpha$ -secretase, has been shown to increase synaptic density (Mucke et al. 1994; Roch et al. 1994; Meziane et al. 1998; Bell et al. 2008). Moreover, gain- or loss-of-function studies with either intraventricular APPs $\alpha$  infusion, down-regulation by antibody infusion or pharmacological inhibition of  $\alpha$ -secretase coherently showed a function for APPs $\alpha$  in spatial memory and for LTP (Turner et al. 2003; Taylor et al. 2008).

Caille et al. provided evidence that APPs $\alpha$  and APLP2s act as cofactors for epidermal growth factor (EGF) to stimulate the proliferation of neurosphere cultures in vitro and neural stem cells in the subventricular zone of adult rodent brain in vivo (Caille et al. 2004). Gakhar-Koppole et al. (2008) and Rohe et al. (2008) also reported that APPs stimulated neurogenesis and neurite outgrowth, but suggested that it is mediated through enhanced ERK phosphorylation and may be dependent on membrane-bound APP. Han et al. (2005) offered yet a different mechanism that the growth promoting property is mediated by the ability of APPs $\alpha$  to down-regulate CDK5 and inhibit  $\tau$  hyperphosphorylation. Of direct physiological relevance, growth and neuronal phenotypes reported in APP deficient mice were shown to be fully restored by expressing only APPs $\alpha$  (Ring et al. 2007), and the lethality of the *C. elegans apl-1* null mutant can be rescued by expressing only the APL-1 extracellular domain (Hornsten et al. 2007; Wiese et al. 2010).

### Axon Pruning and Degeneration

APPs $\alpha$  has shown synaptotrophic and neuroprotective functions, whereas APPs $\beta$  was reported to be much less active or may even be toxic (reviewed in Turner et al. 2003). Recently, employing organotypic slice cultures, Copanaki et al. showed that APPs $\alpha$  (and not APPs $\beta$ ) antagonizes dendritic degeneration and neuron death triggered by proteasomal stress (Copanaki et al. 2010). The most striking difference came from the study of Nikolaev et al. (2009), which reported that soluble APPs $\beta$ , but not APPs $\alpha$ , undergoes further cleavage to produce an amino-terminal  $\sim 35$  kDa APP derivative (N-APP), which in turn binds to the death receptor DR6 and mediates axon pruning and degeneration under trophic withdrawal conditions. The investigators attempted to link this pathway to both axonal pruning during normal development and axon- and neurodegeneration in AD. The APPs $\beta$  isoform specific cleavage and the differential, or opposite activities between APPs $\alpha$  and APPs $\beta$ , are intriguing as there is only 17 amino acids differences between the two isoforms.

## Intracellular Signaling

Besides the  $\gamma$ -secretase cleavage that yields A $\beta$ 40 and A $\beta$ 42, PS-dependent proteolysis also occurs at the  $\epsilon$ -site of the membrane-intracellular boundary to generate AICD (Sastre et al. 2001; Weidemann et al. 2002; Zhao et al. 2005). This cleavage is highly reminiscent of the PS-mediated release of the Notch intracellular domain (NICD) obligatory for Notch signaling (reviewed in Selkoe and Kopan 2003). Accordingly, AICD has been shown to translocate to the nucleus (Cupers et al. 2001; Gao and Pimplikar 2001; Kimberly et al. 2001). AICD is labile but can be stabilized by Fe65 (Kimberly et al. 2001). Using a heterologous reporter system, AICD was shown to form a transcriptionally active complex with Fe65 and the chromatin-remodeling factor Tip60 (Cao and Südhof 2001; Gao and Pimplikar 2001). However, subsequent analyses painted a more complex picture: (1) Follow-up studies by Cao et al. provided a modified model, whereby Fe65 is first recruited to the membrane-anchored APP where it is activated through an unknown mechanism.  $\gamma$ -secretase cleavage then releases Fe65 together with AICD, thereby allowing Fe65 to enter the nucleus and to interact with Tip60 (Cao and Südhof 2004); (2) Hass and Yankner revealed that PS-dependent AICD production is not required for the APP signaling activity as it proceeds normally in PS null cells and on PS inhibitor treatment (Hass and Yankner 2005). Instead, the investigators provided an alternative pathway involving Tip60 phosphorylation; (3) a later report documented that the proposed signaling activity is, in fact, executed by Fe65 independently of APP (Yang et al. 2006). Last, the link of Fe65 to chromatin remodeling instead of transcription suggests that APP may not act on specific genes, but rather modulates the overall transcriptional state of a cell (Giliberto et al. 2008).

Regardless of the molecular mechanisms, a *trans*-activating role of the APP/Fe65/Tip60 complex has been consistently documented, at least in overexpression systems using artificial reporter constructs. Accordingly, effort has been taken to identify the downstream targets,

which reportedly include KAI (Baek et al. 2002), GSK3 $\beta$  (Kim et al. 2003; Ryan and Pimplikar 2005), neprilysin (Pardossi-Piquard et al. 2005), EGFR (Zhang et al. 2007), p53 (Checler et al. 2007), LRP (Liu et al. 2007), APP itself (von Rotz et al. 2004), and genes involved in calcium regulation (Leissring et al. 2002) and cytoskeletal dynamics (Müller et al. 2007). However, the validity of these proposed targets have been either questioned or disputed (Hebert et al. 2006; Yang et al. 2006; Chen and Selkoe 2007; Repetto et al. 2007; Giliberto et al. 2008; Tamboli et al. 2008; Waldron et al. 2008; Aydin et al. 2011). Overall, as attractive as the APP/AICD signaling model is, and regardless of the intense effort devoted to this topic in the past 10 years, neither the molecular pathways nor the downstream targets have been unambiguously established.

## Apoptosis

Interestingly, AICD has been shown to be further cleaved by caspases at amino acid 664 of APP (695 numbering) to release two smaller fragments, Jcasp and C31; the latter contains the last 31 amino acids of APP and has been proposed to mediate cytotoxicity in a full-length APP dependent manner (Bertrand et al. 2001; Lu et al. 2003; Park et al. 2009). In support of a functional role of this pathway, neuronal cultures generated from AICD transgenic mice are found to be more susceptible to toxic stimuli (Giliberto et al. 2008), and impaired synaptic plasticity and learning and memory seen in APP transgenic models were corrected in a mouse line in which the caspase site was mutated despite the presence of abundant amyloid pathology (Galvan et al. 2006). However, a more recent publication challenged these findings (Harris et al. 2010), and the physiological significance of this cleavage event thus requires further investigation.

## IN VIVO LOSS-OF-FUNCTION STUDIES OF APP FAMILY PROTEINS

### *C. elegans* and *Drosophila*

*Drosophila* deficient for the single APPL gene are viable, show a defect in fast phototaxis (Luo et al. 1992), and reduced synaptic bouton

numbers at the neuromuscular junction NMJ. This activity involves a complex between APPL, the cell adhesion molecule fasciclin and *Drosophila* Mint/X11 (Torroja et al. 1999; Ashley et al. 2005). Knockout of the *C. elegans* ortholog APL-1, which is expressed in multiple tissue including neurons and muscle, disrupts molting and morphogenesis and results in laval lethality. Interestingly, this lethality could be rescued by neuronal expression of only the extracellular domain of APL-1, suggesting a key physiological role for this APP $\alpha$  related fragment (Hornsten et al. 2007).

### APP/APLP Single Knockout Mice

Three APP mouse mutants, one carrying a hypomorphic mutation of APP (APP $\Delta$ ) (Müller et al. 1994) and two with complete deficiencies of APP (Zheng et al. 1995; Li et al. 1996) have been generated and revealed comparable phenotypes (Anliker and Müller 2006). APP-KO mice are viable and fertile, showing reduced body weight (about 15%–20% smaller) and brain weight (about 10% less) that was associated with reduced size of forebrain commissures and agenesis of the corpus callosum, consistent with a role of APP for neurite outgrowth and/or axonal pathfinding (Zheng et al. 1995; Magara et al. 1999). APP-KO mice also showed increased brain levels of copper (White et al. 1999), cholesterol and sphingolipid (Grimm et al. 2005). In addition, APP-KO animals showed hypersensitivity to kainate-induced seizures (Steinbach et al. 1998), suggesting a role of APP for neuronal excitation/inhibition balance. Behavioral studies revealed reduced locomotor and exploratory activity, altered circadian activity (Müller et al. 1994; Zheng et al. 1995; Ring et al. 2007), and a deficit in grip strength (Zheng et al. 1995; Ring et al. 2007), indicating compromised neuronal or muscular function (see also NMJ phenotype of double knockouts below). In the Morris water maze, APP-KO mice show impairments, both in learning and spatial memory, that are associated with a defect in long-term potentiation (LTP) (Dawson et al. 1999; Phinney et al. 1999; Seabrook et al. 1999; Ring et al. 2007). However, these

impairments are not caused by a gross loss of neurons or synapses, as stereological quantification revealed normal neuron and synaptic bouton counts in the hippocampus of aged APP null mice (Phinney et al. 1999). Surprisingly, a recent study showed that APP deficiency leads to an increase in spine density in apical dendrites of cortical (layers 3 and 5) neurons (Bittner et al. 2009). The same group had previously reported an increase in synapse density in low-density cultures of self-innervating (autaptic) hippocampal neurons (Priller et al. 2006), but normal synaptic density in adult APP-KO mice (Priller et al. 2006). Thus, adaptive mechanisms (e.g., activity-dependent synaptic elimination) likely counteract early developmental changes. It remains to be seen whether alterations in spine density are also present in other brain areas of APP family KOs, which signaling pathways are involved, and how this may relate to functional changes.

Although basal glutamatergic synaptic transmission and paired pulse facilitation was unaffected in hippocampal slice recordings of APP-KO mice, a deficit in paired pulse depression of GABAergic IPSCs may contribute to the LTP defect of APP-KO mice (Seabrook et al. 1999). This may involve, as hypothesized (Seabrook et al. 1999), a reduction in feedback suppression mediated by presynaptic GABA $_B$  autoreceptors (but see below Yang et al. 2009). Although the molecular mechanisms of these alterations remain to be determined, these studies indicate that defects in Ca $^{2+}$ -handling, synaptic plasticity and/or neuronal network properties, rather than gross structural changes, cause functional impairments of APP knockout mice. Indeed, recently it was shown that APP is involved in the regulation of L-type Ca channel LTCCs level (Yang et al. 2009). APP-KO mice showed increased levels of Ca $v$ 1.2 channels in the striatum that lead to alterations in GABAergic short term plasticity in striatal and hippocampal neurons, such as reduced GABAergic paired pulse inhibition and increased GABAergic posttetanic potentiation (Yang et al. 2009). Moreover, there is recent evidence from overexpression and APP knockdown studies in hippocampal



neurons indicating an A $\beta$  independent role of APP for the regulation of Ca<sup>2+</sup>-oscillations (Santos et al. 2009).

### Combined Knockouts of APP Family Members

To test whether APLPs may functionally compensate for APP deficiency, mice lacking individual or all possible combinations of APP family proteins have been generated (reviewed in Anliker and Müller 2006; Zheng and Koo 2006). APLP1-KO mice revealed a somatic growth deficit as the only abnormality (Heber et al. 2000a), whereas, to date, no abnormalities have been found for APLP2-KO mice (von Koch et al. 1997). It should be kept in mind, however, that APLP deficient mice have not been examined in comparable detail as APP-KOs. In contrast to the subtle phenotypes of single mutants, double knockout mice (DKO) carrying APLP2/APLP1 and APLP2/APP-deficiencies proved lethal shortly after birth (von Koch et al. 1997; Heber et al. 2000). Surprisingly, APLP1/APP-deficient mice turned out to be viable, fertile, and without any additional abnormalities (Heber et al. 2000). These data indicated redundancy between APLP2 and both other family members, and corroborate a key physiological role for APLP2. None of the lethal double mutants, however, displayed obvious histopathological abnormalities (examined at the light microscopic level) in the brain. So far, the postnatal lethality of the APP/APLP2-DKO precluded the analysis of APP/APLP2 mediated functions in the postnatal and adult nervous system. However, organotypic hippocampal slice cultures can be studied in case of early postnatal lethality. Of note, using this technique, APP/APLP2-DKO mutants revealed defects in basal glutamatergic synaptic transmission that were absent in single mutants (Schrenk-Siemens et al. 2008). Thus, a more complete picture of APP/APLP function in the CNS will await the generation of brain specific conditional mutants.

In the peripheral nervous system, APP and APLP2 play a redundant and essential role for neuromuscular synapse formation and

function, as diaphragm preparations from newborn APP/APLP2-DKO mice show excessive nerve growth, a widened endplate pattern, reduced apposition of pre- and postsynaptic components, and severely impaired (spontaneous and evoked) neurotransmission (Wang et al. 2005). Moreover, submandibular ganglia of APP/APLP2-DKO mice showed a reduction in active zone size, synaptic vesicle density, and number of docked vesicles (Yang et al. 2005) pointing to primarily presynaptic defects (but see conditional mutants). Thus, impaired function of the NMJ likely causes early postnatal lethality of combined mutants and defects in grip strength in APP single KOs. Indeed, subsequent analysis of neuromuscular transmission of APP-KO mice showed reduced paired pulse facilitation that was associated with an increase in asynchronous presynaptic transmitter release mediated by N- and L-type Ca<sup>2+</sup> channels (Yang et al. 2007).

Triple KO mice lacking all three APP family members die shortly after birth. Unlike the DKO mutants, which did not display histological alterations in the brain, 80% of all triple knockouts showed cranial abnormalities (Herms et al. 2004). The majority of animals showed focal dysplasia resembling human type II lissencephaly and a partial loss of cortical Cajal-Retzius cells (Herms et al. 2004). Within affected areas, neuronal cells from the cortical plate migrated beyond their normal positions and protruded into the marginal zone and the subarachnoid space, indicating a critical role for APP family members in neuronal adhesion and/or positioning (Herms et al. 2004). Interestingly, a very similar phenotype was detected in mice lacking the APP interactors Fe65 and Fe65L1 (Guénette et al. 2006). These data suggest that APP family proteins may mediate some of their function(s) via an APP/Fe65 signaling complex. A role of APP family members in neuronal positioning/migration is further supported by acute in utero knockdown of APP (Young-Pearse et al. 2007) in rats using shRNA electroporation. In summary, these data corroborate an essential role of the APP gene family for normal brain development.

## APP CONDITIONAL KNOCKOUT

Germline deletion of APP and APLP2 in mice results in a general impairment in pre- and post-synaptic patterning and a specific defect in pre-synaptic targeting of CHT (Wang et al. 2005, 2007). Conditional alleles of APP and APLP2 have been generated (Wang et al. 2009; Mallm et al. 2010). Consistent with the synaptic adhesion property of APP, deletion of APP (on a global APLP2-KO background) in either presynaptic motor neurons or in postsynaptic muscle was shown to lead to similar neuromuscular synapse defects (Wang et al. 2009). Interestingly, postsynaptic APP expression is required to mediate presynaptic CHT targeting and synaptic transmission, suggesting that transsynaptic APP/APP interaction is necessary in recruiting the presynaptic APP/CHT complex and cholinergic synaptic function. Whether APP modulates other synaptic processes through similar recruitment of synaptic proteins is an interesting question requiring further investigation.

## In Vivo Defined Genetic Modifications of APP Proteins

The above knockout animals provide important information concerning the physiological functions of APP proteins, which may be executed either as a full-length protein or as various processing products. The creation of knockin alleles expressing defined proteolytic fragments of APP offers a powerful system to delineate the APP functional domains in vivo. In this regard, knockin mice that express  $\alpha$ - or  $\beta$ -secretase processed soluble APP (APPs $\alpha$  or APPs $\beta$ ) or membrane anchored APP containing mutations of the highly conserved carboxy-terminal sequences have been generated. These alleles are summarized in Figure 2 and will be discussed in this section.

### APPs $\alpha$ and APPs $\beta$ Knockin

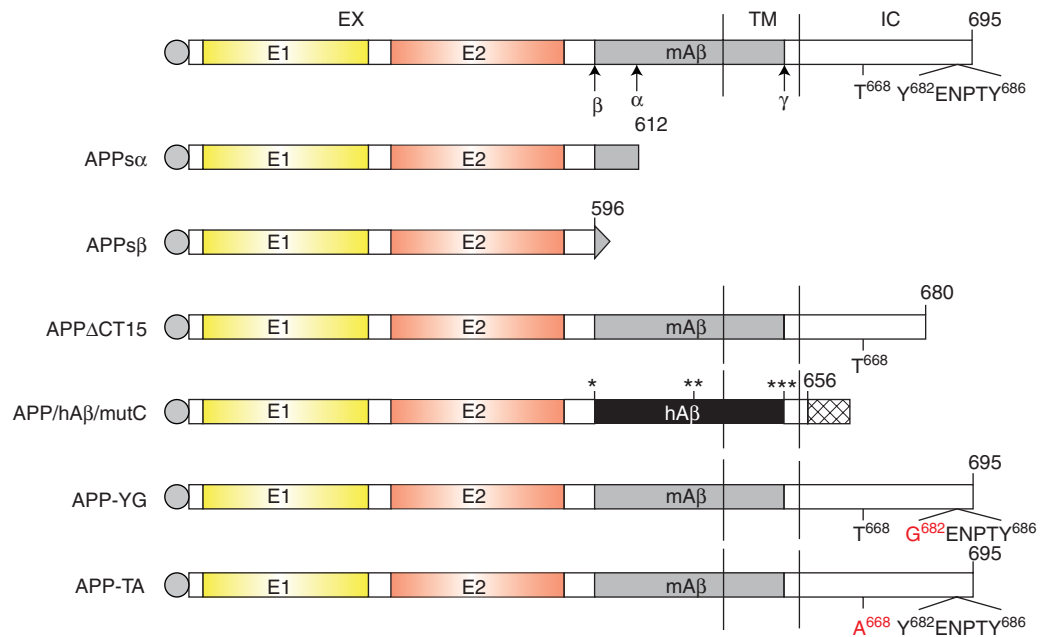
Ring et al. (2007) created a strain of APPs $\alpha$  knockin mice by introducing a stop codon immediately after the  $\alpha$ -secretase cleavage site. Interestingly, all of the phenotypes reported in APP deficient mice including body and brain

weight deficits, grip strength deficits, alterations in locomotor activity, and impaired spatial learning and LTP have been shown to be fully restored by expressing only APPs $\alpha$  (Ring et al. 2007). Consistently, Taylor et al. (2008) showed a requirement for APPs $\alpha$  for in vivo LTP employing infusion of  $\alpha$ -secretase inhibitor or recombinant APPs $\alpha$ , respectively. This crucial function of APPs $\alpha$  for synaptic plasticity and cognition is also of relevance for AD, as reduced CSF levels of APPs $\alpha$  and  $\alpha$ -secretase ADAM10 are prominent features of sporadic AD cases (Lannfelt et al. 1995; Sennvik et al. 2000; Colciaghi et al. 2002; Tyler et al. 2002).

Li et al. (2010a) generated an APPs $\beta$  knockin allele that allows investigation of the stability and possible cleavage of APPs $\beta$  in the absence of APPs $\alpha$ . Contrary to Nikolaev (2009), the APPs $\beta$  protein was shown to be highly stable in vivo and does not undergo further cleavage under regular cell culture conditions in vitro. Crossing the APPs $\beta$  allele to APLP2 null background revealed that APPs $\beta$  failed to rescue the nerve sprouting phenotype of the APP/APLP2 null neuromuscular junction or early postnatal lethality (Li et al. 2010a). These data support the view that APPs $\beta$  exists as a stable protein and that the neuromuscular synapse defects present in APP/APLP2 null mice is not caused by the lack of APPs $\beta$  and, by extension, a defective APPs $\beta$ /DR6 pathway. However, when crossing the APPs $\alpha$  knockin allele (Ring et al. 2007) to an APLP2 null background, most of the combined mutants survived into adulthood (Weyer et al. 2011). These data suggest a distinct functional role of secreted APPs $\alpha$  sufficient to partially rescue the lethality of APP/APLP2-DKO mice, and revealed a synergistic role of both APP and APLP2 for hippocampal function and synaptic plasticity (Weyer et al. 2011).

### Deletion or Mutation of the APP Intracellular Domain

Two APP carboxy-terminal deletion knockin mice have been reported. One deletes the last 15 amino acids of the APP sequence (APP $\Delta$ CT15) (Ring et al. 2007); the other replaces mouse A $\beta$



**Figure 2.** Schematic representation of APP and its knock-in constructs (not drawn to scale). EX, TM, and IC stand for extracellular, transmembrane, and intracellular region, respectively. E1 and E2 domains are marked in yellow and orange, respectively. mAb and hAb represent mouse and human Aβ, respectively. β, α, and γ indicate the cleavage sites by β-, α-, and γ-secretase, respectively. \*\*\* represents signal peptide. \*\*\* symbolizes the FLAG tag. Residue T<sup>668</sup>, and YENPTY motif are labeled to illustrate the corresponding point mutations in APP-YG knock-in and APP-TA knock-in mice. \*, Swedish mutation (K595M596-N595L596); \*\*, Arctic mutation (E618-G618); \*\*\*, London mutation (V642-I618), which are introduced in the APP/hAβ/mutC knock-in allele. All residues are numbered according to APP695 numbering.

with the human Aβ sequence containing the Swedish, Arctic, and London FAD mutations and simultaneously deletes the last 39 residues of the APP sequence (APP/hAβ/mutC) (Li et al. 2010b). Crossing the APP/hAβ/mutC allele to APLP2 null background resulted in similar neuromuscular synapse defects and early postnatal lethality as in mice doubly deficient in APP and APLP2, supporting a functional role of the APP carboxy-terminal domain in these development activities. Nevertheless, Aβ production and amyloid pathology could proceed without the carboxy-terminal sequences (Li et al. 2010b). An essential role of the APP carboxy-terminal domain, specifically the YENPTY motif, in development was shown by the creation of APP knockin mice in which the Tyr<sup>682</sup> residue of the Y<sup>682</sup>ENPTY sequence was changed to Gly (APP<sup>YG</sup>). Crossing the

homozygous knockin mice on APLP2-KO background showed that the APP<sup>YG/YG</sup>/APLP2<sup>-/-</sup> mice show neuromuscular synapse deficits and early lethality similar to APP/APLP2 double KO mice (Barbagallo et al. 2011a). In sharp contrast, similar analysis of the knockin mice with mutation of the highly conserved Thr<sup>668</sup> residue (APP<sup>TA</sup>) showed that this site is dispensable for the APP-mediated development function (Barbagallo et al. 2011b).

## CONCLUDING REMARKS

Because of the central role of APP in AD pathogenesis, a great deal of effort has been devoted to understanding the biological functions of APP since its cloning in 1988. In vitro and in vivo studies have shown important activities of APP in various neuronal and synaptic

processes, which can be executed either as a full-length protein or as one of the processing products. However, the underlying mechanisms remain largely undefined and often controversial. Key questions regarding whether APP is a receptor or a ligand or both, whether APP is by itself a signaling molecule or rather plays a secondary role in gene regulation, how APP function is coordinated between its full-length form and the proteolytic cleavage products and by its many intracellular binding partners awaits further investigation. The creation of the comprehensive panel of APP mouse strains including global inactivation, tissue-specific knockout and defined genetic modifications, combined with modern biological tools such as powerful large-scale experimentation and exciting neuroimaging technology, place us in an excellent position to address these questions.

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