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REVIEW

APP physiological and pathophysiological functions: insights from animal models

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The amyloid precursor protein (APP) has been under intensive study in recent years, mainly due to its critical role in the pathogenesis of Alzheimer's disease (AD). β-Amyloid (Aβ) peptides generated from APP proteolytic cleavage can aggregate, leading to plaque formation in human AD brains. Point mutations of APP affecting Aβ production are found to be causal for hereditary early onset familial AD. It is very likely that elucidating the physiological properties of APP will greatly facilitate the understanding of its role in AD pathogenesis. A number of APP loss- and gain-of-function models have been established in model organisms including *Caenorhabditis elegans*, *Drosophila*, zebrafish and mouse. These *in vivo* models provide us valuable insights into APP physiological functions. In addition, several knock-in mouse models expressing mutant APP at a physiological level are available to allow us to study AD pathogenesis without APP overexpression. This article will review the current physiological and pathophysiological animal models of APP.

Introduction

The amyloid precursor protein (APP) is one of the most intensively studied molecules since it was first cloned more than 20 years ago [1-3]. A notable feature of APP is that it undergoes finely regulated secretase cleavage processing (Figure 1). In general, the α-secretase or β-secretase cleaves the APP extracellular domain generating the soluble N-terminal fragments APPsα or APPsβ and the membrane-associated C-terminal fragments CTFα and CTFβ, respectively. CTFα or CTFβ will further be cleaved by the γ-secretase within the transmembrane domain releasing the p3 peptide or β-amyloid (Aβ) peptide. Following γ-secretase cleavage, both CTFs will also release the APP intracellular domain (AICD) into the cytoplasm. α-Secretase-mediated APP cleavage prevents Aβ production, and thus is called the non-amyloidogenic pathway, whereas the β-secretase-mediated APP cleavage generates Aβ peptides and is called the amyloidogenic pathway. However, recent studies showed that APP proteolytic cleavage could be more complicated than above (reviewed by Chow *et al*. [4]). For instance, besides the γ-site cleavage of the CTFs by γ-secretase, CTFs may also undergo 'ε-site' cleavage near the cytoplasmic membrane boundary of APP [5].

The normal physiological functions of APP have raised curiosity, since it belongs to an evolutionarily conserved protein family (Figure 2) and combined deficiency of APP and its homologs leads to early postnatal lethality in mammals [6, 7]. A number of APP loss-of-function and gain-of-function *in vivo* models have been established and characterized in different model organisms, including *Caenorhabditis elegans*, *Drosophila*, zebrafish and mouse. Studies from different species indicate conservation of function between APP family members, including roles in cell adhesion, neuron migration and synaptogenesis.

Enormous scientific efforts have been put into APP-related studies mainly because of its vital pathophysiological functions in Alzheimer's disease (AD). AD is the leading cause of dementia in the aged population. There

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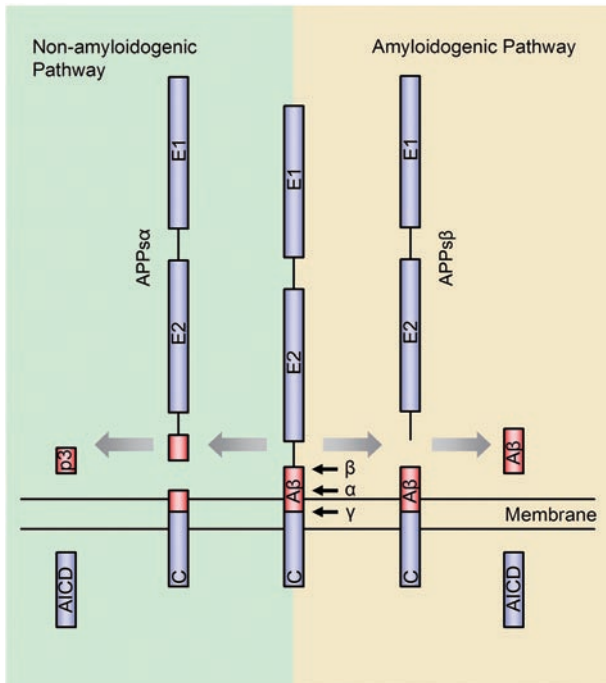E-mail: huiz@bcm.edu

Figure 1 APP amyloidogenic and non-amyloidogenic processing pathways (not drawn to scale). The Aβ domain is highlighted in red. For simplicity, only one cleavage site is shown for each enzyme.

are three pathological hallmarks of AD: extracellular amyloid plaques, intracellular neurofibrillary tangles and neuronal degeneration including synapse loss. Amyloid plaques are extracellular deposits primarily composed of Aβ peptides that are derived from proteolytic cleavage of APP and associated with other components (reviewed by Armstrong *et al*. [8]). The pathogenic mechanism of AD is very controversial, however, the Aβ cascade hypothesis is currently most favored. This hypothesis states that the excessive Aβ peptides generated from mis-regulated cleavage of APP or impaired clearance play a central role in AD [9]. While the vast majority of AD cases occur after 65 years of age, a small percentage of patients (< 5%) develop clinical symptoms before the age of 65 years due to autosomal dominant familial AD (FAD) mutations [10]. These mutations have been discovered in three different genes: *APP*, *Presenilin 1* and *2* (*PSEN1* and *PSEN2*). Most mutations in the *APP* and *PSEN* (or *PS*) genes alter the production of Aβ cleavage profiles, leading to accelerated amyloid pathology. Several lines of knock-in mice (KI) with *APP* FAD mutations have been generated in recent years, either by mutation of *APP* alone or in combination with *PS1* mutations. Compared to traditional AD transgenic mice, which have been used extensively

in the field, these KI models do not suffer from the confounding effects of APP overexpression and offer us a more physiologically relevant platform to investigate AD pathogenesis. We believe a review of these *in vivo* APP animal models will facilitate the understanding of AD pathogenesis in the context of APP physiology.

Non-mammalian APP animal models

C. elegans

The *C. elegans* homolog of APP, APL-1, is structurally similar to its mammalian counterpart and shares sequence homology in the N-terminal E1 and E2 domains and the intracellular C-terminal domain, which has the highest sequence conservation (Figure 2) [11]. There are no known coding region splice variants detected in APL-1, which most closely resembles the neuronal isoform of APP, APP695 [11]. However, APL-1 does not contain the amyloid-β sequence, similar to the functionally redundant mammalian APP homologs APLP1 and APLP2 [11-14].

Nematode development includes four larval stages after each of which is a molt where a new, larger exoskeleton is formed to accommodate the growth of the larvae. In worms, loss of function of the single *apl-1* gene leads to developmental arrest and lethality during the first larval stage (L1), likely due to a molting defect [15, 16]. Many of the *apl-1*-null worms arrest at the L1/L2 transition, unable to shed old cuticle and exhibiting massive internal degradation [15, 16]. RNAi knockdown of *apl-1* also leads to a molting defect, although less severe than the null mutant, as the worms exhibit loose cuticle primarily around the head beginning at the L3/L4 molt [16, 17]. In addition to the molting defect, *apl-1* knockdown leads to hypersensitivity to the acetylcholinesterase inhibitor aldicarb, signifying a defect in cholinergic neurotransmission [16]. The neuronal phenotype and the molting defect were found to be independent of one another, suggesting *apl-1* contributes to multiple functions within the worm. However, more studies are needed to determine the mechanism of *apl-1* regulation in each of these functions.

Structure-function studies have been performed in an attempt to identify functional domains of *apl-1* through rescue of the *apl-1*-null lethality and molting defect. Surprisingly, both phenotypes were found to be rescued by either a C-terminal truncation of *apl-1* or the soluble N-terminus, showing that the highly conserved C-terminus is not required for *apl-1* function in the worm [15, 16]. This differs from the mammalian system in which the APP C-terminus is essential for viability on a nonredundant background (see discussion under 'KI models
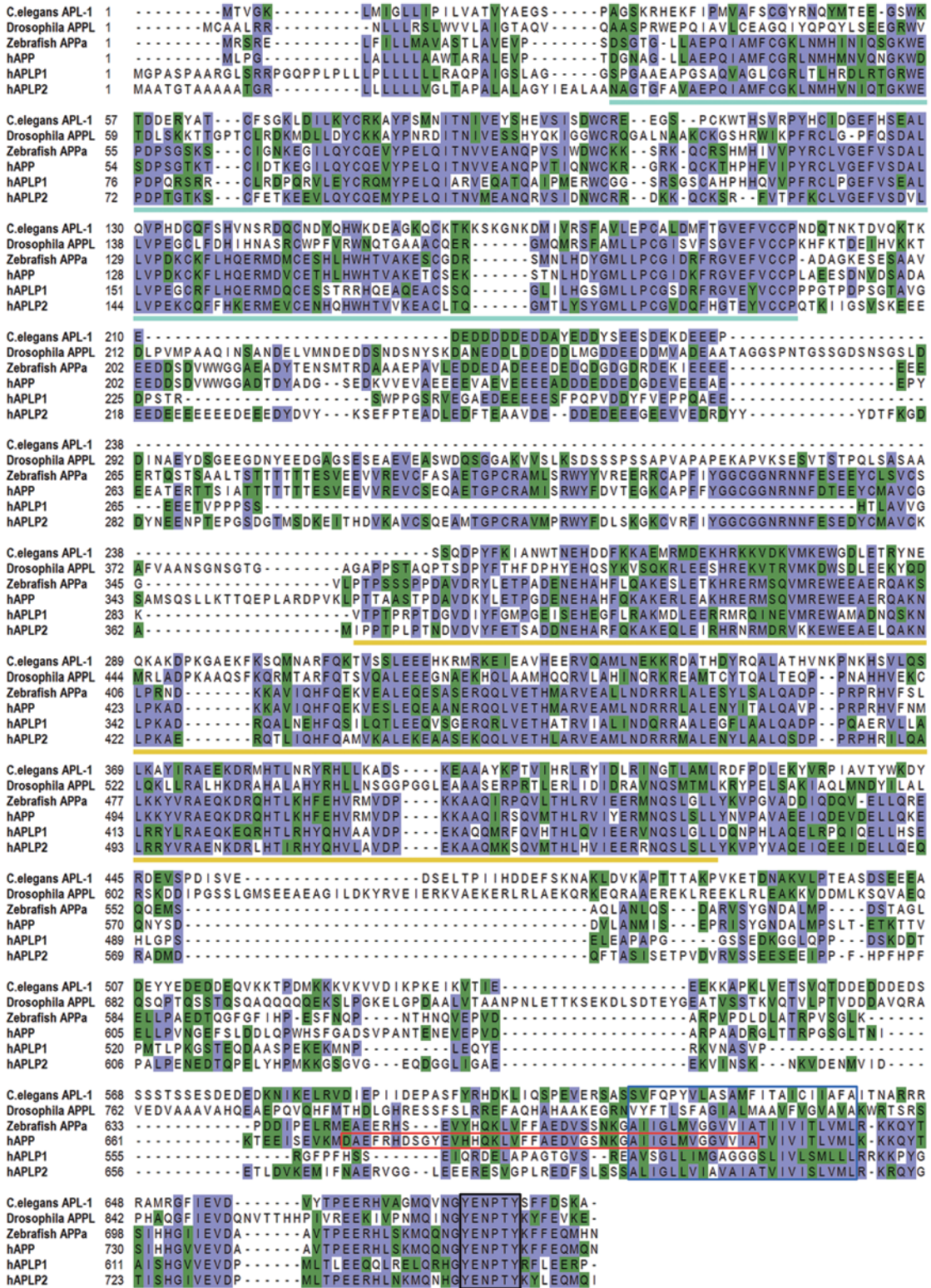
Figure 2 Homology between APP family members. Comparison of protein sequences of *C. elegans* APL-1, *Drosophila* APPL, zebrafish APPa and the human APP family members reveals distinct areas of homology. Purple sequences indicate identical homology while green references similar amino acids. Homologous regions include the E1 domain (pale blue line), E2 domain (yellow line) and sequences within the C-terminus such as the conserved Thr site (arrow head) and YENPTY motif (black box). The transmembrane domain and Aβ sequence are noted by the blue and red boxes, respectively.

expressing distinct *APP* fragments') [18, 19]. Hornsten *et al.* [15] were unable to accomplish rescue using the C-terminus alone, indicating, together with the other rescue experiments, that the N-terminus is the primary functional domain of APL-1 in the worm.

Drosophila

APPL is the APP homolog in *Drosophila*, and, like the worm homolog, does not contain the Aβ sequence (Figure 2). *Appl*-deficient flies are viable and fertile with no obvious phenotypes; however, these flies do have subtle behavioral defects, such as fast phototaxis impairment. This defect can be partially rescued by transgenic expression of either wild-type fly APPL or human APP, which demonstrates the functional conservation of APP between different species [20].

Subsequent loss and gain-of-function studies revealed more specific functions of APPL. APPL plays an important role in axonal transport, since either *Appl* deletion or its overexpression will cause axonal transport defects similar to kinesin and dynein mutants [21]. APPL is required for the development of neuromuscular junctions (NMJs), since *Appl* deletion leads to decreased NMJ bouton number, whereas *Appl* overexpression dramatically increases bouton number [22]. This activity can be explained by the formation of a potential complex including APPL, the APPL-binding protein dX11/Mint, and the cell adhesion molecule FasII, which together can regulate synapse formation [23]. APPL also plays a role in the development of the *Drosophila* peripheral nervous system (PNS) as both *Appl* deletion and *Appl* RNAi leads to the loss of scutellar mechano-sensory organs (MSOs). Overexpression of human APP homologs in the fly leads to MSO Notch gain-of-function phenotypes, possibly through the interaction of the APP YENPTY motif with Numb/Pon and Dab [24].

APPL promotes post-developmental neurite arborization, which may be involved in brain injury response and repair. This function was revealed in a study showing that overexpression of human APP and APPL can induce post-developmental axonal arborization. This phenotype is dependent on the APP C-terminal domain and its interaction with the Abelson tyrosine kinase. Interestingly, similar to mammals, APPL is strongly upregulated after traumatic brain injury (TBI) in flies upon which APPL-deficient flies suffer a higher mortality rate compared to controls [25].

Overall, *Appl* loss and gain-of-function studies in *Drosophila* models offer us important insights into the physiological functions of APP and some of these findings have already been validated in mouse models.

Zebrafish

Zebrafish have two APP homologs, APPa and APPb. APPa is a 738-aa protein and contains the Kunitz protease inhibitor domain in the N-terminus, which resembles the human APP770 isoform. APPb is a 694-aa protein, which is more similar to the human APP695 isoform [26]. The study of a transgenic zebrafish model expressing GFP under the *appb* promoter control reveals that the GFP is mainly expressed in subregions of brain, spinal cord and the developing vasculature of zebrafish embryos. The GFP expression level increases during development, and in adult transgenic zebrafish, GFP was abundantly expressed in the brain [27]. Though *appa* knockdown in zebrafish does not show any significant defects in development, *appb* knockdown animals show several early developmental deficits, including a shortened body axis, a short and curly tail and mild synopthalmia, which can be explained by defective convergent-extension movements [26]. These defects can be largely rescued by human APP695 mRNA, partially rescued by human APPsα mRNA, but cannot be rescued by APP bearing the Swedish mutation, which leads to FAD in humans. It is interesting that this study indicates APP FAD mutations may not only contribute to AD pathogenesis during aging but also affect early development due to APP loss-of-function.

Though there are not many APP physiological function studies employing zebrafish, as a well-established model organism for vertebrate development, we expect to see more novel findings from the zebrafish system in the near future.

APP models in mice

To understand the physiological functions of APP and its family members, individual and combined mouse mutants of *APP*, *APLP1* and *APLP2* have been generated. For *APP*, there are two knockout (KO) alleles [28, 29], one hypomorphic allele [30], two conditional alleles [31, 32], four defined truncation KI alleles [18, 19, 33] and two C-terminal point mutation KI alleles reported [34, 35].

APP, APLP1 and APLP2 single KO mice

APP KO mice are viable and fertile but suffer from various defects and abnormalities, such as reduced body and brain weight, decreased size of forebrain commissures, increased frequency and severity of corpus callosum agenesis [28, 30], reactive gliosis [28], increased sensitivity to kainate-induced seizures [36], increased copper and iron levels in the cerebral cortex and liver [37, 38], upregulated cholesterol and sphingomyelin levels

in the brain [39] and decreased plasma glucose levels as well as hyperinsulinemia [40]. Behavioral studies revealed that *APP* KO mice have decreased locomotor activity, reduced forelimb grip strength and deficits in the Morris water maze task as well as passive avoidance learning [41-43]. Electrophysiology studies show that these mice are also defective in long-term potentiation (LTP), which is associated with attenuated paired-pulse depression of GABA-mediated inhibitory postsynaptic currents [44]. Recently, APP was found to be involved in the regulation of L-type calcium channel function [45]. Loss of APP leads to increased levels of the L-type calcium channel subunit Cav1.2 in the striatum, which is associated with reduced GABAergic paired pulse inhibition and increased GABAergic post-tetanic potentiation [45]. However, these behavioral and electrophysiological impairments may not be caused by a gross loss of neurons or synapses because unbiased stereological quantification failed to detect any significant neuronal or synapse loss in aged *APP* KO mice [42]. Also, attempts to examine dendritic spine density in *APP* KO mice have revealed mixed results. Bittner *et al.* [46] reported that *APP* deletion leads to a twofold higher density of spines in apical dendrites of layer III and layer V neurons of the somatosensory cortex at 4-6 months of age, whereas Lee *et al.* [47] found a significant decrease in spine density in cortical layers II/III and hippocampal CA1 pyramidal neurons in 1-year-old *APP* KO mice compared with wildtype controls. This discrepancy may be due to a region-specific effect, a contribution of aging and/or an adaptive mechanism. Nevertheless, additional study is warranted in this area.

The phenotypes of *APLP2* and *APLP1* single KO mice are also mild [6, 7]. *APLP2* KO mice are viable, fertile and normal in size. No abnormalities were detected in tissues examined by histochemical analysis. *APLP2* KO mice showed no difference from controls in forelimb strength as well as learning and memory performance in the Morris water maze and conditioned avoidance tasks. Similarly, *APLP1* single KO mice are viable, fertile, but have a postnatal growth deficit. However, these mice show normal locomotor activity and forelimb grip strength [6, 7].

Mice with combined KO of APP family members

Though the phenotypes of *APP* family member single KOs are all relatively subtle, *APP/APLP2* or *APLP1/APLP2* double KO (dKO) mice exhibit early postnatal lethality. This strongly indicates that functional redundancy exists among APP family members. Intriguingly, the *APP/APLP1* dKO mice are viable [7], suggesting a unique property specific to *APLP2* that is required when

APP or *APLP1* is absent.

In the PNS, APP and APLP2 play an essential yet redundant role in the formation and function of neuromuscular synapses. *APP/APLP2* dKO mice have poorly formed NMJs, with excessive nerve growth and reduced apposition of pre- and postsynaptic elements. The number of synaptic vesicles at the presynaptic terminals is also reduced in parallel with impaired neurotransmitter release [48]. Moreover, study of the ultrastructure of the submandibular synapse also revealed significant reductions in synaptic vesicle density, active zone size and docked vesicle number per active zone in *APP/APLP2* dKO mice [49].

Mice lacking all three APP family members survive through embryonic development but die shortly after birth [50]. Cranial abnormalities were seen in 81% of the triple KO mice while 68% showed cortical dysplasia resembling human type II lissencephaly. Additionally, the mutant mice showed partial loss of cortical Cajal Retzius cells at E18.5, suggesting a role for APP family members in neuronal migration and/or adhesion [50]. A very similar cortical dysplasia phenotype was also observed in mice that are double deficient for Fe65 and Fe65L1, a family of proteins that have been well known to interact with the APP cytoplasmic domain [51], suggesting an APP/Fe65 signaling complex mediating neuronal migration. The role of APP in neuronal migration/adhesion was further supported by the finding that acute knockdown of *APP* in rats using shRNA electroporation resulted in defective cortical plate entry and migration of neuronal precursor cells in the embryonic cortex [52].

APP conditional KO

To counter postnatal lethality and further dissect APP physiological functions, two conditional alleles of *APP* have been generated [31, 32]. Tissue-specific deletion of *APP* in either neurons or muscle in the *APLP2* KO background resulted in neuromuscular defects similar to phenotypes seen in *APP/APLP2* dKO mice, suggesting that APP expression is required in both motor neurons and muscle cells for the proper formation and function of neuromuscular synapses [31]. The authors propose that the trans-synaptic homotypic interaction of APP promotes neuromuscular synapse development. This was further supported by the synaptogenic properties of APP revealed by hippocampal and HEK293 mixed culture experiments [31]. Interestingly, muscle APP expression is required for the proper pre-synaptic localization of CHT (high-affinity choline transporter) and synaptic transmission, suggesting that trans-synaptic APP interaction is necessary for recruiting the presynaptic APP/CHT complex. Consistent with the role of APP in synaptic plastic-

ity and synaptic function, a recent study that attempted to identify APP-interacting proteins using a tagged APP transgenic approach revealed a list of synaptic proteins including bassoon and neurexin [53]. Whether the trans-synaptic interaction of APP is involved in the recruitment of these presynaptic molecules and how it coordinates with other synaptic adhesion complexes such as neuroligin/neurexin are exciting questions awaiting further investigation.

Knock-in models expressing distinct APP fragments and single amino acid mutations

As mentioned earlier, sequential proteolytic processing of APP generates three fragments: a large soluble ectodomain (APPsα or APPsβ), a short peptide such as P3 or Aβ and the AICD. To investigate physiological functions exerted by these different APP fragments, two research groups have generated four different gene-targeted *APP* mutant KI alleles, in which Aβ and AICD regions, are missing or mutated (Figure 3).

In order to determine the role of APPsα and APP C-terminal domain, the Müller group created two KI mouse lines [33]. The first expresses APPsα (*APPsα*-ki) and the second expresses a C-terminal truncation of APP removing the highly conserved YENPTY motif (*APPΔCT15-ki*) by deletion of the last 15 aa residues (Figure 3B and 3C). Both of the lines have an overall reduction of mutant APP expression in both mRNA and protein levels, which is attributed to an effect of SV40 polyA cassette. In *APPΔCT15*-ki brains, even with a reduction of mutant APP expression of more than 50%, there is a relatively higher percentage of cell surface APP and less Aβ production compared to controls. This is consistent with the previous *in vitro* studies showing that the C-terminal region was involved in APP endocytosis and processing [54, 55].

In comparison with *APP* KO mice, the *APPsα*-ki and *APPΔCT15*-ki mice appear to rescue a variety of phenotypes observed in *APP* KO mice. For instance, the body and brain weight loss phenotype of *APP* KO animals at different ages is largely rescued. Behaviorally, these KI mice do not exhibit any defects in grip strength test and water maze test. Electrophysiology experiments showed that LTP deficits observed in *APP* KO mice at 9-12 months were also corrected in both KI lines. Taken together, these results suggest that, similar to what was observed with APL-1 in the *C. elegans* model [15], the C-terminal region of APP may not be required for certain physiological functions of APP.

To further dissect the function of different APP domains, our research group has also generated two distinct KI lines. The first model expresses APPsβ with a FLAG tag at the C-terminus named *APPsβ*-ki[18] (Figure 3D). The second model, named *APP/hAβ/mutC* [19], expresses APP with a humanized Aβ region, including three FAD mutations (Swedish, Arctic and London) to facilitate Aβ production, and a frameshift mutation in the C-terminus that deletes the last 39 aa residues of APP (Figure 3E). Similar to the *APPsα*-ki and *APPΔCT15*-ki lines, *APPsβ*-ki and *APP/hAβ/mutC*-ki mice do not show any obvious growth or anatomical deficits. As mentioned above, when *APP* KO mice are bred to the *APLP2* KO background, *APP/APLP2* dKO pups exhibit postnatal lethality. Similarly, when crossed with *APLP2* KO mice, neither the *APPsβ*-ki nor *APP/hAβ/mutC*-ki allele is able to rescue the lethality. Moreover, the deficits of NMJs observed in *APP/APLP2* dKO pups, including aberrant apposition of presynaptic proteins with postsynaptic endplates and diffuse synaptic band width, also exist in *APPsβ*[ki/ki]/*APLP2*^{−/−} and *APP/hAβ/mutC*[ki/ki]/*APLP2*^{−/−} pups. Considering that neither of the KI alleles contains a functional APP C-terminal domain, our data clearly demonstrate a critical and indispensable role of the conserved C-terminal region of APP in early development.

Though our KI alleles are not able to rescue lethality, they do rescue certain gene expression changes caused by *APP* KO. In *APP/APLP2* dKO mice, the mRNA level of transthyretin (TTR) and Klotho is reduced [18]. However, the expression of TTR and Klotho is normal in the *APPsβ*[ki/−]/*APLP2*^{−/−} pups. This finding indicates that soluble APP can modulate the expression of certain genes through a currently unknown pathway. TTR has been shown to have Aβ binding ability [56-58] and Klotho has been extensively implicated in the aging process [59-61]. The enhancement of TTR and Klotho expression by APPsβ offers the fascinating possibility of a self-protective mechanism in the APP processing pathway to counter the production and toxicity of Aβ during aging.

When we crossed the *APP/hAβ/mutC* KI line onto the *PS1* M146V (a *PS1* FAD mutation) KI background to accelerate Aβ deposition, we found massive plaques in the hippocampus and cortex in mice over 1 year of age. This implies that amyloid pathogenesis could progress independently without the conserved C-terminal sequence [19].

The essential role of the highly conserved C-terminal region of APP in early development revealed by *APPsβ*[ki/ki]/*APLP2*^{−/−} and *APP/hAβ/mutC*[ki/ki]/*APLP2*^{−/−} mice was further confirmed by a recent study of Barbagallo *et al*. [34]. They generated a KI mouse with a single amino acid mutation at Tyr[682] (Y682G), and showed that, in the absence of *APLP2* functional redundancy, the Y682G mutation resulted in postnatal lethality and neuromuscular synapse defects similar to *APP/APLP2*-double-deficient mice [34,
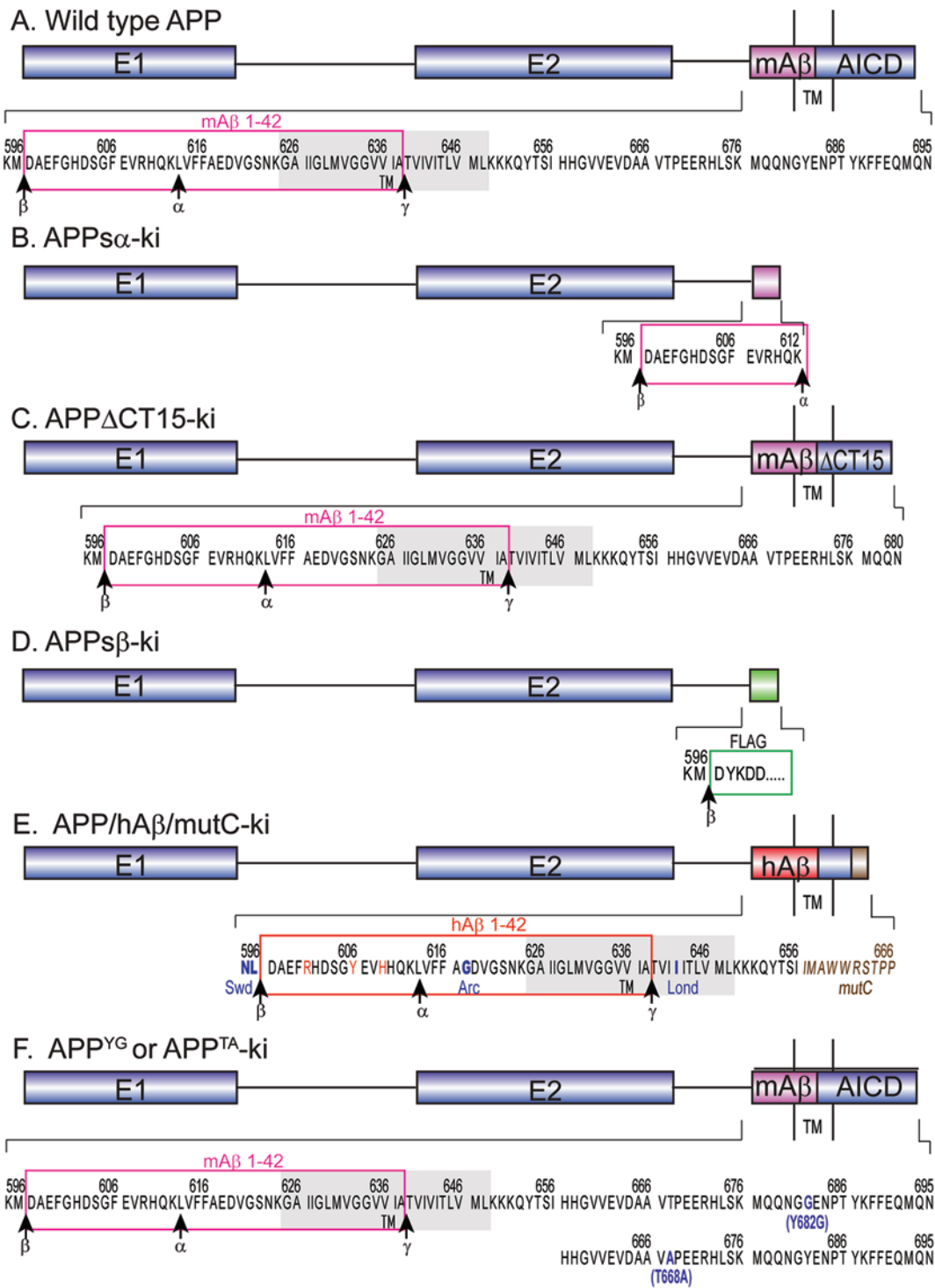
Figure 3 APP knock-in alleles expressing distinct APP fragments. **(A)** Wild-type APP695 with the mouse Aβ region (mAβ) framed in pink, and transmembrane area (TM) shaded in grey. AICD, APP intracellular domain. α, β and γ are cleavage sites of the α-, β-, and γ-secretases, respectively. **(B and C)** APPsα and APPΔCT15 knock-in alleles, respectively, reported by Ring et al. [33]. **(D)** APPsβ knock-in reported by Li et al. [18]. FLAG-tag sequence is framed in green. **(E)** APP/hAβ/mutC knock-in by Li et al. [19]. Humanized Aβ region (hAβ) is framed in red, and the different residues between human and mouse sequence are also shown in red. Additional FAD mutations are bolded in blue. Swd, Arc and Lond, represent Swedish, Arctic and London mutations, respectively. The sequence resulting from a frame-shift mutation at C-terminus is illustrated in brown. **(F)** APP[YG] and APP[TA] knock-ins harbor APP Y682G and T668A mutations, respectively [34, 35].

62]. The importance of this Tyr[682] site is strengthened by a parallel study, which found that a distinct mutation at a different Thr site, Thr668 (T668A), had no effect on both animal survival and formation of neuromuscular synapses [34, 35]. Tyr[682] is an essential residue of the Y[682]ENPTY[687] motif, which is the docking site for numerous cytosolic proteins [63]. Some APP interactors require phosphorylated Tyr[682] [64-67], whereas others require non-phosphorylated Tyr[682] [68]. This leads to questions in regards to how the selective phosphorylation state of Tyr682 is regulated and their corresponding functional consequences.

In summary, these different KI lines expressing different segments of *APP* have revealed that APP is involved in multiple functions. Some of these functions are carried out by APP N-terminal ectodomain alone without the APP C-terminal intracellular domain. However, the AICD, especially the YENPTY motif, which is well conserved from worms to humans, plays a critical role in developmental regulation in mice. Finally, Aβ aggregation and amyloid deposition can proceed without the APP C-terminus, implying that APP physiological functions could be genetically uncoupled with AD pathogenesis.

APP FAD mutant KI models

Many APP transgenic models expressing APP with FAD mutations under the control of a robust exogenous promoter have been generated and have provided us with valuable knowledge about AD pathogenesis, especially Aβ plaque deposition. However, in order to accelerate plaque development, transgenic mice usually overexpress mutant APP at a very high level. For instance, a commonly used transgenic line, Tg2576, overexpresses APP to almost sixfold using the hamster prion promoter [69]. Considering the crucial and various functions of APP in nervous system development, this provokes the question of whether the memory and behavioral defects observed in AD-transgenic mice are due to Aβ pathology or simply APP overexpression.

To avoid the complications of overexpression and build a more physiologically relevant model, several labs generated different FAD *APP* KI lines by introducing human FAD mutations and/or humanized Aβ to the endogenous mouse *APP* gene. Compared to traditional transgenic models, *APP* KI mice exhibit a number of advantages. Since the KI allele is under the native *APP* promoter control, the amount of APP expression remains at physiological level. For the same reason, the temporal and spatial expression patterns of mutant APP are not affected. In contrast to the transgenic lines in which the wild-type mouse *APP* gene is still intact, where mouse APP and Aβ may complicate the phenotype, the KI

models have mouse Aβ replaced with the human Aβ sequence.

One FAD KI model was developed by Reaume *et al.*, [70] who generated *APP*[NLh/NLh] mice bearing the Swedish FAD mutation (K670N/M671L) with a humanized Aβ sequence expressed at endogenous levels. Amyloidogenic β-secretase cleavage of this mutant APP is accurate and also enhanced, while the non-amyloidogenic processing is repressed. Human Aβ production is significantly increased in these mice, a ninefold increase compared to normal human brain tissue [70]. Though this *APP* KI model overproduces human Aβ40 and Aβ42, Aβ plaque deposition was not detectable [71]. Later, the *APP*[NLh/NLh] strain was crossed with the *PS-1*[P264L/P264L] line (a PS1 FAD mutant KI line) to generate the *APP*[NLh/NLh]/*PS-1*[P264L/P264L] double KI (DKI) model [71]. *APP*[NLh/NLh]/*PS-1*[P264L/P264L] DKI animals exhibit elevated levels of Aβ42 and initial Aβ deposition begins at 6 months of age. Aβ deposition at 6 months was predominately seen in the cerebral cortex with the majority of deposits found in the frontal cortex, followed by the parietal cortex [71]. Microarray analysis was performed to compare the gene expression profile changes between *APP*[NLh/NLh]/*PS-1*[P264L/P264L] animals and two transgenic models, Tg2576/PS-1[P264L/+] and Tg2576/PS-1[P264L/P264L], both before and after detectable Aβ deposition [72]. Gene expression difference was analyzed in *APP*[NLh/NLh]/*PS-1*[P264L/P264L] DKI animals and their wild-type littermates at 2 months of age, when plaques in DKI brains are absent, and at 18 months of age, when the DKI cortical plaque load is 3.2%. Similar experiments were also performed to compare expression changes in Tg2576/PS-1[P264L/+], Tg2576/PS-1[P264L/P264L] and non-transgenic controls (non-Tg/PS-1^{+/+}) at 2 months of age, when Tg2576/PS-1[P264L/+] had no plaques and the cortical plaque load of Tg2576/PS-1[P264L/P264L] is 0.09%, and at 12 months of age, when the plaque load of Tg2576/PS-1[P264L/+] and Tg2576/PS-1[P264L/P264L] was 10.6% and 25.8%, respectively. In young animals before Aβ deposition, the DKI animals do not share any altered gene expression when compared with the two transgenic models, which is possibly due to APP overexpression in transgenic models and/or a genetic background difference. However, there are a number of common changes found in the three models with Aβ deposition and many of these changes are also found in human AD brains [72]. This finding strongly supports this DKI animal model as a valuable AD research tool. Chang *et al.* [73] performed electrophysiological and behavioral studies with *APP*[NLh/NLh]/*PS-1*[P264L/P264L] animals. DKI animals showed an age-related decrease of AMPA receptor-mediated evoked currents and spontaneous miniature currents. The downregulation of synaptic AMPA receptor is confirmed by electron

microscopic study. DKI animals also exhibit age-related deficits in bidirectional plasticity seen during analysis of LTP and LTD in these mice as well as memory flexibility when examined by Morris water maze. Additionally, long-lasting and selective impairment in adult hippocampal neurogenesis is found in 9-18 months old $APP^{NLh/NLh}$/$PS-1^{P264L/P264L}$ DKI animals, while the olfactory bulb neurogenic system is not affected. The number of MCM2-positive neural stem and progenitor cells is decreased by 3-fold compared to controls and doublecortin-positive neuroblasts are decreased by twofold as well. This neurogenesis impairment phenotype may be explained by Aβ deposition-induced microglia activation and neuroinflammation [74].

Another similar FAD mutant *APP* KI line was generated by Kohler *et al.* [75], which includes the humanized

Aβ sequence containing both Swedish (K670N/M671L) and London (V717F) mutations. $APP^{SL/SL}$ single KI animals do not show any Aβ deposits. Once this strain was crossed to an FAD *PS1* transgenic strain ($PS1^{M146L-tg}$), then Aβ deposition occurred in a similar pattern to APP transgenic animals. However, the Aβ deposits in $APP^{SL/SL}$/$PS1^{M146L-tg}$ mice began much later and developed more slowly than APP transgenic animals, with no apparent neurodegeneration [75]. Further characterization revealed that $APP^{SL/SL}$/$PS1^{M146L-tg}$ mice have increased levels of Aβ peptides at 10 months of age and amyloid plaques at 14 months of age. Microdialysis showed that the hippocampal extracellular acetylcholine (ACh) levels of 15-27 months old $APP^{SL/SL}$/$PS1^{M146L-tg}$ mice are slightly but significantly reduced. However, there were no major changes found in stimulated ACh release and overall

Table 1 Summary and comparison of APP knock-in mouse models

APP allele	APP knock-in mutation(s)	Mouse model	Aβ Plaque	Major findings
APP^{NLh}	K670N/M671L (Swedish mutation) Humanized Aβ	$APP^{NLh/NLh}$	No	The human Aβ production is 9-fold higher than normal human brain tissue [70].
APP^{NLh}	K670N/M671L (Swedish mutation) Humanized Aβ	$APP^{NLh/NLh}$/$PS-1^{P264L/P264L}$	Yes	1. Aβ deposition begins at 6 m/o [71]. 2. The altered gene expression profile of $APP^{NLh/NLh}$/$PS-1^{P264L/P264L}$ animals after plaque deposition is shared by two transgenic models as well as human AD brains [72]. 3. $APP^{NLh/NLh}$/$PS-1^{P264L/P264L}$ animals show age-related decreasing of AMPA receptor number and activity. They also exhibit age-related deficits in LTP and LTD studies as well as memory impairment in water maze test [73]. 4. $APP^{NLh/NLh}$/$PS-1^{P264L/P264L}$ mice have long-lasting and selective impairment in adult hippocampal neurogenesis at 9-18 m/o [74].
APP^{SL}	K670N/M671L; (Swedish mutation) V717F (London mutation) Humanized Aβ	$APP^{SL/SL}$/$PS1^{M146L-tg}$	Yes	1. $APP^{SL/SL}$ single knock-in animals do not show any Aβ deposits [75]. 2. $APP^{SL/SL}$/$PS1^{M146L-tg}$ mice have increased levels of Aβ peptides at 10 m/o and amyloid plaques at 14 m/o [75]. 3. Microdialysis study indicates that the hippocampal extracellular acetylcholine (ACh) levels of 15-27 m/o $APP^{SL/SL}$/$PS1^{M146L-tg}$ mice are slightly but significantly reduced [76].
APP^{V642I}	V642I	$APP^{V642I/+}$	No	Long-term spatial memory is significantly impaired and acquisition of spatial memory is slightly affected [77].

central cholinergic function was not affected [76].

The KI model attempting to genetically mimic human FAD to the greatest extent was generated by Kawasumi *et al.* [77]. This strain contains an *APP V642I* mutant KI allele without modifying the mouse Aβ sequence, which was then studied in a heterozygous state. *APP V642I/+* animals appear normal compared to their wild-type littermates up to 2.5 years of age. However, their long-term spatial memory is significantly impaired and acquisition of spatial memory is slightly affected. Histologically, the ratio of Aβ42 (43)/Aβ40 is greatly increased in mutants, but there are no plaques or neurofibrillary tangles in the mutant brains.

All the mouse models mentioned above are summarized in Table 1.

Concluding Remarks

In vivo models of APP family members provide us with a considerable amount of insight into the physiological function of APP. It is now well known that APP and its homologs are important for early developmental events, such as mediating cell adhesion, cell migration and synaptogenesis. These models also highlight several intriguing directions for future study. First, full-length APP is vital for neuromuscular synapse assembly and possibly central synaptogenesis. Second, APPsβ, independent of full-length APP, can regulate the expression of certain genes like *TTR* and *Klotho*. The receptor mediating such an event remains to be identified. Third, *APP/ APLP2* germline dKO animals are early postnatal lethal, whereas animals with a conditional deletion of *APP* only in the central nervous system (CNS) on an *APLP2*-null background are viable. This finding strongly suggests that APP has vital, yet undiscovered, functions outside the CNS. Finally, it is known that APP is strongly upregulated in brains that have experienced TBI in both human patients and in mice. TBI-induced APP upregulation may contribute to higher Aβ production and increased risk of AD in later life. Since studies in *Drosophila* demonstrated that the mortality rate of *Appl*-deficient flies is higher than controls after TBI treatment, it will be extremely interesting to know how *APP/APLP2* conditional KO animals survive TBI and to further dissect the functional role of APP in post-TBI brain repair.

All of the FAD *APP* KI AD mouse models share mild phenotypes and pathological manifestations. To generate plaque pathology, both an APP KI mutation and an FAD *PS1* allele are needed. Considering that a heterozygous *APP* FAD mutation is able to trigger early onset AD in humans, the effects of these mutations in mice are considerably weaker. This difference may be due to the short-life span of mice and the intrinsic physiological differences between humans and mice. Nevertheless, these *APP* KI models provide us with an alternative platform and a more physiologically relevant disease model to study human AD. More comprehensive and sensitive assays will be required for better characterizing *APP* KI mice and consequently AD pathogenesis in the future.

Acknowledgments

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