# Regulation of the physiological function and metabolism of APP by APP binding proteins

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**Abstract**

β-Amyloid precursor protein (APP) is a receptor-like, type-I membrane protein that plays a central role in the pathogenesis of Alzheimer's disease. The cytoplasmic domain of APP is important for the metabolism and physiological functions of APP. The cytoplasmic domain contains a GYENPTY motif that interacts with proteins that contain a phosphotyrosine binding (PTB) domain such as X11/Mint, FE65, and the JIP family of proteins. X11 and X11-like proteins are neuronal adaptor proteins involved in presynaptic function and the intracellular trafficking of proteins. Recent studies in X11s knock-out mice confirmed findings from in vitro studies that X11 proteins affect APP metabolism and the generation of β-amyloid peptide. FE65 proteins are involved in transactivation in coordination with the intracellular domain fragment of APP, and/or in cellular responses to DNA damage. Neurodevelopmental defects observed in FE65s double knockout mice suggest that FE65 proteins cooperate with APP to play a role in neuronal cytoskeletal regulation. c-Jun N-terminal kinase (JNK) interacting protein-1 (JIP-1), a scaffolding protein for the JNK kinase cascade, has been suggested to mediate the intracellular trafficking of APP by molecular motor kinesin-1. This article reviews some of the recent findings regarding the regulation of physiological function and metabolism of APP by APP binding proteins.

## Introduction

β-amyloid precursor protein (APP) is the precursor of β-amyloid (Aβ) peptide, which is a principal component of senile plaques and is implicated in the cause and progression of Alzheimer's disease (AD) [1]. Physiologically, APP and its two paralogues, β-amyloid precursor-like protein 1 (APLP1) and 2 (APLP2), play essential roles in brain development and neuromuscular synapse formation, and are necessary for survival during the early postnatal stages in mice [2–4]. APP is a receptor-like, type-I membrane protein, which has a large extracellular domain, a transmembrane domain, and a short cytoplasmic domain. Decades of research have demonstrated that the cytoplasmic region of APP is important for the protein interactions that affect its metabolism and physiological function. This review provides a brief overview of the APP binding proteins and their involvement in the regulation of APP processing and functions. Then we focus on three families of proteins, X11/Mint, FE65 and JIP. Recent findings on the relation between the protein interactions and phosphorylation of APP are also discussed.

## The cytoplasmic domain of APP and its binding proteins

The cytoplasmic domain of APP is composed of 47 amino acid residues that are highly conserved among species. From the time that the APP gene was cloned in the late 1980's, a number of proteins that interact with the cytoplasmic domain of APP have been identified by yeast two-hybrid screening and biochemical screening (Fig. 1).

The cytoplasmic domain of APP contains a 681-GYENPTY-687 sequence (numbering for the APP695 isoform), which consists of an internalization signal sequence and a consensus binding motif for a phosphotyrosine binding (PTB) domain. The PTB domain, also referred to as a phosphotyrosine interaction (PI) domain, is a protein-protein interaction domain similar to the classical Src homology 2 (SH2) domain. At present, approximately 60 human proteins have been found to contain PTB domains [5]. Many proteins that contain a PTB domain have been shown to bind to APP: X11/X11α/Mint1, X11-like (X11L)/X11β/Mint2, and X11-like2(X11L2)/X11γ/Mint3 [6, 7] [8, 9]; FE65, FE65-like (FE65L1), and FE65-like2 (FE65L2) [6, 7, 10–13]; c-Jun N-terminal kinase (JNK) interacting protein-1b/Islet brain 1 (JIP-1b/IB1) and JIP-2 [14–18]; disabled-1 (Dab1) and Dab2 [19]; ShcA and ShcC [20, 21]; Numb and Numb-like (Nbl) [22]; autosomal recessive hypercholesterolemia (ARH) [23]; and APP intracellular domain associated protein-1

(AIDA-1) [24, 25]. Some proteins without a PTB domain such as c-Abl [26], growth factor receptor-bound protein 2 (Grb2) [27], UV-DDB [28], and sorting nexin 17 (SNX17) [19] also bind to the GYENPTY motif of APP. The SH2 domains of c-Abl and Grb2 are involved in the binding of these proteins to the GYENPTY motif of APP.

The PTB domain was originally discovered as a domain that recognizes phosphotyrosine in the context of a NPXpY sequence. However, there are many proteins with a PTB domain that bind to NPXY sequences in the absence of tyrosine phosphorylation [5]. Phosphorylation of the GYENPTY motif of APP is not necessary for the binding of APP to most of its binding proteins that contain a PTB domain. The only exception to this is Shc, which requires the phosphorylation of Tyr682 for binding to APP [21, 29]. Tyr682 phosphorylation is also necessary for the binding of APP to the SH2 domains of Abl and Grb2, and might regulate downstream signal transduction cascades [29, 30]. Structural studies have revealed that the binding interface of APP and the PTB domain of X11 involves residues within the GYENPTY motif [31]. Recently, the structures of FE65 PTB2 bound to the cytoplasmic domain of APP and of FE65L1 PTB2 bound to the APP peptide in solution were revealed by crystallography and NMR, respectively [32, 33]. These studies suggest that the PTB domains of these APP binding proteins bind to the GYENPTY motif in a manner that is similar to other proteins with PTB domain, however, the presence of additional binding interfaces might allow for additional specificity.

Regions of the APP cytoplasmic domain that mediate protein interactions are not limited to the GYENPTY motif. Other regions of the APP cytoplasmic domain are known to bind to proteins such as GTPase Go [34], kinesin light chain (KLC) [35], protein interacting with the APP tail 1 (PAT1) [36], caveolin-3 [37], a human homologue of yeast amino-terminal acetyltransferase (hARD1) [38], FKBP12 [39], peptidyl-prolyl isomerase (Pin1) [40, 41], and APP-binding protein 1 (APP-BP1) [42]. APP-BP1, which is a regulatory subunit of the activating enzyme for the small ubiquitin-like protein NEDD8, binds to the 31 amino acid C-terminus of APP. APP-BP1 is co-localized with APP in lipid rafts and has been suggested to mediate the neuronal apoptosis that is induced by APP [42–44].

**Regulation of APP metabolism by APP binding proteins**

Newly synthesized APP passes through the protein secretory pathway, is transported to the plasma membrane, internalized by endocytosis, and delivered to the endosomes and lysosomes (Reviewed in [45]). During the secretory process, APP is

*N*-glycosylated in the endoplasmic reticulum (ER) and *O*-glycosylated in the Golgi apparatus. The "mature" glycosylated APP is subjected to two-step proteolytic cleavages. First, cleavage at the extracellular juxtamembrane position by α- or β-secretase produces a large N-terminal ectodomain (sAPPα or sAPPβ) and a truncated C-terminal fragment with a transmembrane region (CTFα or CTFβ). Second, CTFα is cleaved by γ-secretase presenilin complex [46], which generates a non-amyloidogenic p3 fragment and an APP intracellular domain (AICD). CTFβ is cleaved by the same γ-secretase to generate amyloidogenic Aβ peptides (Aβ40 and Aβ42) and an AICD. Although the subcellular compartment in which each proteolytic cleavage occurs is not known completely, it is likely that the short cytoplasmic domain is critical for the regulation of intracellular APP trafficking and processing. Indeed, deletion of the cytoplasmic domain of APP impairs the internalization of APP from the plasma membrane and decreases the secretion of Aβ peptides [47, 48]. Substitutions of residues in the GYENPTY motif with Ala also result in impaired internalization of APP and decreased secretion Aβ peptides in cultured cells [49]. In the knock-in mice, in which endogenous APP is replaced with truncated APP lacking residues around the GYENPTY motif, turnover of APP and Aβ levels are strongly reduced in brain [50]. These results suggest that the interaction of proteins with the GYENPTY motif of APP is important for the regulation of APP metabolism.

Many studies have examined the effects of different APP binding proteins on APP processing in cultured cells using protein overexpression and knock-down techniques [51]. Result from such studies have shown that the X11 family of proteins stabilizes cellular APP and decreases the secretion of sAPP and Aβ peptides [8, 52–55], whereas the FE65 family of proteins promote the secretion of sAPP and Aβ peptides [12, 56–59]. JIP-1b stabilizes immature APP and decreases the secretion of sAPP and Aβ peptides [15]. Recent findings on these three protein families are further described below. Dab1 regulates neuronal migration in mammals by binding to the cytoplasmic domain of the apoE receptor [60]. Dab1 increases levels of sAPP and CTFs in transfected cell lines and primary neuronal cultures. Treatment with reelin protein, an extracellular ligand for the apoE receptor, significantly enhanced the interaction of APP and Dab1, resulting in the increased cleavage of APP and the decreased production of CTFβ and Aβ peptides [61]. Dab2 regulates APP endocytosis at the cell surface [19]. Numb, an endocytic adapter protein that is important for cell fate determination during development, has been shown to affect APP metabolism in isoform-dependent manner [62]. Expression of Numb with a short PTB domain resulted in the accumulation of APP in early endosomal and recycling compartments. In contrast, expression of Numb with a

PTB domain containing an extra insertion resulted in lower levels of cellular APP and cleaved fragments. Numb might alter the intracellular trafficking and sorting of APP to the recycling and degradation pathways [62]. ARH is expressed mainly in skeletal muscle and liver, and regulates cholesterol uptake. Down-regulation of ARH by RNA interference has been shown to increase cellular levels of APP [23]. AIDA-1 is an adaptor protein expressed in brain; however, its function remains unknown. AIDA-1a, a shorter splice variant of AIDA-1 composed of two N-terminal SAM domains and a C-terminal PTB domain, binds to APP and decreases secretion of Aβ40 by inhibiting γ-secretase. In contrast, AIDA-1b, a longer isoform of AIDA-1 with an additional N-terminal region containing ankyrin repeats, does not bind to APP [24, 25]. SNX17 is a member of the sorting nexin family that binds low density lipoprotein receptors and regulates the endocytic trafficking of these receptors. Although SNX17 lacks a PTB domain, it binds to the GYENPTY motif of APP. Suppression of SNX17 decreases the half-life of APP and increases the production of Aβ peptides [19].

In addition to the protein interactions at the GYENPTY motif, the metabolism of APP is regulated by protein interactions at other APP motifs. For example, a human homologue of the yeast N-terminal acetyltransferase hARD1, binds to a novel motif (658-HGVVEVD-664) in the cytoplasmic domain of APP. hARD1 acts as acetyltransferase in association with its co-factor, hNAT1. Co-expression of hARD1 and hNAT1 suppresses the secretion of Aβ40 in cell culture [38]. PAT1 is associated with microtubules and binds to a region of the cytoplasmic domain of APP that contains a basolateral sorting signal (649-KKKQYTSIHHG-659) [36]. PAT1a, a polymorphic variant of PAT1, facilitates the trafficking of APP to the cell surface and increases the proteolytic cleavage of APP, which promotes the secretion of Aβ peptides [63].

As mentioned above, the metabolism of APP is influenced by the network of protein interactions *via* the cytoplasmic domain. But the contribution of each binding protein on the metabolism may not be equal. Only a few binding proteins such as X11 and FE65 families have already been tested their roles on APP *in vivo*. Some binding proteins may not directly regulate the metabolism of APP but just display secondary effects by competing with other binding proteins. Further quantitative studies will be required to compare the significance of each binding protein for the metabolism of APP.

**X11/Mint family of proteins**

X11 proteins are adaptor proteins that contain a conserved C-terminal PTB domain that binds to APP and PDZ domains, and divergent N-terminal regions that bind

to synaptic proteins such as CASK and Munc-18. X11 and X11L are expressed in neurons, whereas X11L2 is expressed ubiquitous (Reviewed in [64, 65]). X11 binds to the molecular motor KIF17 to regulate intracellular transport in neurons [66]. X11 proteins are involved in synaptic function such as presynaptic neurotransmitter release, and the regulation of APP metabolism [67–69].

In cultured cells, the overexpression of an X11 protein (X11, X11L, or X11L2) stabilizes intracellular APP and suppresses the production of cleaved fragments including Aβ peptides [8, 52–55]. Based on these *in vitro* observations, the effects of X11 proteins on APP metabolism were examined *in vivo* using murine models of AD. The effects of X11 protein overexpression were examined using transgenic mice that express APP containing a familial AD mutation (APPswe) that results in high levels of Aβ production. The amount of Aβ40 and the number of amyloid plaques were deceased in double-transgenic mice expressing X11 and APPswe compared to control [70]. There were also low levels of Aβ peptides and plaque formation in the cerebrum of double-transgenic mice expressing X11L and APPswe [71]. In contrast, high levels of Aβ peptides and CTFβ have been observed in the hippocampi of X11L knock-out mice [72], suggesting that X11L regulates the amyloidogenic metabolism of APP. In double knock-out mice lacking both X11 and X11L, the β-cleavage of APP and the accumulation of Aβ peptides were increased in brain. One possible mechanism underlying these effects is that members of the X11 family of proteins might regulate the β-cleavage of APP by anchoring APP outside of the detergent-resistant membrane fraction, thereby protecting APP from active β-secretase β-site-cleaving enzyme (BACE) [73] (Fig. 2A). Taken together, these studies support the idea that the X11 family of proteins functions as stabilizers of APP metabolism.

Recently, Ho et al. reported that the deletion of each of the three X11 genes in transgenic mice decreased the production of amyloid plaques and resulted in lower levels of Aβ40 and Aβ42 peptides by reducing the β-secretase cleavage of APP [74]. These results, although apparently at odds with those described above, might be attributable to the different genetic backgrounds used in the different studies: the studies described above examined endogenous APP and Aβ peptides in the brains of knock-out mice [72, 73]. The studies conducted by Ho et al. examined human Aβ peptides in double-transgenic mice in which human mutant APP and mutant γ-secretase presenilin1 were overexpressed, thereby resulting in a greater production of Aβ peptide in the brains of those mice [74]. Thus, the precise role of X11 proteins in the regulation of APP processing in human brain remains unknown; however, it is likely that these proteins play a critical role. Among the X11 proteins, X11L is likely to be the most

important regulator for the processing of APP in brain because mice with defective X11L display the most striking changes in APP processing compared to other members of the X11 family [73, 74]. This might be a result of the higher affinity of X11L for APP or the broader distribution of X11L in brain compared to X11.

Results from the in vitro and in vivo studies described above suggest that the interaction of APP and X11 proteins is a potential therapeutic target for AD. Therefore, it is important to understand what factors influence the APP/X11 interaction (Fig. 2B). The expression of the PTB domain of X11 alone is sufficient to mimic the effects of full-length X11 in cultured cells [75]. However, data from this laboratory has shown that X11L also contains an N-terminal regulatory region (X11L221–250) that is separate from the PTB domain. Phosphorylation of Ser236 and Ser238 in the regulatory region is critical for increasing the association of X11L with APP. This region and the putative phosphorylation sites are conserved in X11, but not in the non-neuronal protein X11L2 [76].

The effects of X11 proteins on APP metabolism are also affected by other proteins that bind to the X11 proteins. For example, X11L interacts with Alcadein, a type I membrane protein, and forms a tripartite complex composed of APP, X11L, and Alcadein. The formation of this tripartite complex has been shown to suppress the metabolic cleavage of APP [77]. X11L binding protein of clone number 51 (XB51), also known as NIP1 or NECAB3, has been shown to regulate the formation of Aβ peptides in an isoform-dependent manner; the association of XB51 with X11L and APP can increase the formation of Aβ, whereas XB51 alone suppresses the formation of Aβ in an X11L-independent manner [78, 79]. The ADP-ribosylation factor (ARF) GTPase, which binds to the PTB and PDZ domains of X11 proteins, is essential for vesicle budding and is important for X11 protein-mediated increases in cellular APP [80]. The synaptic protein Munc18-1 has been reported to enhance the effects of X11 to decrease APP processing and Aβ peptide secretion in vitro [81]. Interaction of X11 or X11L with Munc18-1 mediates the association of APP with syntaxin 1-containing microdomains. This association inhibits the APP-BACE interaction and β-cleavage *via* microdomain segregation [82].

**FE65 family of proteins**

The human and murine FE65 family of proteins consists of FE65, FE65L1, and FE65L2 (reviewed in [83]). These proteins are expressed in brain and share an N-terminal WW domain and two C-terminal PTB domains, PTB1 and PTB2. The

interaction of FE65 proteins with the cytoplasmic domain of APP is mediated by the PTB2 domain, whereas the PTB1 domain binds proteins such as the low density lipoprotein receptor-related protein ApoER2, the histone acetyltransferase Tip60, and the transcription factor CP2/LSF/LBP1 [84–86].

FE65 is localized in the cytoplasm and nucleus of the cell. Nuclear translocation of FE65 is regulated by its interaction with APP and/or AICD [86-89]. Data from many laboratories have shown that FE65 forms a complex with AICD and nuclear proteins such as Tip60 and CP2/LSF/LBP1 to regulate transcriptional activity [83, 85, 86]; however, the physiological relevance of the activity remains unclear. Recent reports also showed that FE65 involved in the response of cell stress, especially to DNA damages [88, 96]. FE65 and APP have also been shown to regulate neuronal membrane motility by interacting with Mena, a regulator of actin dynamics that is important for neurite growth and synapse modification [90, 91]. The physiological roles of the FE65 proteins have been studied *in vivo* using knock-out mice. Isoform-specific FE65 knock-out mice exhibit impaired learning and memory [92]. FE65 and FE65L1 double-knock-out mice display neuroanatomical abnormalities in the cortex and hippocampus that resemble the abnormalities that occur in mice lacking APP family members or Mena/Vasp proteins [93]. These results imply the possibility that FE65 proteins cooperate with APP to play a role in neural development.

FE65 proteins also play a role in the metabolism of APP. A number of studies have reported that overexpression of FE65, FE65L1, and FE65L2 promoted the production of Aβ peptides and the secretion of sAPP in cultured cells [12, 56–59], whereas the effects of FE65 to stabilize immature APP and inhibit the secretion of Aβ peptides in HEK293 cells have also been shown [94]. In APP/FE65 double-transgenic mice, lower levels of Aβ42 and decreased accumulation of Aβ peptides in the cerebral cortex were observed compared to controls [95]. In primary neuronal cultures from transgenic mice in which human mutant APP was expressed and FE65 was partially knocked out (the 97 kDa isoform of FE65 was absent, but the 60 kDa isoform of FE65 was increased), secretion of Aβ was decreased compared to control [92]. The effects of FE65 protein deficiency on endogenous APP processing was examined in double knock-out mice lacking both FE65 and FE65L1. In these mice, levels of Aβ42 were significantly reduced in young adult males compared to control, although the steady-state levels of APP were unchanged [93].

**JIP family of proteins**

JIP proteins are scaffold proteins that bind the components of the JNK kinase cascade to facilitate the signal transduction [97–99]. JIP proteins also associate with kinesin-1 motor proteins to mediate its active transport of cargo proteins along microtubules [100, 101]. In the mouse, JIP-1 plays an important role in neuronal stress responses involving the JNK pathways, and is necessary for the kinesin-1-dependent promotion of axonal growth [102, 103]. Among the JIP family of proteins, JIP-1 and JIP-2 contain an N-terminal JNK-binding domain and a C-terminal Src homology 3 domain and PTB domain. The PTB domain of JIP-1b, a splice variant of JIP-1 also known as Islet Brain 1 (IB1) [104], binds to the GYENPTY motif of APP. Both JIP-1a, the other variant that lacks a part of the PTB domain, and JIP-2 have weak binding affinities for APP [14–17, 105].

APP undergoes fast anterograde axonal transport [106]**.** APP can be transported not only as cargo in transport vesicles but also as a cargo receptor associated with a motor complex. It was previously thought that the association of APP with the motor complex involved the direct binding of the cytoplasmic domain of APP to the KLC subunit of the kinesin-1 motor [35, 107]. However, the existence of a direct interaction between the KLC and APP is not universally accepted [108]. JIP-1 contains a binding motif for KLC at the C-terminal end adjacent to the PTB domain. Recent reports support an alternative model in which JIP-1 serves as a bridge between the KLC and APP (Fig. 3) [14, 18, 109]. In cultured cells, the interaction of JIP-1b with APP stabilized immature cellular APP and inhibited the secretion of Aβ peptides [15]. The precise relationship between axonal transport and the processing of APP in neurons remains unclear. Kamal et al. proposed that the proteolytic processing of APP occurs in an axonal membrane compartment that contains APP, β-secretase BACE, and γ-secretase presenilin-1, and that the transport of APP occurs via kinesin-1 [107]. However, this model is somewhat controversial as results from several laboratories have failed to support its predictions [108]. Alcadein is an X11L binding protein that also binds the KLC and competes with JIP-1 for binding to the kinesin-1 motor. In the presence of Alcadein, the APP-JIP-1 complex is released from the KLC, the transport of APP-containing vesicles is inhibited, and the generation of Aβ peptides is increased (Fig. 3) [110]. These results suggest that the transport of APP-containing vesicles by kinesin-1 suppresses the formation of Aβ peptides, whereas the accumulation of stationary or aggregated APP-containing vesicles increases the formation of Aβ peptides.

In addition to its role in APP trafficking, JIP-1 is also reported to be involved in AICD-dependent transcriptional regulation. JIP-1b has been shown to transport AICD

to nuclei and dock AICD with Tip60 in similar manner as FE65 [89]. However, in contrast to the effects of FE65 on gene activation, data from a Gal4-dependent reporter gene assay suggests that the gene activation by JIP-1 is Tip60-independent [111].

**Phosphorylation of APP at Thr668 and interactions with APP binding proteins**

The Thr668 residue in the middle of the cytoplasmic domain of APP is constitutively phosphorylated by CDK5 and GSK3β in neurons. It is also transiently phosphorylated by JNK during the stress response and by CDC2 in dividing cells. The phosphorylation state of Thr668 is an important modulator of APP metabolism and function (Reviewed in [112]). The APP binding proteins JIP-1b and X11L can affect the phosphorylation state of Thr668. JIP-1b enhances the phosphorylation of Thr668 by JNK by scaffolding of APP and JNK kinases [14, 18, 113]. X11L (but not X11 or X11L2) also facilitates the phosphorylation of Thr668, possibly through a conformational change of APP upon its binding to X11L [114].

The interactions of APP binding proteins are also modulated by Thr668 phosphorylation. Thr668 is located within the 667-VTPEER-672 motif, which forms a type I β-turn and N-terminal helix-capping box structure to stabilize its C-terminal helix structure. The phosphorylation of Thr668 results in a significant conformational change in the cytoplasmic domain of APP [115] and disrupts the interaction of APP with FE65 [94]. Further evidence for this "molecular switch" model is provided by structural analysis of AICD bound to the PTB2 domain of FE65 [32]. The binding interface of the cytoplasmic domain of APP to the PTB2 domain of FE65 consisted of two alpha-helices in addition to the typical NPTY motif. The phosphorylation of Thr668 disrupted the N-terminal alpha helix resulting in a dissociation of the complex as suggested previously. Dissociation of APP and FE65 as a result of phosphorylation might be responsible for the decreased effects of FE65 on the secretion of Aβ peptides in cells, on nuclear transport, and on gene transactivation mediated by AICD [88, 95, 116]. JIP-1 is reported to interact with phosphorylated APP and to exhibit greater co-localization with phosphorylated APP than with non-phosphorylated APP. JIP-1 might facilitate the localization of phosphorylated APP to the terminals of neurites [105]. Pin1 has been proposed to be a phosphorylation-dependent regulator of APP conformational change. Pin1 is a peptidyl-prolyl isomerase that specifically recognizes phosphorylated S/T-P bonds and facilitates the cis-trans interconversion of the peptidyl-prolyl bond, thereby affecting protein folding. Pin1 binds to the phosphorylated Thr668-Pro motif of APP and accelerates its isomerization, resulting in a conformation change of the APP

cytoplasmic domain [40, 41]. This interaction might be important for the regulation of Aβ production. Pastorino et al. reported that Pin1 knock-out mice exhibit increased amyloidogenic APP processing and elevated levels of insoluble Aβ42 peptide in brain in an age-dependent manner [40]. However, in another study, Akiyama et al. observed decreased production of Aβ peptides in Pin1-deficient mice [41]. There is ongoing debate over the importance of Thr668 phosphorylation with regard to the production of Aβ peptides in brain. The treatment of neurons with kinase inhibitors, which decrease Thr668 phosphorylation, has been shown to decrease the secretion of Aβ peptides [117]. However, the levels and subcellular distribution of APP and its metabolic products including Aβ peptides were unaffected in APP T668A knock-in mice in which the Thr668 residue was substituted to Ala and could not be phosphorylated [118].

## Conclusion

Decades of research have identified nearly thirty proteins that bind to the cytoplasmic domain of APP. To play physiological roles APP may act together with and/or regulate binding proteins such as FE65 and JIP-1 as reviewed in this issue. The gene disruption studies have revealed that FE65 family and JIP-1 are involved in neuronal development [93, 103]. However, it still remains unclear whether the interactions with APP are critical for their functions. APP knock-out mice display modest brain developmental and behavior defects, and double or triple knock-out of APP family proteins causes much severer phenotype [2–4]. Ring et al. reported that the cytoplasmic domain of APP was dispensable to rescue gross phenotypes observed in APP knock-out mice [50]. This result implies that the major functions of APP may not be mediated by the interactions through the cytoplasmic domain. Alternatively, since many APP binding proteins also bind to APLP1 and 2, APP binding proteins may be involved in the core roles of APP, for which the cytoplasmic domain of APLP1 or 2 functions redundantly. More detailed studies are necessary to evaluate the physiological meaning of the interactions between APP and the binding proteins that are predicted from *in vitro* observations.

In contrast, the roles of APP binding proteins to regulate the metabolism of APP are well analyzed. Without protein interactions through the cytoplasmic domain, the intracellular trafficking or proteolytic cleavages of APP is not disrupted completely but severely disturbed both *in vitro* and *in vivo* [47, 48, 50]. Recent gene ablation studies support the model that X11 and FE65 family proteins are involved in the regulation of APP metabolism [72–74, 93, 94]. Future studies will be required to evaluate the roles of

other binding proteins that have been suggested by *in vitro* studies. It will also be important to examine the relationships among the numerous binding proteins with regard to their effects on the metabolism of APP. As highlighted in this review, each APP binding protein behaves differently on the APP metabolism. Basically APP binding proteins with PTB domain may compete each other for the binding to the short GYENPTY motif of APP. Therefore the regulation of binding affinity and the local protein concentration of each molecule in intracellular microdomains may be important for precise metabolism of APP [73]. The phosphorylation at Thr668 may function as a molecular switch that changes the binding preference of APP [112]. And the perturbation of the balance of protein interactions is likely to be a putative cause of AD. From therapeutic perspective, the manipulation of the protein interaction of APP is a potential approach to reduce Aβ production. For example, the interaction of X11L with APP can be promoted by stimulating N-terminal regulatory region of X11L to decrease the production and deposit of Aβ [76]. The development of new tools and compounds that allow for the specific manipulation of individual protein interactions will be valuable for exploring therapeutic strategy and also contribute to a deeper understanding of the function and metabolism of APP at the molecular level**.**

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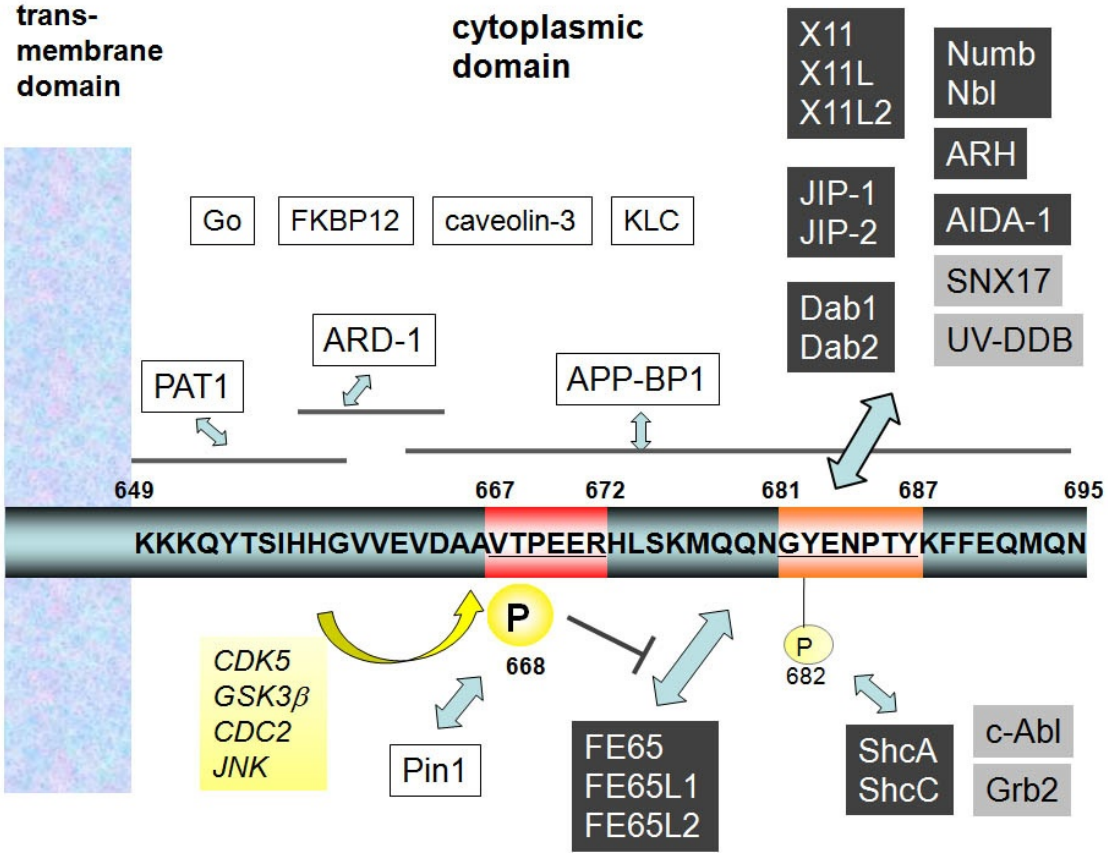


**Figure 1.**

**The cytoplasmic domain of APP and its binding proteins.**

Shown here is the amino acid sequence of the APP cytoplasmic domain. Two functional motifs, 667VTPEER672 and 681GYENPTY687, are marked (numbers indicate amino acid positions of the APP695 isoform). Proteins that contain PTB domains and bind the GYENPTY motif of APP are indicated with white letters in black boxes. Proteins that do not have a PTB domain and bind the GYENPTY motif are indicated with black letters in gray boxes. Other binding proteins are indicated with black letters in white boxes. The Thr668 residue is the major phosphorylation site of APP in brain. Cdk5, GSK3$\beta$, JNK, and CDC2 are protein kinases responsible for the phosphorylation of Thr668. Thr668 phosphorylation affects the interaction of proteins such as FE65 and Pin1. Tyr682 is a putative phosphorylation site, the phosphorylation of which is required for interaction with Shc, Abl, and Grb2.

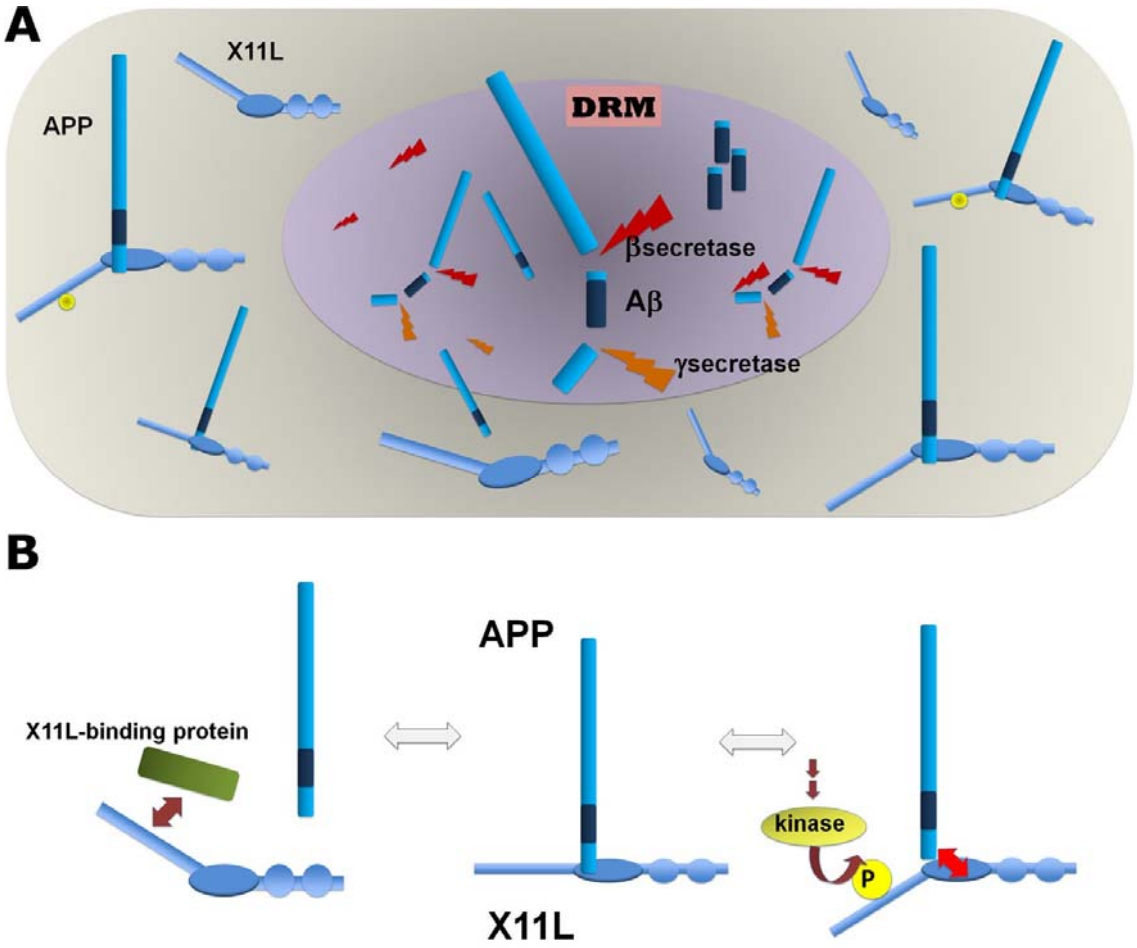


**Figure 2**

**A model for the regulation of APP metabolism by X11 proteins.**

**A.** X11 proteins interact with the cytoplasmic domain of APP and anchor APP outside of a compartment corresponding to the detergent-resistant membrane (DRM) fraction. Once APP dissociates from X11 proteins, APP is translocated to the DRM, where β-secretase BACE is activated. In the DRM, APP is cleaved by BACE and γ-secretase presenilin to generate Aβ peptides. **B.** Proposed machinery that might modulate the binding between APP and X11 proteins. The PTB domain of X11 proteins is sufficient for APP binding (middle); however, protein interactions or modifications of other regions can affect binding. For example, the protein interactions of X11L with X11L-binding proteins such as XB51 might inhibit APP binding (left). In contrast, the phosphorylation of the N-terminal region of X11 or X11L by unknown protein kinases might induce a conformational change and facilitate the interaction with APP (right).

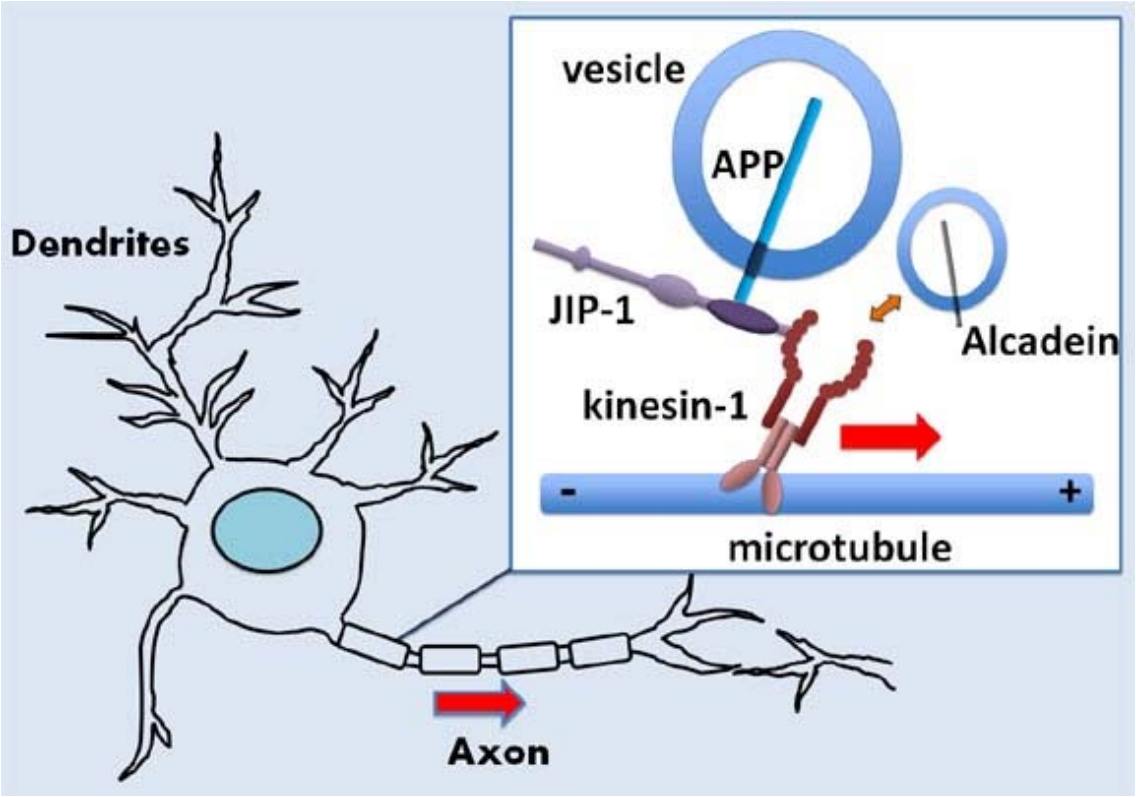


**Figure 3**

**A model for the fast axonal transport of the APP–JIP-1 complex by kinesin-1 in neurons.** Vesicles containing APP are subject to anterograde transport by the molecular motor kinesin-1 from the soma to the nerve terminals along microtubules in axons. JIP-1 binds APP via the PTB domain as well as kinesin-1 *via* its C-terminal binding motif, and might serve as a bridge between APP and kinesin-1 during transport. The axonal transport of APP might be closely related to APP processing. For example, Alcadein can compete with the APP–JIP-1 complex for binding to kinesin-1 and inhibit APP transport. APP vesicles released from kinesin-1 can enter the amyloidogenic pathway to produce Aβ peptide.