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Cytoplasmic Tail Adaptors of Alzheimer's Amyloid-β Protein Precursor

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Abstract

Alzheimer's disease is characterized pathologically by senile plaques in the brain. The major component of senile plaques is amyloid-β (Aβ), which is cleaved from Alzheimer's Aβ protein precursor (AβPP). Recently, information regarding the cytoplasmic tail of AβPP has started to emerge, opening up various insights into the physiological roles of AβPP and its pathological role in Alzheimer's disease. The cytoplasmic domain of AβPP shares the evolutionarily conserved GYENPTY motif, which binds to a number of adaptor proteins containing the phosphotyrosine interaction domain (PID). Among the PID-containing proteins, this article focuses on four groups of adaptor proteins of AβPP: Fe65, X11, mDab1, and c-Jun *N*-terminal kinase-interacting protein 1b/islet-brain 1.

Index Entries: AβPP(APP); the GYENPTY motif; PID/PTB; Fe65; X11/Lin-10/Mint; mDab1; JIP; JIP1b/IB1; G protein.

Introduction

Alzheimer's disease is the most prevalent neurodegenerative disease. An established hallmark of this disease is extracellular neuritic plaques, whose major constituent is amyloid-β (Aβ), which is cleaved from a large precursor, termed Aβ protein precursor (AβPP). The three members in the mammalian AβPP family are AβPP, Aβ precursor-like protein (APLP)-1, and APLP-2. All of these are single-transmembrane, receptor-type molecules with a large glycosylated extracellular domain and a short cytoplasmic domain. Regardless of the species, all members of the AβPP family contain the conserved GYENPTY motif in the cytoplasmic domain (Fig. 1). The cytoplasmic domain of

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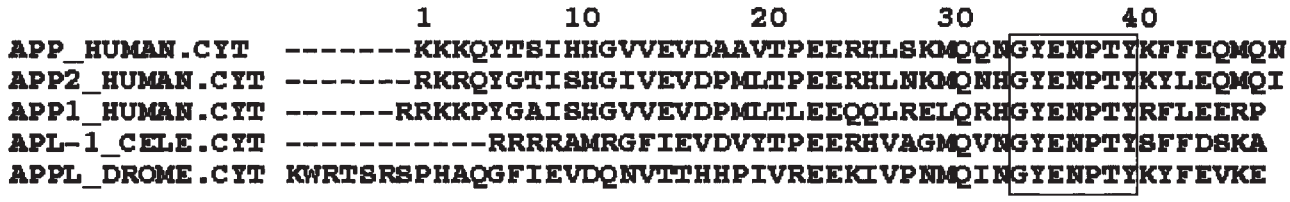

Fig. 1. The cytoplasmic domains of the AβPP family proteins. The alignment of the cytoplasmic domains of human AβPP (APP_HUMAN.CYT), human APLP-2 (APP2_HUMAN.CYT), human APLP-1 (APP1_HUMAN.CYT), *C. elegans* apl-1 (APL-1_CELE.CYT), and *Drosophila* APPL (APPL_DROME.CYT). All members of the AβPP family from different species share the GYENPTY motif (boxed). The numbers on the top are the amino acid positions relative to the cytoplasmic domain of human AβPP. The amino acid sequences of the AβPP cytoplasmic domains are identical among human, squirrel monkey, pig, chicken, mouse, rat, guinea pig, and electric ray. The cytoplasmic domains of APLP-2 are identical between human and mouse. The accession numbers of the sequences used in this alignment are human AβPP, P05067; human APLP2, Q06481; human APLP1, P51693; *C. elegans* apl-1, Q10651; *Drosophila* APPL, P14599.

AβPP ($A\beta PP_{CD}$) has the unexpected function of interacting with a number of cytoplasmic proteins. Two different groups of these proteins bind to $A\beta PP_{CD}$. The first is pertussis toxin-sensitive heterotrimeric GTPase G_o, which binds to the middle cytoplasmic region His^{657}-Lys^{676} *(1,2).* The second is comprised of cytoplasmic adaptors without enzyme activity. They interact with the extreme cytoplasmic tail Met^{677}-Asn^{695}, whose interaction core is the GYENPTY region. This article focuses on the proteins belonging to the latter group.

The NPXY motif (X denotes any amino acid) contained in the GYENPTY AβPP signature can be found in a number of receptors and signaling molecules. The NPXY motif originally was identified in the cytoplasmic domain of the low-density lipoprotein receptor (LDLR) in 1990. NPXY was proposed as the internalization motif via clathrin-coated pits *(3).* The NPXY motif can now be found in a wide range of proteins, including all members of the LDLR family, AβPP, and the inositol polyphosphate 5′ phosphatase Ship *(4).*

The phosphotyrosine interaction domain (PID), also termed phosphotyrosine binding (PTB) domain, binds to the NPXY motif. The PID domain was originally noted in Shc, which interacts with the NPXY motif in the cytoplasmic portion of the epidermal growth factor (EGF) receptor (EGFR) *(5,6).* The PID of Shc is distinct from SH2 but still binds to NPXY in a phosphotyrosine-dependent manner *(5–7).*

The Four Groups of AβPP Adaptor Proteins

Since its discovery in 1987, much attention has been directed to AβPP. Numerous possible functions of AβPP have been proposed based on AβPP-interacting molecules, which metabolize, transport, modulate, or possibly transduce the potential AβPP signals. Because the proposed functions of AβPP are too diverse to be addressed in this article, we focus on four groups of intracellular adaptors of AβPP: (a) Fe65, (b) X11, (c) mouse disabled-1 (mDab1), and (d) c-Jun N-terminal kinase (JNK)-interacting protein (JIP) 1b. All of these use their PID to bind to the evolutionarily conserved GYENPTY motif in $A\beta PP_{CD}$.

Fe65: The First Identified AβPP Adaptor

Fe65 originally was identified in 1991 as a brain-specific transcriptional activator with unknown functions *(8).* In 1995, using yeast

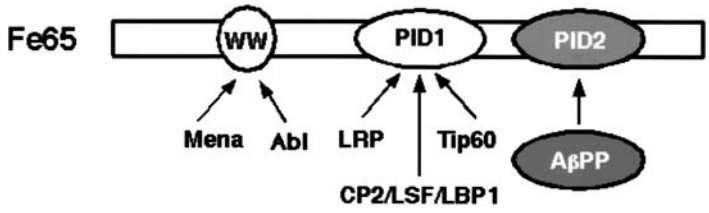

Fig. 2. Interaction of Fe65 with cytoplasmic proteins. Fe65 has one WW domain, which binds to Mena *(18)* and Abl *(19)*. Fe65 has two PIDs: the first PID binds to CP2/LSF/LBP1 transcriptional factor *(13),* LRP *(14),* and Tip60 *(21)*; the second PID binds to AβPP *(9).*

two-hybrid screening, AβPP was found to interact with PID of Fe65 *(9).* Subsequently, other members of the Fe65 family were identified as AβPP-binding proteins (Fe65L *[10]* and Fe65L2 *[11]*). Fe65 has one WW domain and two PIDs (Fig. 2). The second PID binds to the GYENPTY region of AβPP, in a phosphotyrosine-independent manner *(12).* Fe65 can also bind to other proteins. The first PID of Fe65 binds to the transcription factor CP2/LSF/LBP1 *(13).* The first PID also binds to the NPXY motif of LDLR-related protein (LRP) *(14).* Because the second PID binds to AβPP, Fe65 is able to bridge LRP and AβPP *(14,15).*

Two novel isoforms of Fe65L2, designated I-214 and I-245, were recently identified *(16).* They are produced by alternative splicing of the messenger RNA (mRNA) and lack PIDs. Secretion of AβX-40 (Aβ-40) and Aβ-42 is increased by overexpression of Fe65L2 but not by that of I-214, suggesting that Fe65L2 affects Aβ production and a possible regulation of this function of Fe65L2 by alternative splicing. In addition, Chang et al. *(17)* recently reported that Fe65L2 increased generation of $A\beta PP_{CD}$ and Aβ-40. This modulation did not occur as the direct effect of Fe65L on γ-secretase activity.

The WW domain of Fe65 binds to several proteins in mouse brain. The binding proteins include Mena *(18),* the mammalian homologs of *Drosophila* Enabled, which is involved in the Abl tyrosine kinase pathway. This WW domain also binds to the active form of Abl itself, which can phosphorylate AβPP on Tyr^{682}, which in turn becomes the binding site of the Src homology 2 (SH2) domain of Abl *(19).* Therefore, there is a possibility that AβPP–Fe65 interaction is involved in the Abl tyrosine kinase pathway. AβPP, Fe65, Mena, and actin are colocalized in focal complexes of lamelipodia, suggesting that the AβPP-Fe65-Mena complex is involved in actin-based cell movement *(20).*

Accumulated evidence indicates that Fe65 transduces signals upon cleavage of AβPP. It has been reported that Fe65 activates the reporter gene transcription induced by artificial DNA-binding domain fused to the C-terminus of AβPP *(21).* Tip60, a component in histone acetyltransferase complex, binds to the first PID of Fe65. Therefore, Fe65 can function as a signal transducer tethered on the membrane by AβPP *(21,22)*, which is released by the proteolytic cleavage of AβPP, moves to the nucleus, and activates downstream transcription factors with associated Tip60. The LRP cytoplasmic domain (LRPICD) markedly inhibits $A\beta P\text{-}P_{CD}$–Fe65 transactivation mediated by Tip60 *(23).* LRPICD has a close interaction with Tip60 in the nucleus, as demonstrated by a fluorescence resonance energy transfer (FRET) assay.

Kimberly et al. *(24)* demonstrated that $A\beta P\text{-}P_{CD}$, which is a highly labile fragment, is stabilized by forming complexes with Fe65 and can then enter the nucleus in neurons and non-neuronal cells. Kinoshita et al. *(25)* directly demonstrated that the AβPP carboxyl-terminal domain generated by γ-secretase translocates to the nucleus and continues to interact with Fe65 in the nucleus, determined by subcellular colocalization of both proteins and FRET assays.

Wang et al. *(26)* recently generated a line of Fe65 knockout mice in which the 97-kDa

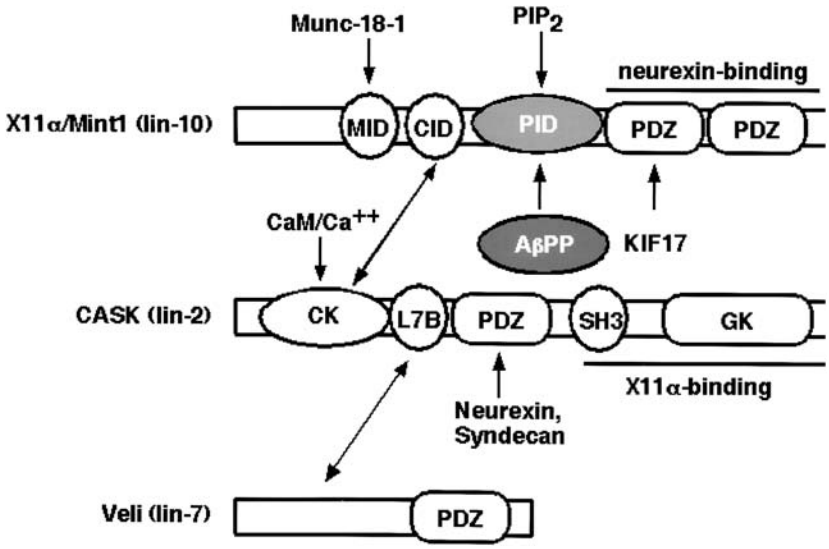

Fig. 3. X11 interactors and the X11-α/Ca^{2+}/calmodulin-dependent membrane-associated kinase (CASK)/Veli (Lin-10/Lin-2/Lin-7) complex. X11-α (Mint1), the lin-10 homolog, contains Munc 18-interacting domain (MID), CASK-interacting domain (CID), PID, and two PDZ domains. X11-γ (Mint3) lacks MID. MID binds to Munc-18-1 *(33);* CID binds to CaMKII domain (CK) of CASK *(32);* PID binds to AβPP *(15)* and PIP_2 *(33);* and the first PDZ domain binds to KIF17 *(119).* The region containing two PDZ domains binds to neurexin *(120).* CASK, the Lin-2 homolog, contains the CK domain, Lin-7-binding domain (L7B), the PDZ domain, SH3, and the guanylate kinase domain (GK). The CK domain binds to calmodulin *(32)* as well as to CID of X11-α; L7B binds to Veli *(41);* and the PDZ domain binds to neurexin and syndecan *(121).* The region containing SH3 and GK binds to X11-α (32). Veli, the lin-7 homolog, binds to CASK and contains one PDZ domain (41).

full-length Fe65 (p97) was ablated. Expression of an N-terminus-truncated Fe65 isoform (p60) remained. The p97Fe65 −/− mice were viable and showed no obvious physical impairments or histopathological abnormalities. However, p97Fe65 −/− demonstrated poorer performances than wild-type mice on a passive avoidance task, impaired hidden-platform acquisition, and a severe reversal-learning deficit. These findings suggest that Fe65 plays an important role in learning and memory.

X11/Lin-10/Mint: The Second Identified Adaptor of AβPP

X11-α *(27)* and its homologs, X11-β *(28)* and X11-γ *(29),* bind to the GYENPTY motif of AβPP via the PID. X11-α does not require tyrosine phosphorylation of the NPTY region in $AβPP_{CD}$ *(30).* X11s have a variable N-terminal region but share a constant C-terminal region that contains one PID and two PDZ (PSD95, disc-large, ZO-1) domains (Fig. 3). X11-α and -β are neuron-specific, but X11-γ is ubiquitously expressed *(29,31,32).* The PID of X11-α also binds to phosphatidylinositol biphosphate (PIP_2) *(33).* Munc-18-1, also known as Munc18a or nSec1, binds to the N-terminal Munc-18-1 binding domain (MID) of X11-α and -β but not to X11-γ, which lacks MID *(31,33).*

X11 has three different names corresponding to its different origins: X11, Lin-10, and Mint. *Caenorhabditis elegans* lin-10 was found in a lineage abnormality mutant *(34).* Human X11 was accidentally discovered in Friedreich's ataxia-susceptible locus *(35)* but was not related to the disease *(36).* Mints were identified as the adaptor proteins of synaptic protein Munc-18-1 *(33).* Therefore, mammalian X11-α is also termed

Mint1 *(33);* X11-β is also termed X11L *(28)* or Mint2 *(33);* and X11-γ is also termed X11L2 *(29)* or Mint3 *(31).*

X11 is a part of the conserved complex involved in basolateral protein sorting. This physiological function of X11 has been unraveled through the genetic analysis of its *C. elegans* homolog, *Lin-10*, which was identified in a mutant worm harboring a defect in its vulval development *(34).* In the loss-of-function mutant worm, the vulval cell lineage cannot be induced properly, and instead it becomes the hypodermal cell lineage. This defect in vulval induction is caused by the mislocalization of Let-23 (nematoda EGFR) in the vulval precursor cell. The Let-23 tyrosine kinase receptor normally is transported to the basolateral membrane of the vulval precursor cell, where it meets the ligand Lin-3 (nematoda EGF), situated on the outer surface of the anchor cell. The correct transportation of Let-23 requires Lin-2, -7, and -10 *(37,38).*

Although the initial identification of the *lin-10* gene *(34)* was determined to be incorrect, the correct identification revealed that the Lin-10 gene product forms a tripartite complex with *Lin-2* and *-7* gene products *(38).* The Lin-10-mediated basolateral sorting appears to be shared between polarized epithelial cells and neurons, because GLR-1, a glutamate receptor of *C. elegans*, also requires Lin-10 for correct postsynaptic delivery in neurons. The mislocalization of GLR-1 in the Lin-10-defective worm is suppressed when the C-terminus of GLR-1 is mutated to harbor another type of PDZ-binding motif, suggesting the presence of similar, PDZ-dependent, alternative mechanisms of GLR-1 transportation *(39).* The mammalian counterparts of Lin-2 (CASK), -7 (Veli), and -10 (X11) also form a tripartite complex *(40,41).* However, it remains unclear whether the delivery of AβPP-related protein (APL-1) (the AβPP homolog in *C. elegans*) is altered in the *Lin-10* mutant worm in vivo or whether the proteins of the AβPP family are mislocalized in X11-deficient mice in vivo.

Because X11 is a part of the general basolateral sorting machinery, it is reasonable to assume that X11 may influence the metabolism of AβPP during its *de novo* synthesis. In fact, X11-α slows the metabolism of AβPP and prolongs its half-life, resulting in the reduction of soluble AβPP, Aβ-40, and Aβ-42 *(42,43).* Munc-18-1, which interacts with the N-terminal MID of X11, suppresses Aβ-40 secretion by X11-α *(44).* Interestingly, to stabilize wild-type AβPP and Swedish mutant K595N/M596L-AβPP, not only PID (which is the AβPP-binding site of X11) but also the two PDZ domains are required *(28,45).* These findings suggest that the additional players that bind to the PDZ domains are involved in this effect of X11 on the AβPP metabolism. The transcription factor nuclear factor (NF) κB/p65 induces increased secretion of amyloidogenic Aβ-42 but not Aβ-40 *(46).* The Aβ-42 production is suppressed by binding of NFκB/p65 to the PDZ domain of X11-β.

X11 has other functions, the most interesting of which is transinhibition by X11. Biederer et al. *(47)* reported that X11-α and -β, but not -γ, strongly inhibited transactivation by AβPP-Gal4/VP16, a fusion protein of AβPP linked to the potent transcription factor Gal4/VP16, whereas Fe65 enhanced transactivation as mentioned earlier.

It should be emphasized that despite the fact that both X11 and Fe65 bind to similar regions of AβPP, they have opposing effects on cellular Aβ production, as discussed earlier. It has been reported that AβPP, Fe65, and X11-β display overlapping distributions in both transfected cells and primary neurons and that the Fe65 and X11 compete for binding to AβPP *(48).* Therefore, elucidation of the mechanism that converts the AβPP binding from that for Fe65 to that for X11 would provide valuable information for the future therapeutic intervention in Alzheimer's disease.

mDab1: The Third Identified Adaptor of AβPP

mDab1 binds to the cytoplasmic GYENPTY motif of AβPP through its PID (Fig. 4). mDab1 also binds to Ship and the cytoplasmic tail of APLP-1, LRP, and LDLR *(14,49,50).* The

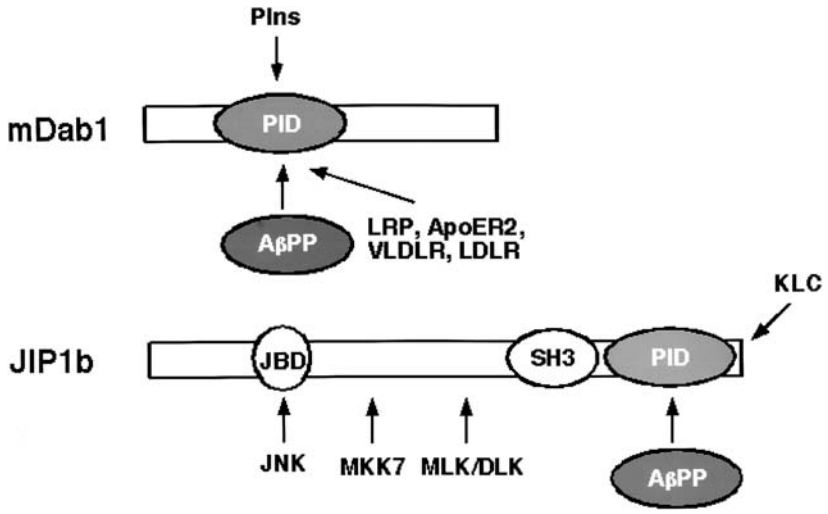

Fig. 4. Interaction of cytoplasmic adaptors with mDab1 or JIP1b. mDab1 contains one PID, which binds to AβPP, apoER2, LRP, VLDLR, LDLR, PI(4)P, and PI(4,5)P_2 *(14,49,69).* JIP1b has an N-terminal JNK-binding domain (JBD) *(73),* SH3, and PID, which binds to AβPP *(63).* The extreme C-termini conserved between JIP1 and -2 bind the kinesin light-chain (KLC) *(85).*

phosphorylation of either tyrosine contained in GYENPTY motif is not required for or, rather, weakens the binding of mDab1 to the GYENPTY motif *(49).* The PID of mDab1 also binds to PI(4)P or PI(4,5)P_2. The bound lipids do not interfere with mDab1–AβPP interaction *(49).*

It is generally accepted that mDab1 is the downstream transducer of the reelin pathway that determines the migration of developing neurons. The Abl tyrosine kinase is localized in *Drosophila* central nervous axons. *Drosophila* Disabled (dab) was identified as one of the genes screened for its ability to exacerbate the defects in the axonal development of Abl-disrupted files *(51).* Yeast two-hybrid screening using Src tyrosine kinase identified mDab1 *(52),* the mouse homolog of *Drosophila* dab. During embryogenesis, mDab1 is tyrosine-phosphorylated in neuronal cells, but the tyrosine phosphorylation disappears after nerve tracts are established. This tyrosine-phosphorylated mDab1 associates with SH2 domains of Src, Fyn, and Abl *(52).* Surprisingly, the phenotype of mDab1-disrupted mice is very similar to that of reeler mice *(53).*

A large extracellular matrix protein known as reelin is disrupted in naturally occurring reeler mice that show cerebellar ataxia with massive abnormal migration of cerebral and cerebellar neurons *(54).* Two other mutant mouse strains, scrambler and yotari, which show phenotypes indistinguishable from reeler mice, have mutations in mDab1 *(55),* further confirming that mDab1 and reelin are operating in the same functional pathway.

Multiple lines of evidence show that very-LDLR (VLDLR) and apolipoprotein E receptor 2 (apoER2) constitute the reelin receptor and transduce reelin signals through tyrosine phosphorylation of associated mDab1 *(56–59).* Other receptors (the cadherin-like neuronal receptor *[60]* and integrins *[61]*) have also been proposed as reelin receptors. In summary, mDab1 is an essential component of the reelin-VLDLR/apoER2-mDab1 pathway involved in neuronal migration.

An interesting issue is whether mDab1 is involved in the AβPP signaling pathway. Although mDab1 clearly is involved in the reelin pathway, its relationship with the AβPP

signaling pathway is elusive. Knockout mice deficient in either AβPP or APLP-1 or -2 do not manifest severe anatomical or neurological abnormalities. Double-knockout mice of either two of the three mammalian AβPP family members have displayed mysterious phenotypes. The double-knockout mice of APLP-1/-2 and of AβPP/APLP-2 are lethal early postnatally, whereas those of AβPP/APLP-1 are viable without any serious defects. The lethal double-knockout mice of AβPP/APLP-2 or of APLP-1/-2 do not display any obvious anatomical or neurological phenotype, with the exception that those mice die within 5–15 h after birth *(62).* Although the cause of death is still unclear, the absence of abnormal migration of neurons in the double-knockout mice suggests that mDab1 is not involved, at least not in the early postnatal lethality.

JIP1b: The Fourth Identified Adaptor of AβPP

JIP1b binds to the GYENPTY motif of AβPP using its PID (*see* Fig. 4; *[63,64]*). JIP1b is a rodent molecule, and islet-brain 1 (IB1) is the human homolog. They are termed JIP1b/IB1. JIP1 has two mammalian homologs: JIP2 *(65)* and JIP3 *(66).* All three homologs bind to three members of the JNK family, including JNK1, -2, and -3, to varying degrees. JIP1 and -2 are structurally similar, but JIP3 is distantly related to JIP1 and -2. The PID of JIP1b also binds to rhoGEF *(67),* apoER2, Megalin, and LRP *(68,69).*

JIP1b is a scaffolding protein of the JNK pathway kinases. JNK has been implicated in various signaling pathways, including the pathway triggering neuronal apoptosis *(70).* The single-knockout mice of either JNK1, -2, or -3 or the double-knockout mice of JNK1/-3 or JNK2/-3 survive normally, but the double-knockout mice of JNK1/-2 are embryonic lethal, with drastic impairment of neuronal apoptosis in the brain *(71).* Therefore, JNK1 and -2 would be redundant kinases required for developmental programmed cell death in the neural system. Although JNK3-deficient mice mature to adulthood, they exhibit significant resistance to kainic acid-induced apoptosis of hippocampal neurons. This observation shows that JNK3 is involved in apoptosis caused by extracellular insults *(72).*

JIP1 was initially identified as a cytoplasmic inhibitor of the JNK signal transduction cascade *(73).* JIP1 scaffolds the JNK cascade kinases JNK, MKK7, MLK3 or DLK, and HPK-1 *(74).* Coexpression of JIP1 and JNK with MKK7 or MLK3 increases JNK activation, indicating that JIP1 facilitates activation of the JNK cascade by scaffolding the necessary components together *(74,75).* JIP1b is a subsequently reported isoform of JIP1 *(76–79)* that seems to be the major form of JIP1 in the rat brain *(80).* JIP1b contains a 47-amino acid residue insertion in the C-terminal portion of JIP1 that completes the PID. It is this PID-containing isoform JIP1b, and not JIP1 (which has an incomplete PID), that interacts with the conserved GYENPTY motif of AβPP *(63).*

Recently, JIP1-deficient mice with contradictory phenotypes were reported from two laboratories. One strain of JIP1-deficient mice showed lethality at the two-cell stage *(81).* The other strain showed viability to adulthood without any gross abnormality. The neuronal cells of the latter mice showed significant resistance to neuronal toxicity caused by oxygen and glucose deprivation or kainic acid *(82).*

The most established function of JIPs is to act as cargoes of kinesin-1-mediated axonal transport. The sunday driver protein, the *Drosophila* homolog of JIP3, forms a complex with kinesin-1 and mediates axonal transport *(83).* Unc-16, the *C. elegans* homolog of JIP3, genetically interacts with kinesin mutants unc-104 and unc-116. Unc-16 is required for the correct synaptic vesicle localization *(84).* In mammalian systems, JIP1, -2, and -3 are also involved in axonal transport through binding to the tetratricopeptide repeat (TPR) of the kinesin-I light-chain (Klc1). Kinesin forms a complex with JIPs loaded with the JNK pathway kinases and apoER2 *(85).* Taken together, it is highly likely that JIPs function

as the evolutionarily conserved cargo for kinesin-mediated axonal transport.

Interestingly, the complex containing AβPP is axonally transported via kinesin in both *Drosophila* and mice *(86–88).* This AβPP transportation appears to be mediated by the direct binding of the kinesin light-chain to AβPP *(87).* However, it was also demonstrated that the AβPP–Klc1 interaction is not direct but is mediated by JIP1 *(89).* The PID of JIP1 binds the $A\beta PP_{CD}$ *(62,63),* whereas the JIP1 C-terminal region interacts with the TPR of Klc1.

Rohn et al. *(90)* and our group *(91)* independently noted that treatment with anti-AβPP antibody causes cell death in primary neurons and neuronal cells overexpressing AβPP. This was confirmed by Mbebi et al. *(92).* We also found that (a) anti-AβPP antibody exerts its toxic function by binding to the cell surface AβPP *(91);* (b) the AβPP region responsible for antibody-induced neuronal death is His^{657}-Lys^{676} but not Met^{677}-Asn^{695} *(93);* and (c) G_o, JNK, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and caspases are involved in this toxic pathway *(94).* In this mechanism, JIP1b functions as an essential adaptor that binds JNK pathway kinases to AβPP. The signals caused by antibody/AβPP binding (G_o activation) initially occur at the neighboring area of AβPP and are then transmitted to the cytoplasmic signals (JNK activation). We also revealed that dimerization of $A\beta PP_{CD}$ triggers apoptosis signal-regulating kinase (ASK)-1/JNK-mediated neuronal cell death *(95).* ASK-1 formed a complex with $A\beta PP_{CD}$ via JIP1b. Thanks to AβPP-bound JIP1b, which also associates with JNK pathway kinases, the signal from AβPP efficiently arrives at JNK. Scheinfeld et al. *(96)* found that the threonine 668 within the $A\beta PP_{CD}$ is phosphorylated by JNK1. Although JIP1 can facilitate this phosphorylation, it is not required for this process. Considering that JIP1b also functions to stabilize the activity of JNK *(73,94),* JIP1b can also act as an AβPP adaptor that maximizes the signal/noise ratio of the associated JNK activity.

The expression of JIP1b stabilizes immature AβPP and suppresses the production of a secreted large extracellular amino-terminal domain of AβPP, the generation of a cleaved intracellular carboxyl-terminal fragment of AβPP, and the secretion of Aβ-40 and Aβ-42 *(97).* Regulation of AβPP metabolism by JIP1b is dependent on its direct interaction with carboxyl-terminal fragment of AβPP rather than on an interaction with JNK. JIP2, a weaker AβPP-binding protein, does not influence the processing of AβPP, although it is known that both JIP1b and JIP2 equally regulate the JNK signaling cascade.

AβPP and JIP1 are able to cause reporter gene activation in a γ-secretase cleavage-dependent manner that depends on presenilin 1 (PS1) *(98).* This activation seems to occur by a mechanism different from that seen with an Fe65–AβPP complex, as evidenced by microscopy and its independence of Tip60 coexpression. Although Fe65 enters the nucleus in the absence of full-length AβPP, JIP1 does not. Strong activation occurs only for the combination of JIP1 and AβPP but not for JIP–APLP-1 or JIP–APLP-2, which does cause strong activation in combination with Fe65.

What Does AβPP Actually Do Through These Adaptors?

As described earlier, multiple functions of the four adaptors have been proposed. Fe65, together with Mena, might be involved in the Abl tyrosine kinase pathway; it could be a bridge between AβPP and the proteins of the LDLR family, or it could be a signaling molecule in itself that transduces the signal in the cytoplasm or that translocates to the nucleus and acts as a transcriptional activator. X11 is a component of the basolateral sorting machinery. mDab1 is a transducer of reelin signals that is required for neuronal migration. JIP1b is a scaffolding protein of the JNK cascade kinases and kinesin cargo simultaneously. These different functions of the AβPP adaptors reflect the difference in the functions of AβPP in developing and postmitotic neurons.

The mature neurons are polarized with distinct subcellular domains, such as dendrites, cell body, axons, and synapses. Because of this polarity, proteins produced in the soma must be appropriately transported along axons or dendrites to different subcellular locations to exert their proper functions. The adaptors of AβPP could be involved in different categories of activity in polarized neurons. One such activity is the transport of synthesized AβPP; another is the cytoplasmic mediation of the function of AβPP after it is delivered to the predetermined positions. Because the binding domain (the conserved GYENPTY motif) is shared among these adaptors, the adaptor protein used as a cargo during the transport may be replaced with another adaptor used in the signaling process. Considering that the binding of Fe65 to AβPP is the tightest and the binding of the other three adaptors is moderately weaker, with the transient nature of the JIP1b–AβPP interaction *(63),* the different binding characteristics of these adaptor proteins might be important in their exchange. Most recently, Tarr et al. *(99)* reported that Shc binds exclusively to the phosphorylated NPTY region of AβPP in vivo. Considering the fact that the affinity of AβPP for mDab1 and Fe65 decreases when $A\beta PP_{CD}$ is phosphorylated (Tyr phosphorylation for mDab1 *[49]* and Thr phosphorylation for Fe65 *[100]*), phosphorylation of $A\beta PP_{CD}$ might drastically change the profiles of the binding proteins. It also should be noted that in native brain membranes, AβPP–G_o interaction, mediated by the His^{657}-Lys^{676} domain of AβPP, is strengthened by the addition of the isolated Met^{677}-Asn^{695} peptide of AβPP *(1),* suggesting that endogenous adaptors bound to the C-terminal Met^{677}-Asn^{695} domain of AβPP may modulate His^{657}-Lys^{676}-mediated AβPP–G_o interaction.

Relevance of the Adaptors to Alzheimer's Disease

Fe65 expression is widespread in neurons in the adult brain *(101).* The areas with the highest expression include regions of the hippocampus in which the earliest abnormalities of Alzheimer's disease are detectable. Because overexpression of Fe65 in cultured cells promotes translocation of AβPP to the cell surface and increases secretion of Aβ *(102),* overexpression of Fe65 in the hippocampus might be relevant to abnormal AβPP metabolism and Aβ production in the same area. Expression of mRNA for a neuron-specific version—the exon 9-containing isoform—of Fe65 is reduced in the frontal and temporal cortices in brains, with Alzheimer's disease *(103).* Fe65 colocalizes with neither AβPP nor Aβ deposits in the brain with Alzheimer's disease but associates with abnormally phosphorylated tau, another pathological hallmark of Alzheimer's disease, pointing to an interesting linkage around Fe65 between AβPP dysfunction and abnormal tau phosphorylation in brains with Alzheimer's disease *(104).*

Hu et al. *(105)* reported that a trinucleotide deletion polymorphism in intron 13 of the *Fe65* gene may be protective for sporadic Alzheimer's disease and suggested that this deletion may modify the splicing between exon 13 and 14—the two exons encoding the interaction domain of Fe65 with AβPP. In addition, they revealed that a protective (minor) allele alters the splicing of the terminal exon by selection of an alternative acceptor site, resulting in an isoform (FE65a2) with an altered C-terminal region lacking part of an AβPP-binding site *(106).* Pull-down assays confirmed that the FE65a2 isoform binds to AβPP less efficiently, suggesting that an attenuated binding of Fe65 with AβPP is, in part, responsible for resistance to the very late-onset dementia of the Alzheimer type. However, another research group detected the protective effect of the deletion only in a population over age 75 yr *(107).*

On the other hand, Guenette et al. *(108)* failed to show an association of the Fe65 intron 13 polymorphism with Alzheimer's disease in both a family-based and a case-control sample. Cousin et al. *(109)* did not find any statistically significant difference between the control and the populations with Alzheimer's disease in

another case-control study. Adjusting for age and sex, however, they found a slight risk for early-onset Alzheimer's disease associated with the deletion. *Fe65* gene remains a potential candidate for a genetic association with Alzheimer's disease, but conclusions by many research groups are conflicting.

X11-β colocalizes with APP in primary cortical neurons and in transfected Chinese hamster ovary (CHO) cells *(110).* Moreover, X11-β is associated with the neuritic plaques found in Alzheimer's disease but not with neurofibrillary tangles.

Investigating the role of Dab1 in tau phosphorylation, Brich et al. *(111)* found that wild-type Dab1, but not a mutant lacking tyrosine phosphorylation sites, protected mice from the hyperphosphorylation of tau. However, the absence of Dab1 was not sufficient to cause tau hyperphosphorylation, because hyperphosphorylation was manifested only when Dab1 was mutated in specific mouse strain backgrounds.

Multiple observations suggested that activation of JNK, which is strictly regulated by AβPP-associated JIP1b, is relevant to Alzheimer's disease pathomechanisms. First, JNK activation could influence AβPP processing by increasing nonamyloidgenic α-secretase cleavage of AβPP and the secretary form of AβPP (sAβPP). Mudher et al. *(112)* found that disheveled-1 expression augments sAβPP via the mechanism involving JNK. Although the consequence of JNK-induced sAβPP increase remains unclear, this increase might affect Aβ production. Furthermore, sAβPP has biological functions in process extension of neurons and proliferation of astrocytes *(113–116),* suggesting that augmented sAβPP by JNK could potentially contribute to gliosis observed in Alzheimer's disease.

Second, neuronal JNK is activated in the brains of sporadic cases of Alzheimer's disease, depending on its stages of severity. Zhu et al. *(117)* examined the chronological and spatial relationship between activated extracellular signal-regulated kinase (ERK), JNK, and p38 mitogen-activated protein kinase (MAPK) during progression of Alzheimer's disease and found that; (a) all three kinases are activated in the same damaged neurons in mild and severe cases; (b) in nondemented cases with limited pathology, ERK and JNK are activated, but p38 MAPK is not, and (c) in nondemented cases lacking any sign of pathology, either ERK alone or JNK alone can be activated. Zhu et al. *(117)* also reported that phosphorylated JNK is significantly increased in cases of Alzheimer's disease over control cases.

Similar observations were obtained from rodent models of Alzheimer's disease. Savage et al. *(118)* reported that JNK and p38 MAPK pathways were significantly activated in the cortex in the model mice of both ages 7 and 12 mo, which were K595N/M596L-AβPP-overexpressing mice crossed with P264L-PS1-knock-in mice. Consistent with the lack of neuronal loss, activation of c-Jun, the nuclear target of JNK, was not observed in the brain of the mice age 12 mo. The observed JNK activation was localized in the neurites containing phosphorylated tau, which were close to extracellular Aβ deposits.

Further studies of molecular mechanisms underlying the AβPP adaptors will unravel the functions of AβPP in physiological situations and also in the pathological process of Alzheimer's disease.

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