Proteolytic processing of amyloid β protein precursor (AβPP) generates peptides that regulate normal cell signaling and are implicated in Alzheimer’s disease pathogenesis. AβPP processing also occurs in nerve processes where AβPP is transported from the cell body by kinesin-I, a microtubule motor composed of two kinesin heavy chains and two kinesin light chain (Klc) subunits. AβPP transport is supposedly mediated by the direct AβPP-Klc1 interaction. Here we demonstrate that the AβPP-Klc1 interaction is not direct but is mediated by JNK-interacting protein 1 (JIP1). The phosphotyrosine-binding domain of JIP1 binds the cytoplasmic tail of AβPP, whereas the JIP1 C-terminal region interacts with the tetra-tricopeptide repeats of Klc1. We also show that JIP1 does not bridge the AβPP gene family member AβPP-like protein 2, APLP2, to Klc1. These results support a model where JIP1 mediates the interaction of AβPP to the motor protein kinesin-I and that this JIP1 function is unique for AβPP relative to its family member APLP2. Our data suggest that kinesin-I-dependent neuronal AβPP transport, which controls AβPP processing, may be regulated by JIP1.

Alzheimer’s disease is characterized by the deposition of Aβ peptides, which are cleaved off from AβPP (1, 2). AβPP is transported along axons via conventional kinesin-I-dependent fast anterograde transport (3, 4), which might be involved in processing of AβPP (5). It has been proposed that the kinesin-dependent transport of AβPP is mediated by the direct binding of AβPP to the TPR domain of Klc1 (4). The TPR domain of Klc1 has been shown to bind JIP proteins (6). The JIP protein family, members of which were initially isolated as scaffolds for kinases of the JNK cascade, consists of three proteins: JIP1 (7), JNK-interacting protein 2 (JIP2) (8), and JSAP1/Sunday Driver/JNK-interacting protein 3 (JIP3) (9–11). JIP1 has two alternatively spliced forms in mouse, JIP1a, and JIP1b. JIP1b (referred to as JIP1 in this manuscript) and JIP2, which are conserved between human and mouse, contain a C-terminal Src homology region 3 and a PTB domain. JIP1a, which has not been found in humans, lacks a complete PTB domain. JIP3 is structurally unrelated (11). Of interest, JIP1 has also been identified in yeast two-hybrid screenings performed to isolate AβPP-interacting proteins. The PTB domain of JIP1 binds to the YENPTY motif found in the AβPP intracellular domain (AICD/AID) (12, 13). The biological relevance of the JIP1-AβPP interaction is underlined by the evidence that these two proteins interact in mouse brain (13).

The direct binding of Klc to AID has been a puzzle to us because Klc has never been cloned as an AID-interacting protein, despite extensive yeast two-hybrid screenings performed in many laboratories, including ours. On the contrary, Klc1 was one of the predominant proteins isolated in yeast two-hybrid screening using the C terminus of JIP1 as bait (data not shown). This apparent paradox can be resolved by postulating that the interaction between Klc and AβPP is indirect and that JIP proteins are required to form a Klc-JIP-AβPP tripartite complex. Contrary to the published results (4), here we demonstrate that JIP1 is necessary for Klc-AβPP association. Furthermore, JIP1 does not bridge APLP2 to Klc1. These findings provide a novel insight into the function of JIP proteins in the transport of AβPP.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—The following antibodies were used: αFLAG (mouse monoclonal M2, Sigma), αgreen fluorescent protein (αGFP) (rabbit polyclonal, Clontech), αMye (mouse monoclonal 6E10, Santa Cruz Bio-technology), αAβPP2 (rabbit polyclonal 171616, Calbiochem), αAβPP (mouse monoclonal 22C11, Chemicon), αT7 (mouse monoclonal, Novagen), and rabbit polyclonals αJIPN and αJIPC and their pre-immune sera (12).

**Plasmids and Yeast Two-hybrid Screenings**—For yeast expression, AβPP, JIP1, and JIP2 regions were cloned in pGBK7T and/or pGADT7 (Clontech), for Gal4 binding domain (BD) and Gal4 activation domain (AD) fusions, respectively. Klc1 constructs were directly cloned by yeast two-hybrid screening using a mouse brain library (Clontech) and JIP1 (540–707) as bait. The sequences of the Klc1 clones were identical to Klc1 (GenBank™ accession numbers X925743 and AK351309) except for the region coded by the last 10 amino acids, which were identical to mouse expressed sequence tags (GenBank™ accession numbers AA270658, AW108197, BF494177, BE859872, BM945454, BU706278, and CB248553) and rat Klc1b (GenBank™ accession number M75147), indicating that the clones are mouse Klc1b. Other control vectors, pGBK7T–53 (BD fusion of p53), pGBK7–Lam (BD fusion of nuclear lamin), pGADT7–T (AD fusion of large T antigen), and pCCL1 (Gal4), are from Clontech. No yeast constructs displayed auto-activation, unless otherwise noted. The yeast constructs were transformed in AH109 yeast strain (Clontech) on plates lacking tryptophan and leucine. Patches of yeast transformants were replica-plated on plates lacking tryptophan and leucine, adenine, and histidine to monitor the interaction. Four independent patches of transformed yeast showed identical results, unless otherwise noted. LacZ activity of yeast transformants were assayed using O-nitrophenyl-β-D-galactopyranoside (Sigma) as substrate. Bacterial expression vectors of glutathione S-
transferrase (GST), GST-AID, GST-ΔNPTY, and His/T7-JIP1 are described (12). His/T7-Klc1 was created by cloning the yeast Klc1 insert into pET28c (Novagen). For bacterial expression of GST-11aa, the C-terminal 11 amino acids of JIP1 were cloned in pGEX-2Tm (12). GST-11aa were immobilized on 30 μl of glutathione beads in 500 μl of Buffer B (20 mM Tris/HCl, pH 8.0, 1 mM EDTA, and 1 mM dithiothreitol) by incubating for 1 h at 4 °C. The beads were washed once with Buffer C (20 mM Tris/HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 150 mM NaCl, 0.1% (w/v) Triton X-100). The washed beads were incubated with purified His/T7-Klc1 at various concentrations in the presence or absence of His/T7-JIP1 in a total volume of 200 μl of Buffer C for 2 h at 4 °C. After the incubation, the beads were washed three times with Buffer C and boiled in 40 μl of 2× SDS sampling buffer. Ten μl were used to detect the bound GST fusion proteins by Coomassie Brilliant Blue staining and to detect the bound Klc1 and JIP1 by Western blotting with αT7 antibody.

RESULTS AND DISCUSSION

In Yeast, Klc1 Does Not Interact with AID although AID-JIP1/2 and Klc1-JIP1/2 Interactions Are Readily Detectable.—To characterize the interaction among ApβP, JIP, and Kcl1, we initially used the yeast two-hybrid system. Various domains of each gene (Fig. 1A) were fused to BD and AD and tested for their interaction. Two independent Klc1 constructs, identified in a yeast two-hybrid screening using JIP1PTβ+K as bait (data not shown), did not interact with AID (Fig. 1B, upper panel). This is significant, because both Klc1 constructs contain all of the six TPRs (Fig. 1A) that have been reported to be sufficient for the interaction between Klc1 and ApβP (4). At the same time, JIP1 (1PTβ+K) interacted with AID but not with ΔNPTY, an AID mutant lacking the PTB-binding motif.
FIG. 2. JIP1 bridges Klc1 to AβPP. A, illustrations of mammalian expression vectors used. FLAG, Myc, and GST tags are indicated. Other designations are the same as in Fig. 1A. B, HEK293 cells were transfected with AβPP together with the indicated GST proteins. The cell lysates were precipitated with glutathione beads. AβPP in total lysates (TL) and GST precipitants (ppt) were detected by Western blotting with αAβPP antibody. Similar amounts of GST fusions were detected in the precipitants (data not shown). C, HEK293 cells were transfected with the same GST fusions together with FLAG-Klc1. Klc1 in total lysates and precipitants were detected by Western blotting with αFLAG antibody. Similar amounts of GST fusions were detected in the precipitants (data not shown). D, HEK293 cells were transfected with the indicated combinations of AβPP, FLAG-Klc1, and GST-JIP1 fusion proteins, and analyzed as in C, E, HEK293 cells were transfected with various combinations of plasmids indicated above each lane: without (−) or with (+) FLAG-Klc1; without (−) or with (+) Myc-JIP1α (1a), Myc-JIP1 (1); without (−) or with (+) AβPP (FL), or AβPP lacking C-terminal 15 amino acids (AβPPΔ). The cell lysates were immunoprecipitated by FLAG M2 beads, and Klc1, JIP1α/JIP1, and AβPP/AβPPΔ in the total lysates, and the precipitants (IP αFLAG) were analyzed by Western blotting by αFLAG, αMyc, and αAβPP antibodies, respectively. E, HEK293 cells were transfected with the indicated combinations of FLAG-Klc1, Myc-JIP1α/JIP1, and AβPP. The cell lysates were precipitated with FLAG M2 beads, and Klc1, JIP1α/JIP1, and AβPP in the total lysates and the precipitants were detected by Western blotting as in D, G, HEK293 cells were transfected with the indicated combinations of Myc-JIP1α/JIP1 and AβPP. The lysates were immunoprecipitated by αJIP1 serum (C) or its pre-immune serum (P). JIP1α/JIP1 and AβPP in total lysates and the precipitants were detected with αMyc and αAβPP antibodies, respectively.
YENPTY, confirming the YENPTY motif-dependent interaction between JIP1 and AβPP (12, 13). Next, we analyzed whether JIP1 proteins interact with Klc1. The JIP1 (1PTB+K) interacted with both Klc1 constructs. Interestingly, 1PTB, lacking the C-terminal 11 amino acids of JIP1, lost any interaction with Klc1 but retained the YENPTY motif-dependent interaction with AID, indicating that Klc1-JIP1 interaction is independent of JIP1-AβPP interaction (Fig. 1B, lower panel). Lastly, we studied the JIP2-AβPP and JIP2-Klc1 interactions. As summarized in Fig. 1C, JIP2 interacted with AID and Klc1 in the same manner as did JIP1. We could not test the two-hybrid interaction between the C-terminal 11 amino acids of JIP1 and Klc1 or AID, because the BD fusion of the 11 amino acids was auto-activating. In conclusion, in the yeast two-hybrid system, Klc1 does not interact with AID, whereas JIP1/2 interacts with both Klc1 and AID.

The C-terminal 11 to 9 Amino Acids of JIP1 Are Necessary and Sufficient for Klc1-Klc1 Interaction—The data obtained in the yeast two-hybrid suggest that the C-terminal 11 amino acids of JIP1 are necessary for Klc1-Klc1 binding but are not required for AβPP interaction. To test whether this JIP1 C-terminal region is sufficient for Klc1 binding, we transfected HEK293 cells with either Klc1 or AβPP together with the constructs expressing GST fused to 11, 9, 7, or 0 C-terminal amino acids of JIP1 (GST-11aa, -9aa, -7aa, and -0aa). Although none of these GST fusion JIP1 proteins interacted with AβPP (Fig. 2B), the C-terminal 11, 9, and 7 amino acids of JIP1 did bind Klc1, with the 11-amino-acid fusion being the strongest (Fig. 2C). It is noteworthy that the last nine amino acids of JIP1 and JIP2 are identical.

To further clarify the role of the PTB domain and the C-terminal 11 amino acids of JIP1, GST pull-down experiments were performed using HEK293 cells transfected with AβPP and Klc1 together with GST fusion of JIP1 portions (Fig. 2D). As expected, GST-JIP1-C, which contains both the PTB domain and 11 amino acids interacted with AβPP and Klc1, whereas GST-1PTB, which does not have C-terminal 11 amino acids, binds to AβPP but not to Klc1.

The above data confirm that the domains mediating JIP1-AβPP and Klc1-JIP1 interactions are distinct and not overlapping. Specifically, the PTB domain of JIP1 interacts with the PTB-binding motif (YENPTY) present in AID, whereas the C-terminal 11–9 amino acids of JIP bind to Klc1. Thus, JIP1/2 could potentially bridge Klc1 to AβPP.

JIP1 Bridges AβPP, but Not the Family Member APLP2, to Klc1 in Transfected Cells—It is possible that AβPP and Klc1 interact in mammalian cells, although a direct binding cannot be observed in yeast. To test this possibility, HEK293 cells (in which endogenous JIP1 expression is below detection) were transfected with Klc1/JIP1/AβPP coding plasmids, and the Klc1-AβPP complex was analyzed by immunoprecipitating Klc1. As shown in Fig. 2E, Klc1 did not precipitate AβPP, a finding that is in agreement with the yeast two-hybrid results. However, Klc1 did precipitate AβPP in the presence of JIP1. Notably, JIP1a, which lacks a complete PTB domain and does not bind to AβPP (12, 13), still interacts with Klc1 but failed to promote AβPP-Klc1 interaction. Moreover, AβPPΔ4, which lacks the YENPTY motif and does not bind to JIP1, also did not form Klc1-AβPP-Klc1 interaction. All of the evidence confirms that the JIP1-AβPP binding is necessary for the association between Klc1 and AβPP. In a paper published during the preparation of this manuscript, Inomata et al. (14) have observed that some interaction between AβPP and Klc1 was significantly enhanced by transfecting JIP1. The minimal Klc1-AβPP interaction that they observed in the absence of JIP1 transfection could be mediated by endogenous JIP1, assuming that the cells they used express higher level of JIP1 than ours. Alternatively, it might be attributable to the difference of expression of a distinct linker protein that functions similarly to JIP1.

We further tested JIP2 and JIP3 for this bridging effect. JIP2 was much less efficient than JIP1 in bridging Klc1 to AβPP, and sometimes this effect was undetectable (Fig. 2F). This correlates with the weaker JIP2-AβPP binding as compared with JIP1-AβPP interaction (Fig. 2G). As expected, JIP3, which does not contain a PTB domain, did not bridge AβPP to Klc1 (Fig. 2F).

Finally, we determined whether JIP1 could link another AβPP family member APLP2 to Klc1. To this end, we transfected HEK293 cells with AβPP or APLP2 together with Klc1. These transfections were performed with or without JIP1. After Klc1 precipitation, we found that AβPP associates with Klc1 when JIP1 is expressed, whereas APLP2 did not interact with Klc1 even in the presence of JIP1 (Fig. 3). These data are consistent with our previous finding that JIP1 preferentially interacts with AβPP (12, 15).

Altogether, our data indicate that JIP1-AβPP and Klc1-JIP1 interactions are independent and that JIP1, and at a much lower degree JIP2, bridges Klc1 to AβPP in HEK293 cells. Moreover, this bridging effect is specific for AβPP and is not shared by the other family member, APLP2.
were detected as in AID in the presence or absence of JIP1. Bound Klc1, JIP1, and GST-AID concentration was precipitated by glutathione beads immobilizing GST-AID. The C-terminal 11 amino acids of JIP1 (GST-11aa) were precipitated by glutathione beads immobilizing GST or GST fused to His/T7-JIP1. Bound Klc1, JIP1, and GST-AID in the precipitants (Fig. 5B) were visualized by Western blotting with T7 antibody. C, purified Klc1 of the indicated concentration was precipitated by glutathione beads immobilizing GST or GST fused to the C-terminal 11 amino acids of JIP1 (GST-11aa). Bound Klc1 (upper panel) was detected as in B. Similar amount of GST proteins was precipitated as indicated by Coomassie Brilliant Blue staining of SDS-PAGE (lower panel). D, similarly, purified Klc1 at the indicated concentration was precipitated by glutathione beads immobilizing GST-AID in the presence or absence of JIP1. Bound Klc1, JIP1, and GST-AID were detected as in C.

**Fig. 5.** Purified Klc1 interacts with AID only in the presence of JIP1. A, illustrations of the bacterial constructs. His/T7 and GST tags are indicated. Other designations are the same as in Fig. 1A. B, purified His/T7-JIP1 (JIP1) and/or His/T7-Klc1 (Klc1) at indicated concentrations were precipitated by glutathione beads immobilizing GST; GST fused to AID or to ΔNPTY, as indicated under each lane. Bound JIP1 and Klc1 in the precipitants (ppt) were visualized by Western blotting with T7 antibody. C, purified Klc1 of the indicated concentration was precipitated by glutathione beads immobilizing GST or GST fused to the C-terminal 11 amino acids of JIP1 (GST-11aa). Bound Klc1 (upper panel) was detected as in B. Similar amount of GST proteins was precipitated as indicated by Coomassie Brilliant Blue staining of SDS-PAGE (lower panel). D, similarly, purified Klc1 at the indicated concentration was precipitated by glutathione beads immobilizing GST-AID in the presence or absence of JIP1. Bound Klc1, JIP1, and GST-AID were detected as in C.

**Purified Klc1 Interacts with Purified AID Only in the Presence of Purified JIP1—**Our data apparently conflict with those published by Kamal et al. (4), who have observed the direct interaction between AID and the TPR domain of Klc using purified recombinant proteins. To directly solve the discrepancy between our results and theirs, we used purified recombinant proteins. Kamal and colleagues used His-tagged GFP-AID fusion and GST-TPR fusion proteins (4). We noticed that some GFP vectors express GFP proteins that bind to Klc1 in transfected cells (Fig. 4A). HEK293 cells were transfected with different GFP vectors together with Klc1. When Klc1 was precipitated, the GFP protein expressed from pEGFP-C2 was precipitated. The GFP protein expressed from pEGFP-C3 was also precipitated, although the efficiency was much lower. On the contrary, those GFP proteins expressed by pEGFP-N1 and pEGFP-C1 were not. All of pEGFP-C1, -C2, and -C3 distinctly express (fused to the C-terminal of GFP) 26 amino acids coded by the polylinker regions (Fig. 4B). pEGF-N1 codes only for the GFP protein. Therefore, some amino acid sequences fused to GFP may cause artificial interactions between GFP fusion proteins and Klc1. Thus, we performed our in vitro experiments using GST-AID, His/T7-Klc1, and His/T7-JIP1 recombinant purified proteins (Fig. 5A).

As expected, JIP1 binds to GST-AID but not to GST or GST-ΔNPTY. Again, Klc1 did not show any binding to GST-AID, but it associated with GST-AID in the absence of recombinant JIP1 (Fig. 5B). That the Klc1-AβPP interaction is JIP1-dependent is again confirmed by the finding that Klc1 did not bind to GST-ΔNPTY even in the presence of JIP1 (Fig. 5B). We further confirmed that the Klc1-JIP1 interaction is direct using GST fused to the C-terminal 11 amino acids of JIP1 (GST-11aa). As shown in Fig. 5C, Klc1 binds to GST-11aa with a half-maximum binding approximately at 0.2 μM, which is similar to the JIP1-AβPP interaction (12). In the presence of 1 μM JIP1, Klc1 binding to AID was detectable at 0.5 μM or above (Fig. 5D). Even 2 μM Klc1 (which is more than the concentration used to show saturated binding of Klc to AID by Kamal et al. (4)) did not interact with AID in the absence of JIP1 (Fig. 5D). Thus, purified recombinant Klc1 does not bind to AβPP, whereas the Klc1-JIP1 or Klc1-JIP1-AβPP complexes are readily detectable.

Here we show that AβPP does not directly bind Klc1 in three different systems: the yeast two-hybrid, transfected cells, and purified proteins. Importantly, we demonstrate that JIP1 bridges AβPP to Klc1 by binding each protein partner through distinct regions of JIP1, which do not overlap with each other. The bridging effect of JIP1 is demonstrated in vivo by immunoprecipitation of transfected cells and in vitro using recombinant purified proteins. This finding suggests that JIP1 acts as a scaffold protein essential for linking AβPP to the kinesin-I motor protein (6, 16), thereby regulating AβPP transport along axons. This hypothesis alludes to engaging consequences given the close relationship existing among transport, processing, and pathological/biological roles of AβPP. In fact, neuronal transport of AβPP is intrinsically connected to AβPP cleavage (5), and peptides derived from this proteolysis (Aβ and AID) are involved in both Alzheimer’s disease pathogenesis and normal cell signaling. Recent evidence suggests that AID plays a role in apoptosis (17, 18), Ca2⁺ release (19), and transcriptional regulation (20–22) and that JIP1 modulates some of these functions after AID is liberated (15). Together, these data predict a dual role for JIP1 in AβPP pathophysiology: as a scaffold protein that facilitates the delivery of AβPP to specialized sites where AβPP is processed to Aβ(AID) peptides and as a cofactor for AID signaling.

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