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Association of the Kinesin Motor KIF1A with the Multimodular Protein Liprin-α*

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Liprin-α/SYD-2 is a multimodular scaffolding protein important for presynaptic differentiation and postsynaptic targeting of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptors. However, the molecular mechanisms underlying these functions remain largely unknown. Here we report that liprin-α interacts with the neuron-specific kinesin motor KIF1A. KIF1A colocalizes with liprin-α in various subcellular regions of neurons. KIF1A coaccumulates with liprin-α in ligated sciatic nerves. KIF1A cofractionates and coimmunopreciptates with liprin-α and various liprin-α-associated membrane, signaling, and scaffolding proteins including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, GRIP/ABP, RIM, GIT1, and βPIX. These results suggest that liprin-α functions as a KIF1A receptor, linking KIF1A to various liprin-α-associated proteins for their transport in neurons.

The liprin-α/SYD-2 family of proteins was originally identified as a cytosolic binding partner of the LAR family of receptor protein-tyrosine phosphatases (1). Liprin-α contains various domains for protein interactions including a long coiled coil region in the N-terminal half, three SAM domains in the middle, and a PDZ-binding motif at the C terminus. The N-terminal coiled-coil region of liprin-α mediates homomultimerization (1) and interacts with GIT/Cat/p95-APP/PKL (2), a family of multidomain proteins with GTPase-activating protein activity for ADP-ribosylation factor small GTP-binding proteins (3–7), as well as RIM1 (Rab3-interacting molecule) (8), a scaffolding protein at the presynaptic active zone regulating neurotransmitter release (8, 9). The SAM domains of liprin-α interact with the intracellular domain of LAR (1). The C terminus of liprin-α interacts with the GRIP/ABP family of multi-PDZ proteins, which are known to bind various membrane, cell adhesion, and signaling proteins including AMPA[1] glutamate receptors (GluRs) (10–14), ephrin ligands, and receptors (15–17) and GRASP-1, a neuronal Ras-specific guanine nucleotide exchange factor (18). These results suggest that liprin-α may function as a multimodular scaffolding protein.

Functionally, genetic deletion of *syd-2* (for synaptic defective-2), a *Caenorhabditis elegans* homolog of mammalian liprin-α, results in a diffuse distribution of presynaptic markers, lengthening of the presynaptic active zone, and impairment of synaptic transmission (19). Similarly, genetic deletion of *Dliprin-α*, a Drosophila homolog of liprin-α, leads to an alteration of the size and shape of active zones (20). In addition, disruption of the interaction between liprin-α and GRIP eliminates surface clustering of AMPA receptors in dendrites of neurons (21). These results suggest that liprin-α/SYD-2 regulates presynaptic differentiation of active zone as well as postsynaptic targeting of AMPA receptors. However, it remains largely unknown how liprin-α regulates presynaptic differentiation and postsynaptic receptor targeting. Importantly, liprin-α distributes to various nonsynaptic structures in axons and dendrites (21), suggesting that liprin-α may have novel functions at extrasynaptic sites in addition to its suggested role as an organizer of synaptic multiprotein complexes.

The kinesin superfamily (KIF) of motor proteins transports cargo vesicles or organelles on microtubule tracks (22, 23). KIF1A, a member of the KIF1/Unc104 family of proteins (24), is a neuron-specific kinesin motor known to transport synaptic vesicle precursors containing synaptophysin, synaptotagmin, and Rab3A (24, 25). In support of this, genetic deletion of *unc-104*, a *C. elegans* homolog of KIF1A (26), results in the accumulation of clear vesicles in the cell body (27). Mutation in the KIF1A gene in mice leads to a similar accumulation of vesicles in the cell body and neuronal death (28). Recently, fast and processive movements of Unc104/KIF1A were observed in living *C. elegans* and mammalian neurons (29, 30), and molecular mechanisms underlying the processive movement of Unc104/KIF1A have been extensively characterized (31–34). However, relatively little is known about whether KIF1A transports cargoes other than synaptic vesicle precursors and about the manner in which KIF1A interacts with specific cargoes.

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[1] The abbreviations used are: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluR, glutamate receptor; aa, amino acid(s); LBD, liprin-α-binding domain; EM, electron microscopy; GST, glutathione *S*-transferase.

We report here a direct interaction between KIF1A and liprin-α, which links KIF1A to various liprin-α-associated proteins including AMPA receptors, GRIP, RIM, GIT1, and βPIX. Our results suggest that liprin-α functions as a KIF1A "receptor" linking the KIF1A motor to a cargo of liprin-α-associated proteins.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen and Assay—Liprin-α1 (aa 351–1105) and liprin-α4 (aa 1–863) were amplified by PCR and subcloned to pBHA bait vector (LexA DNA binding domain). The following deletions of KIF1A were amplified by PCR and subcloned in frame into the pGAD10 prey vector (GAL4 activation domain; Clontech): 501–800, 501–937, 501–1105, 657–1105, 717–937, 717–1105, and 923–1105. Deletions of liprin-α1 were subcloned into pGAD10 vector: aa 1–221, 1–673, 1–848, 217–553, 221–673, 351–553, 351–673, 513–673, and 668–1202. KIF1Bα (aa 666–1150) and KIF1Bβ (aa 697–1223) were amplified by reverse transcription-PCR and subcloned into pGAD10. All yeast two-hybrid constructs were confirmed by DNA sequencing.

Coimmunoprecipitation in Heterologous Cells—For the KIF1A expression construct, the full-length KIF1A cDNA was amplified by reverse transcription-PCR, digested with *Hin*dIII and *Eco*RI, and subcloned into GW1 (British Biotechnology). HEK293T cells transfected with GW1-KIF1A and pMT2-HA-liprin-α1 were extracted with Tris-buffered saline containing 1% Triton X-100 and incubated with HA antibodies (mouse monoclonal; 4 μg/ml) or mouse IgG (4 μg/ml), followed by incubation with protein A-Sepharose (Amersham Biosciences). Immunoprecipitates were immunoblotted with HA (rabbit polyclonal; 1 μg/ml) and KIF1A (1131; 1 μg/ml) antibodies.

Antibodies—To generate fusion protein immunogens, regions of KIF1A (aa 657–937 for 1131 antibodies) and liprin-α1 (aa 351–673 for 1120 and 818–1202 for 1127 antibodies) were amplified by PCR and subcloned into pRSETB (vector for H6 fusion protein; Invitrogen). Fusion proteins were purified using Probond resin (Invitrogen). Affinity purification of specific antibodies was performed using immunogen immobilized on polyvinylidene difluoride membrane (Amersham Biosciences). The specificity of KIF1A (1131) antibodies was determined by Western blot analysis on GST-KIF1A (aa 657–937) and GST-KIF1Bβ (aa 697–1223). To examine the reactivity of liprin-α antibodies (1120 and 1127) against the liprin-α family members, COS cell lysates transfected with HA-liprin-α1 or HA-liprin-α2 were immunoblotted with liprin-α (1120, 1127) and HA (mouse monoclonal; Roche Molecular Biochemicals) antibodies. Other antibodies used include GRIP (C8399; pan-GRIP antibody) (10, 11), GST (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), GluR1 (Chemicon), PSD-95 (K23/58; Upstate Biotechnology, Inc., Lake Placid, NY), GluR2/3 (Chemicon), synaptotagmin (Sigma), syntaxin (Sigma), MAP2 (Sigma), neurofilament-H (neurofilament 200; Sigma), cortactin (Upstate Biotechnology), mouse monoclonal HA (Roche Molecular Biochemicals), rabbit polyclonal HA (Santa Cruz Biotechnology), GIT1 (2), and RIM (Transduction Laboratories) antibodies.

Immunohistochemistry and Immunoelectron Microscopy—For immunofluorescence staining of rat brain sections, adult rats were perfused with 4% paraformaldehyde, and brain sections were cut using a vibratome. Brain sections were permeabilized with phosphate-buffered saline containing 50% ethanol at room temperature for 30 min, incubated overnight with combinations of KIF1A (1131; 3 μg/ml), liprin-α (1127; 3 μg/ml), MAP2 (1:200), neurofilament-H (1:100), and GRIP (C8399; 3 μg/ml) antibodies, followed by incubation with Cy3-, fluorescein isothiocyanate-, or Cy5-conjugated secondary antibodies at dilutions of 1:1000, 1:300, and 1:500, respectively (Jackson Immunoresearch). Images were captured using an LSM510 confocal laser-scanning microscope (Zeiss). Postembedding immunogold electron microscopy with KIF1A (1131; 3 μg/ml) antibodies was performed and quantified as described previously (35).

Subcellular Fractionation—Subcellular fractions of whole rat brain were prepared as previously described (11). In brief, rat brain homogenates (*H* in Fig. 6) were centrifuged at 900 × *g* to remove nuclei and other large debris (*P1*). The supernatant was centrifuged at 12,000 × *g* to obtain a crude synaptosomal fraction (*P2*). The supernatant (*S2*) was centrifuged at 250,000 × *g* to obtain light membranes (*P3*) and cytosolic fraction (*S3*). In parallel, the P2 fraction was subjected to hypotonic lysis and centrifuged at 25,000 × *g* to precipitate synaptosomal membranes (*LP1*). The supernatant (*LS1*) was further centrifuged at 250,000 × *g* to obtain a crude synaptic vesicle-enriched fraction (*LP2*) and soluble fraction (*LS2*). For immunoblotting, nitrocellulose or polyvinylidene difluoride membranes were incubated with antibodies against KIF1A (1131; 1 μg/ml), liprin-α (1120; 1 μg/ml), GRIP (C8399; 1 μg/ml), GluR1 (1 μg/ml), PSD-95 (1:1000), and synaptotagmin (1:500).

Flotation Assay and Coimmunoprecipitation—For the flotation assay, brains of 6-week-old rats were homogenized in homogenization buffer (20 mM HEPES, 100 mM potassium acetate, 40 mM KCl, 5 mM $MgCl_2$, 320 mM sucrose, pH 7.4) supplemented with proteinase inhibitors and 5 mM EGTA. Homogenate was centrifuged at 900 × *g* for 10 min, and the supernatant was centrifuged at 12,000 × *g* to obtain crude synaptosomes. Crude synaptosomes were suspended in the homogenization buffer and hypotonically lysed by adding nine volumes of H_2O to release synaptic vesicles. After centrifugation at 200,000 × *g* for 1 h, the membrane pellet was then adjusted to 2 M sucrose and loaded onto the bottom of a discontinuous sucrose gradient of 1.6, 1.0, and 0.3 M sucrose. The sucrose gradient was centrifuged at 350,000 × *g* for 3 h. Fractions were taken from the top of the gradient to the bottom. For coimmunoprecipitation, deoxycholate lysates (10) of the top three fractions were incubated with antibodies against KIF1A (1131; 3 μg/ml), liprin-α (1127; 3 μg/ml), GluR2/3 (Chemicon; 3 μg/ml), or control IgG (rabbit or guinea pig; 3 μg/ml) followed by precipitation with protein A-Sepharose (Amersham Biosciences). Immunoprecipitates were analyzed by immunoblotting with KIF1A (1131; 1 μg/ml), liprin-α (1120; 1 μg/ml), GRIP (C8399; 1 μg/ml), GluR2/3 (1 μg/ml), synaptotagmin (1 μg/ml), RIM (1 μg/ml), GIT1 (1 μg/ml), βPIX (1 μg/ml), syntaxin (1:400), and cortactin (1:1000) antibodies.

Nerve Ligation Assay—Sciatic nerves of anesthetized adult rats were ligated for 60 min followed by perfusion with 4% paraformaldehyde. After a brief postfixation for 30 min, sections (20 μm) of sciatic nerves were cut using a cryotome and incubated overnight with primary antibodies at 4 °C, followed by room temperature incubation with Cy3- or fluorescein isothiocyanate-conjugated secondary antibodies for 3 h. Primary antibodies used include KIF1A (1131; 3 μg/ml), liprin-α (1127; 3 μg/ml), and syntaxin (1:200). Immunofluorescence images were captured using an LSM510 confocal laser-scanning microscope (Zeiss).

Neuron Culture and Immunostaining—Low density hippocampal primary cultures were prepared from E18 rat embryos as described previously (36). Neurons were maintained in the neurobasal medium supplemented with B27 (Invitrogen). For immunofluorescence staining, neurons were fixed and permeabilized with cold methanol (−70 °C) and incubated with KIF1A (1131; 3 μg/ml) and MAP2 (1:200) antibodies, followed by incubation with Cy3- or fluorescein isothiocyanate-conjugated secondary antibodies.

RESULTS

Characterization of the Interaction between KIF1A and Liprin-α by the Yeast Two-hybrid Assay and Coimmunoprecipitation—To better understand the functions of liprin-α, we performed a yeast two-hybrid screen of rat brain cDNA using liprin-α4, a member of the liprin-α family, as bait. Out of ~1 × 10^6 yeast colonies, a cDNA fragment of KIF1A (aa 455–1105) containing roughly the middle third of the protein was isolated. The minimal regions required for the interaction were determined by characterizing deletion variants of KIF1A and liprin-α1, a member of the liprin-α family for which a full-length cDNA was available. The minimal liprin-α1-binding region in KIF1A was narrowed down to aa 657–1105, which we termed the liprin-α-binding domain (LBD) (Fig. 1*A*). The minimal KIF1A-binding region in liprin-α1 was localized to a region (aa 351–673) largely within the N-terminal coiled-coil region (aa 1–650; Fig. 1*B*).

We then tested the specificity of interactions between members of the KIF1 and liprin-α families. In addition to liprin-α4, liprin-α1 (aa 351–1202, a region corresponding to the liprin-α4 bait) also interacted with the KIF1A deletions that showed positive interaction with liprin-α4 (Fig. 1*A*). In contrast, liprin-α1 and liprin-α4 did not interact with KIF1B (both KIF1Bα and KIF1Bβ splice variants), another member of the KIF1 family (37–40) (Fig. 1*C*).

To test whether full-length KIF1A and liprin-α interact in a mammalian cell environment, we performed coimmunoprecipitation experiments using HEK293T cells doubly transfected with KIF1A and HA-tagged liprin-α1 (HA-liprin-α1) (Fig. 1*D*). Immunoprecipitation with HA antibodies, but not control mouse IgG, immunoprecipitated HA-liprin-α1 and coprecipi-

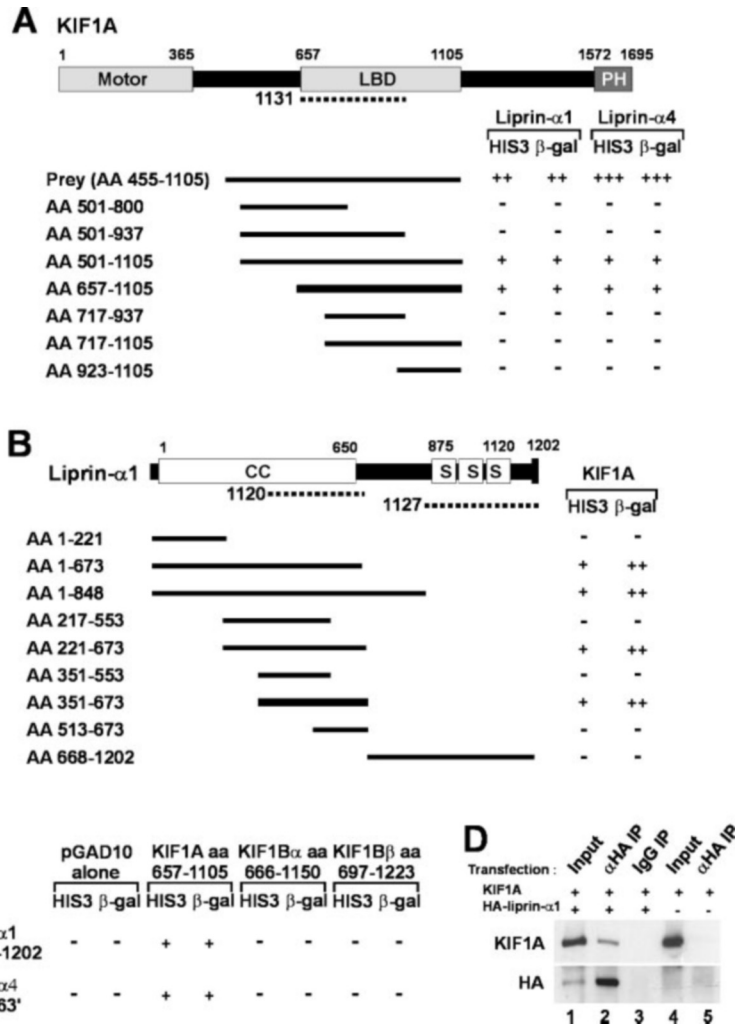

FIG. 1. **Characterization of the interaction between KIF1A and liprin-α by the yeast two-hybrid assay and coimmunoprecipitation.** *A*, minimal liprin-α-binding region in KIF1A. Deletions of KIF1A were tested for binding to liprin-α1 and liprin-α4 in the yeast two-hybrid assay. The minimal liprin-α-binding region in KIF1A (aa 657–1105) is indicated by the *thicker line*. The region that the KIF1A (1131) antibodies were raised against is indicated by a *dotted line* underneath the schematic of the domain organization of KIF1A. *Motor*, motor domain; *LBD*, liprin-α-binding domain; *PH*, pleckstrin homology domain. HIS3 activity was as follows: +++ (>60%), ++ (30–60%), + (10–30%), − (no significant growth). β-Galactosidase activity was as follows: +++ (<45 min), ++ (45–90 min), + (90–240 min), − (no significant β-galactosidase activity). *B*, minimal KIF1A-binding region in liprin-α1. Deletions of liprin-α1 were tested for binding to KIF1A in the yeast two-hybrid assay. The minimal KIF1A-binding region in liprin-α1 (aa 351–673) is indicated by the *thicker line*. The regions that 1120 and 1127 liprin-α antibodies were raised against are indicated. *CC*, coiled-coil domain; *SAM*, sterile α motif. A PDZ domain-binding motif at the C terminus of liprin-α1 is indicated by a *vertical black line*. *C*, specificity of the interactions between members of the KIF1 and liprin-α families. Both liprin-α1 and liprin-α4 interact with KIF1A, whereas only KIF1A (not KIF1Bα or KIF1Bβ) interacts with liprin-α. 1′–863′ in liprin-α4 (full aa sequence is not available) is a region used as bait in the two-hybrid screen and corresponds to aa 351–1202 in liprin-α1. *D*, coimmunoprecipitation between KIF1A and liprin-α in HEK293T cells. HEK293T cell lysates doubly transfected with KIF1A and HA-tagged liprin-α1 (HA-liprin-α1) or singly transfected with KIF1A were immunoprecipitated with mouse monoclonal HA antibodies and immunoblotted with rabbit polyclonal HA and KIF1A (1131) antibodies. KIF1A is specifically coimmunoprecipitated by HA antibodies (*lane 2*) but not by control mouse IgG (*lane 3*). Singly transfected KIF1A does not cross-react with HA antibodies (*lane 5*). *Input*, 5%.

tated KIF1A. HA antibodies did not bring down KIF1A in the absence of HA-liprin-α1.

KIF1A and Liprin-α Distribute to Both Dendrites and Axons in Brain and Cultured Neurons—To characterize the distribution of KIF1A and liprin-α *in vivo*, we generated specific antibodies against fusion proteins of KIF1A (aa 657–937; termed 1131 antibodies) and liprin-α1 (aa 351–673 for 1120 antibodies; aa 818–1202 for 1127 antibodies) (Fig. 1, *A* and *B*). KIF1A (1131) antibodies specifically recognized KIF1A but not KIF1Bβ in immunoblot analysis (Fig. 2*A*). The two liprin-α (1120 and 1127) antibodies reacted equally with HA-liprin-α1 and HA-liprin-α2 (Fig. 2*B*). Liprin-α3 and liprin-α4 were not tested because full-length cDNAs of these isoforms were not available. However, since members of the liprin-α family share similar aa sequences in the regions where the antibodies were raised, it is likely that the liprin-α antibodies recognize all liprin-α isoforms. When tested against rat brain samples, the KIF1A and liprin-α antibodies recognized single bands with molecular masses of ~200 and ~160 kDa, respectively, which are comparable with those of the same proteins transiently expressed in heterologous cells (Fig. 2, *C* and *D*).

Since the yeast two-hybrid results indicated that KIF1A interacts with liprin-α, a protein that localizes to both dendrites and axons (21), we first determined the subcellular distribution of KIF1A proteins in rat brain and cultured neurons by immunofluorescence staining (Fig. 2, *E–H* and *M*). Interestingly, we detected KIF1A in both dendrites and axons. KIF1A overlapped with MAP2, a dendritic marker, in cortex (Fig. 2*E*) and hippocampus (Fig. 2*F*). Consistent with its known axonal localization (25), KIF1A also colocalized with neurofilament H (NF-H), an axonal marker, in the white matter region of cerebellum (Fig. 2*G*) and in axon bundles of spinal cord (Fig. 2*H*). In

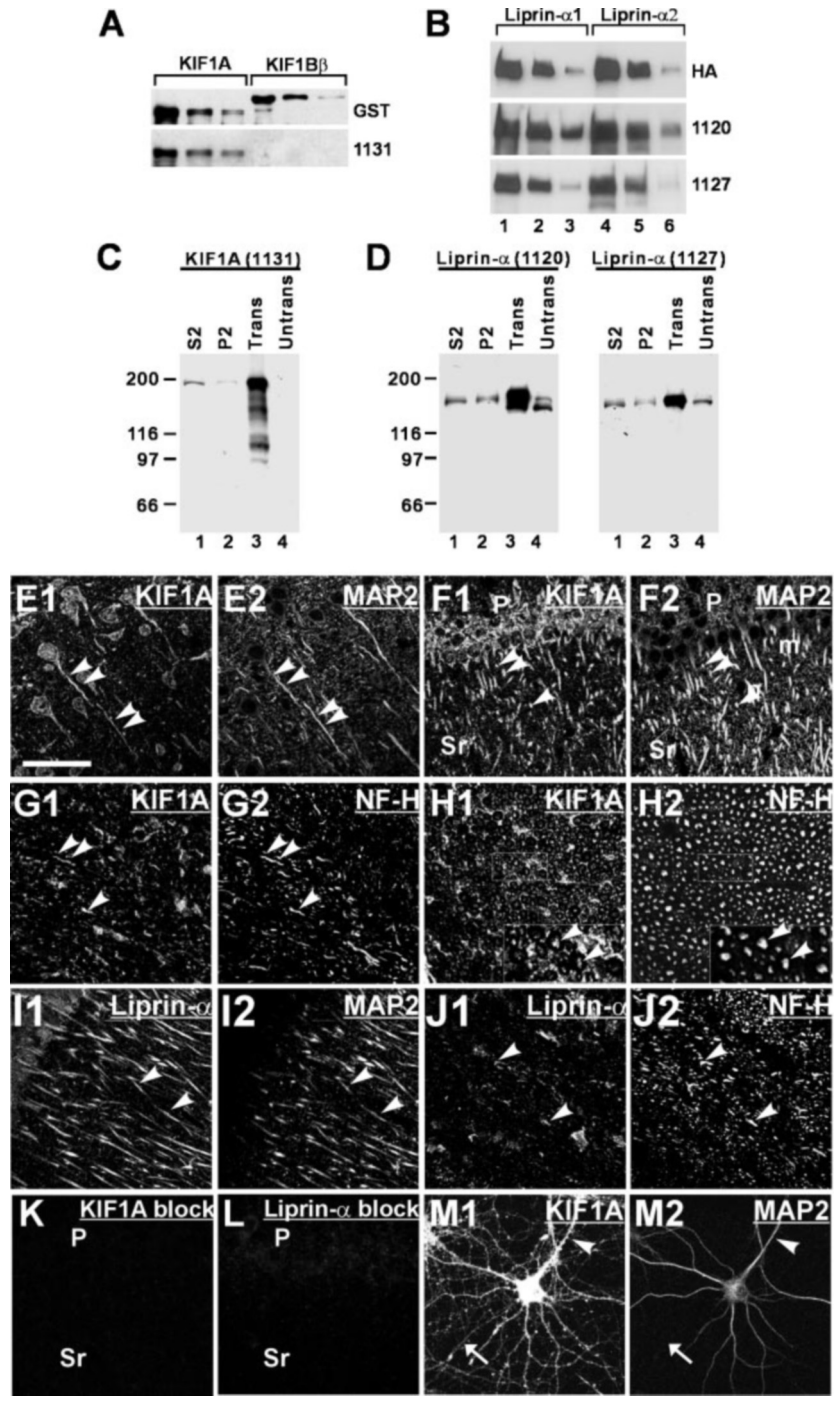

FIG. 2. **KIF1A and liprin-α distribute to both dendrites and axons in rat brain and cultured hippocampal neurons.** *A*, specificity of KIF1A antibodies. Equal amounts (100, 30, and 10 ng for lanes 1–3 and 4–6) of GST fusion proteins containing KIF1A (aa 657–937) and KIF1Bβ (aa 697–1223, equivalent region) were immunoblotted with KIF1A (1131) and GST antibodies. KIF1A antibodies specifically recognize KIF1A but not the closely related KIF1Bβ. *B*, specificity of liprin-α (1120 and 1127) antibodies. HA-liprin-α1 and HA-liprin-α2 expressed in COS cells were immunoblotted with HA and liprin (1120 and 1127) antibodies. Liprin-α antibodies equally recognize liprin-α1 and liprin-α2. HA-liprin-α proteins with a ratio of 10:3:1 were loaded in *lanes 1–3* and *4–6*. *C* and *D*, characterization of KIF1A (1131) and liprin-α (1120 and 1127) antibodies. Subcellular fractions of rat brain (S2 and P2 fractions) and lysates of COS cells transfected with KIF1A and liprin-α-1, respectively, were immunoblotted with the KIF1A and liprin-α antibodies indicated. *P2*, crude synaptosomes; *S2*, supernatant after the removal of the P2; *Trans*, transfected; *Untrans*, untransfected. A small amount of endogenous liprin-α proteins is detected in the untransfected lanes. *E–M*, immunodistribution of KIF1A and liprin-α *in vivo*. Rat brain slices, spinal cord sections, and cultured hippocampal neurons (DIV 21) were labeled by immunofluorescence staining with combinations of KIF1A (1131), liprin-α (1127), MAP2, and neurofilament H (NF-H) antibodies. KIF1A colocalizes with MAP2, a dendritic marker, in cortex (*E*, examples of colocalizations are indicated by *arrowheads*) and hippocampus (*F*). KIF1A colocalizes with NF-H, an axonal marker, in the white matter of cerebellum (*G*) and axon bundles of spinal cord (*H*). Preincubation of KIF1A antibodies with immunogen eliminates the staining (*K*, hippocampal CA1 region). In cultured hippocampal neurons, KIF1A distributes to MAP2-positive dendrites (*M*, *arrowhead*) as well as MAP2-negative axons (*M*, *arrow*). Similar to KIF1A, liprin-α distributes to MAP2-positive dendrites (*I*, hippocampal CA1) and NF-H-positive axons (*J*, cerebellar white matter). Preincubation of liprin-α antibodies with immunogen eliminates the staining (*L*, hippocampal CA1). *P*, pyramidal cell layer; *Sr*, stratum radiatum. The *arrowheads* indicate the sites of colocalization. *Size bar*, 30 μm (*E–M*).

cultured neurons, KIF1A was found in MAP2-positive dendrites as well as MAP2-negative axons (Fig. 2*M*). Preincubation of KIF1A antibodies with immunogen eliminated the KIF1A staining (Fig. 2*K*, an example from the CA1 region of hippocampus). Similar to endogenous KIF1A, exogenous KIF1A was localized to both dendrites and axons of cultured hippocampal neurons (data not shown). Consistently, KIF1A immunogold particles distributed to both the pre- and postsynaptic sides in electron microscopic (EM) analysis (Fig. 4; details described below). Taken together, these results suggest that KIF1A plays a role in both dendritic and axonal transport in neurons.

Similar to KIF1A, liprin-α (1127 antibody) distributed to both dendrites and axons as evidenced by colocalization with MAP2 (Fig. 2*I*, the CA1 region of hippocampus) and NF-H (Fig. 2*J*, the white matter of cerebellum). The other liprin-α (1120) antibodies showed essentially the same distribution pattern (data not shown). Liprin-α staining was eliminated by preincubation of the antibodies with immunogens (Fig. 2*L*, the CA1 region of hippocampus). In cultured hippocampal neurons, both endogenous (21) and exogenous (data not shown) liprin-α distribute to dendrites and axons, similar to the subcellular distribution of liprin-α in brain.

KIF1A Colocalizes with Liprin-α and GRIP in Rat Brain—We tested colocalization between KIF1A, liprin-α, and GRIP (a liprin-α-associated protein) by double or triple label immunofluorescence staining on rat brain sections (Fig. 3). In rat brain, the distribution of KIF1A overlapped that of both liprin-α (Fig. 3*A*, an example from the CA1 dendrites of hippocampus) and GRIP (Fig. 3*B*, hippocampal CA1 dendrites). Triple labeling of KIF1A, liprin-α, or GRIP and NF-H (axons) revealed that KIF1A colocalizes with liprin-α (Fig. 3*C*) and

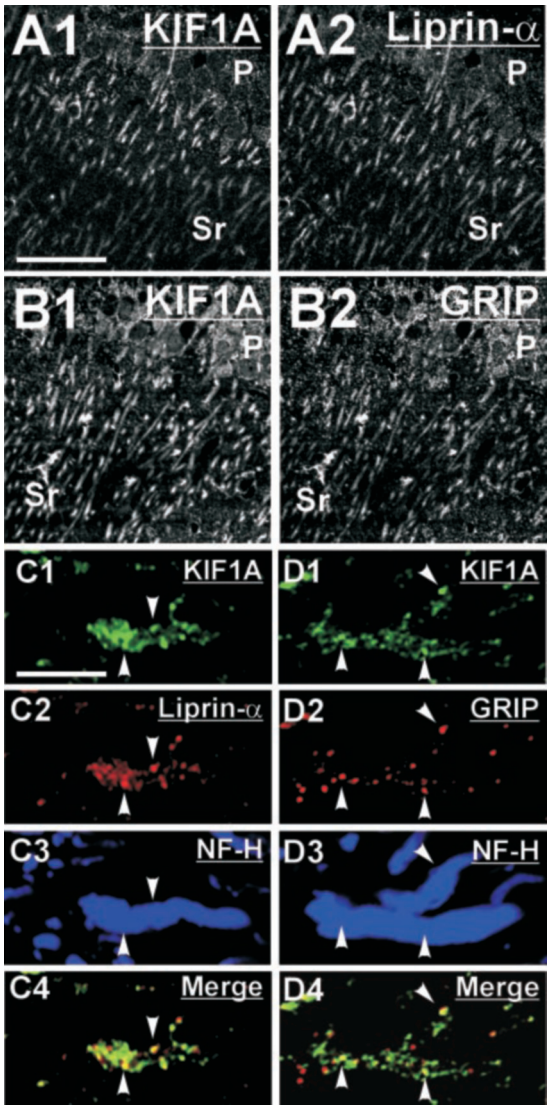

FIG. 3. **KIF1A colocalizes with liprin-α and GRIP in rat brain.** *A* and *B*, rat brain sections were doubly labeled by immunofluorescence staining for KIF1A and liprin-a (*A*), or KIF1A and GRIP (*B*). The distribution of KIF1A largely overlaps that of liprin-α (*A*) and GRIP (*B*) (examples from the CA1 dendrites of hippocampus). *C* and *D*, rat brain sections were triply labeled for KIF1A (*green*), liprin-α (*red*) or GRIP (red), and NF-H (*blue*). KIF1A colocalizes with liprin-α (*C*) or GRIP (*D*) in punctate subcellular structures (*arrowheads*) of axons (examples from axons of cerebellar white matter). *Merge*, *green* plus *red*. *Size bar*, 80 μm (*A–B*) and 10 μm (*C–D*).

GRIP (Fig. 3*D*) in punctate subcellular structures in axons of cerebellar white matter. These results indicate that KIF1A colocalizes with liprin-α and GRIP *in vivo*.

Ultrastructural Localization of KIF1A in Rat Brain by Immunoelectron Microscopy—To further characterize the distribution of KIF1A in central neurons, we determined the subcellular localization of KIF1A by immunogold EM analysis of sections of rat neocortex (Fig. 4). KIF1A immunogold particles were observed in various subcellular sites of the neurons including microtubules (Fig. 4*A*), consistent with the function of KIF1A as a kinesin motor moving along microtubule tracks. KIF1A particles were observed at both pre- and postsynaptic sites (Fig. 4*B*, example of an asymmetric spine synapse). Quantitative analysis revealed that KIF1A immunogold particles were concentrated close to the pre- and postsynaptic membranes (Fig. 4*C*, *left panel*). The density of KIF1A particles was constant along the lateral plane of the synaptic membrane (Fig. 4*C*, *right panel*). Similar to KIF1A, liprin-α is distributed in pre- and postsynaptic sites of neurons at the EM level (21). These results provide further evidence that KIF1A distributes to dendritic and axonal sites.

KIF1A Coaccumulates with Liprin-α in Ligated Sciatic Nerves—Okada *et al.* (25) showed that KIF1A accumulates with synaptophysin but not with syntaxin in ligated sciatic nerve fibers, suggesting that KIF1A selectively transports synaptophysin-containing vesicles. Since liprin-α is detected in sciatic nerve fibers by immunoblot analysis (data not shown), we tested whether KIF1A comigrates with liprin-α in axons of motor neurons by the nerve ligation assay (Fig. 5). In rat sciatic nerves ligated for 60 min, KIF1A and liprin-α accumulated and precisely colocalized on the proximal (cell body) side of the ligation (Fig. 5*A*). Syntaxin also accumulated proximally but did not colocalize with KIF1A (Fig. 5*C*), verifying the specificity of KIF1A/liprin-α coaccumulation. KIF1A, liprin-α, and syntaxin did not accumulate on the distal side of the ligation (Fig. 5, *B* and *D*). These results suggest that KIF1A may anterogradely transport liprin-α along axonal microtubules.

KIF1A Cofractionates and Forms a Complex with Liprin-α and Liprin-α-associated Proteins in Brain—If liprin-α is a KIF1A receptor linking KIF1A to its vesicular cargoes, KIF1A and liprin-α should cofractionate into the subcellular fractions of neurons enriched with light membranes and synaptic vesicles. To test this, we determined fractionation patterns of KIF1A and liprin-α in subcellular fractions of rat brain (Fig. 6*A*). Both KIF1A and liprin-α were detected in the P3 (light membranes) and LP2 (synaptic vesicles) fractions. In addition, proteins associated with liprin-α such as GRIP and GluR1 were also detected in the P3 and LP2 fractions.

To further characterize the association of KIF1A and liprin-α with membranes, we performed the sucrose density flotation assay (Fig. 6*B*). When samples enriched with membranes (see "Experimental Procedures" for details) were loaded onto the bottom of a discontinuous sucrose gradient, KIF1A and liprin-α floated and cofractionated into the light fractions (*lanes 1–3*), along with GRIP, GluR2/3, and synaptotagmin, but not with cortactin (Fig. 6*B*, *left panel*). Detergent treatment of the samples prior to centrifugation eliminated the floating (Fig. 6*B*, *right panel*), suggesting that intact membranes are required for flotation.

To determine whether KIF1A biochemically associates with liprin-α in the floated membranes, we performed coimmunoprecipitation experiments on detergent extracts of the pooled light membranes (fractions 1–3). KIF1A antibodies immunoprecipitated KIF1A and coprecipitated liprin-α, GRIP, GluR2/3, and synaptotagmin, but not syntaxin and cortactin (Fig. 6*C*). The liprin-α (1120) antibody recognizes both liprin-α1 and liprin-α2 (Fig. 2*B*), and the GRIP (C8399) antibody recognizes both GRIP1 and GRIP2/ABP (10, 11). Thus, further study will be required to identify the specific isoforms of liprin-α and GRIP that bind to KIF1A *in vivo*. The coimmunoprecipitation of GRIP and GluR2/3 is presumably due to their association with liprin-α (21). The coimmunoprecipitation of synaptotagmin suggests that KIF1A is biochemically associated with synaptotagmin and is similar to the reported association between synaptotagmin and the closely related KIF1Bβ (40). The lack of coimmunoprecipitation of syntaxin that floated together with KIF1A in the flotation assay (Fig. 6*B*) indicates the specific association of KIF1A with its cargoes, and the lack of coimmunoprecipitation of cortactin with KIF1A is consistent with their differential floating (Fig. 6*B*). Control immunoprecipitation with guinea pig IgG did not bring down any of these proteins. Interestingly, in an independent coimmunoprecipitation experiment on detergent lysates of the floated samples, KIF1A antibodies coimmunoprecipitated two additional liprin-α-binding

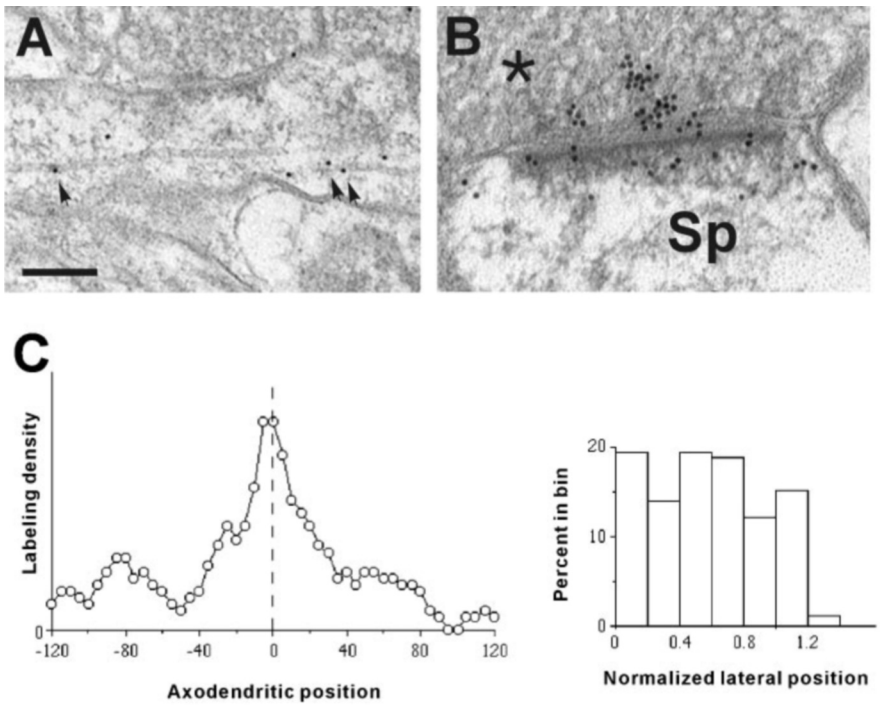

FIG. 4. **Ultrastructural localization of KIF1A by immunoelectron microscopy.** Sections of rat cerebral cortex were stained for KIF1A by immunogold EM. Some of the KIF1A immunogold particles associate with microtubules (*A*, *arrows*). KIF1A distributes to both pre- and postsynaptic sites (*B*, example from an axospinous synapse). *, presynaptic nerve terminal. *Sp*, postsynaptic spine. In a quantitative analysis, KIF1A gold particles are concentrated at pre- and postsynaptic membrane-proximal regions (*C*, *left panel*; the *right side* from the *midline* corresponds to the postsynaptic region). The distribution of gold particles remains consistent along the lateral plane of the synapse (*C*, *right panel*; *0* corresponds to the center, and *1.0* corresponds to the edge of the synapse). *Size bar*, 0.25 μm.

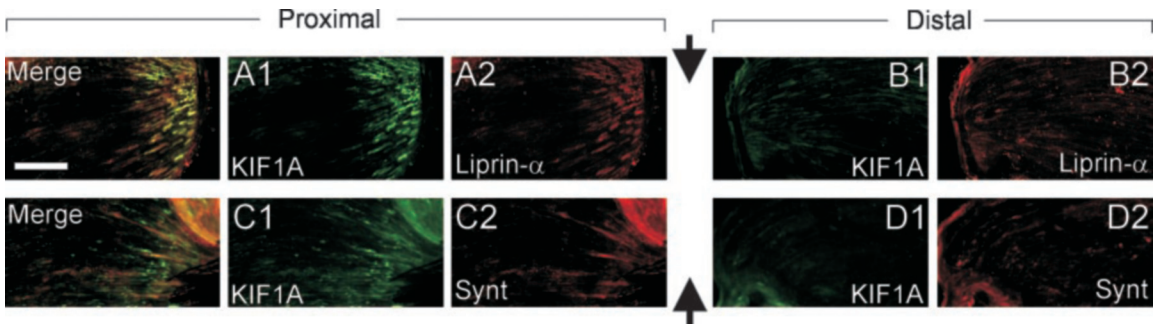

FIG. 5. **KIF1A and liprin-α coaccumulate in ligated sciatic nerve fibers.** Rat sciatic nerves were ligated for 60 min and labeled by double immunofluorescence staining for KIF1A + liprin-α (*A* and *B*), or KIF1A + syntaxin (*C* and *D*, negative control). KIF1A specifically coaccumulates with liprin-α (*A*) but not with syntaxin (*C*) in the proximal side of the ligation. The site of nerve ligation is indicated by *two vertical arrows*. Proximal (cell body) (*A* and *C*) and distal (nerve terminal) (*B* and *D*) sides of the ligation site are indicated. *Images* on the proximal side of the ligation were merged for better comparison. *Size bar*, 220 μm.

proteins, RIM (a scaffolding protein at active zones) and GIT1 (a multimodular scaffolding protein with an ADP-ribosylation factor GTPase-activating protein activity) (Fig. 6*D*). In addition, KIF1A antibodies pulled down the βPIX/Cool-1 (Fig. 6*D*), a Rho-type guanine nucleotide exchange factor that directly interacts with GIT1 (5, 6, 41).

In further coimmunoprecipitation experiments in a reverse orientation, liprin-α antibodies immunoprecipitated liprin-α and coprecipitated KIF1A and other liprin-α-associated proteins including GRIP and RIM (Fig. 6*E*). In addition, GluR2/3 antibodies brought down GluR2/3 and coprecipitated GRIP, liprin-α, and KIF1A (Fig. 6*F*), strongly suggesting that KIF1A and GluR2/3 are biochemically associated in the floated membranes. Importantly, GluR2/3 antibodies did not bring down RIM (Fig. 6*F*), suggesting that the KIF1A cargo vesicles containing postsynaptic proteins may not contain presynaptic proteins. Taken together, these results indicate that KIF1A biochemically associates with liprin-α and various liprin-α-associated membrane, signaling, and scaffolding proteins in the brain.

DISCUSSION

Cargo-binding Domain in KIF1A—We have shown that part of the tail region of KIF1A, termed the LBD domain (aa 657–1105), interacts with liprin-α (Fig. 1). The closely related KIF1Bβ (1770 aa long) requires its tail region (aa 885–1770) for association with vesicles containing synaptophysin, synaptotagmin, and SV2 (40). The C terminus of KIF1Bα, a shorter splice variant of KIF1B, interacts with the PSD-95, SAP97, and S-SCAM PDZ domain-containing proteins (42). KIF1C (1103 aa long), the third member of the KIF1 family, uses its middle (aa 714–809) and C-terminal (last 60 aa residues) regions to interact with protein-tyrosine phosphatase D1 and 14-3-3, respectively (43, 44). Taken together, these results suggest that members of the KIF1 family of kinesin motors use various regions in their tails to associate with specific cargoes.

It has been reported that the C-terminal pleckstrin homology domain of Unc104 plays an important role in the recognition of phospholipids in cargo vesicle membranes (45, 46). Our results demonstrate the LBD domain of KIF1A that is located in the middle the molecule interacts with liprin-α, a multimodular protein that is linked to various proteins including membrane proteins. Considering these results, it is conceivable that the LBD and pleckstrin homology domains of KIF1A may associate with cargo vesicles in a parallel fashion. In this model, the pleckstrin homology domain of KIF1A may bind to the membrane of a cargo vesicle, whereas liprin-α may associate with

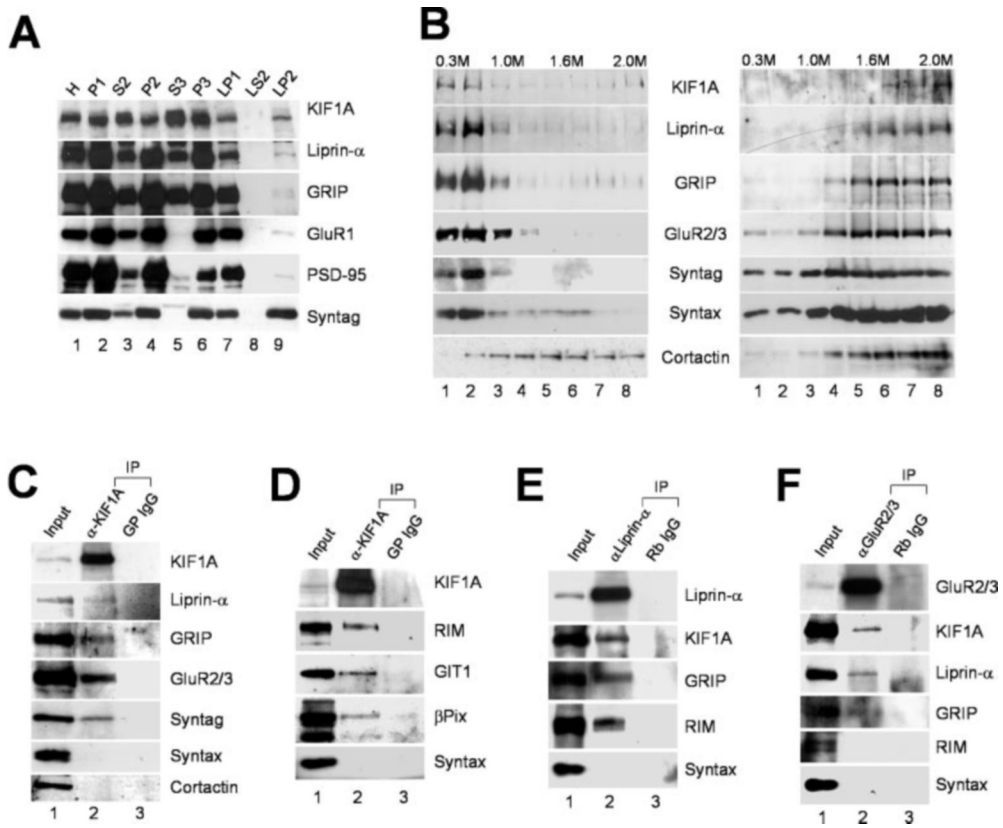

FIG. 6. **KIF1A cofractionates and forms a complex with liprin-α and liprin-α-associated proteins in brain.** *A*, KIF1A and liprin-α are detected in light membrane and synaptic vesicle fractions of brain. Subcellular fractions of adult rat brain were immunoblotted with the antibodies indicated. KIF1A and liprin-α, along with GRIP and AMPA receptors (*GluR1*), distribute to the light membrane (*P3*) and synaptic vesicle (*LP2*) fractions. PSD-95 and synaptotagmin (a presynaptic vesicle protein) were visualized for comparison. *H*, rat brain homogenates; *P1*, nuclei and other large debris; *P2*, crude synaptosomes; *S2*, supernatant after the removal of the P2; *S3*, cytosolic fraction; *P3*, light membranes; *LP1*, synaptic membrane-enriched fraction; *LS2*, synaptic cytosol; *LP2*, synaptic vesicle-enriched fraction. *B*, cofractionation of KIF1A and liprin-α in the sucrose density flotation assay. Membrane-enriched samples from rat brain (see "Experimental Procedures" for more details) were loaded onto the bottom of a discontinuous sucrose density gradient. KIF1A, along with liprin-α, GRIP, and AMPA receptors (GluR2/3), but not cortactin, cofractionate in light fractions (*lanes 1–3*, *left panels*). The floating was eliminated (*right panel*) by the addition of detergent to the samples before flotation (*right panels*). *C*, coimmunoprecipitation of KIF1A with liprin-α- and liprin-α-associated proteins in light membranes. Light membrane fractions (*1–3*) from *B* were solubilized with detergent, immunoprecipitated with KIF1A (1131) antibodies or guinea pig IgG and immunoblotted with the antibodies indicated. KIF1A coimmunoprecipitates with liprin-α, GRIP, GluR2/3, and synaptotagmin but not syntaxin and cortactin. *Input*, 2%. *D*, in an independent coimmunoprecipitation experiment similar to *C*, KIF1A selectively coimmunoprecipitates with RIM, GIT1, and βPIX. *Input*, 1%. *E* and *F*, detergent extracts of the floated light membranes were also immunoprecipitated with liprin-α (1127), GluR2/3 (Chemicon) antibodies, or rabbit IgG (negative control) and immunoblotted with the antibodies indicated. Both liprin-α and GluR2/3 coimmunoprecipitate with KIF1A.

the proteins on the same cargo vesicle. This parallel binding may help to determine the specificity or affinity of the association of KIF1A with its cargoes.

KIF1A-mediated Transports in Dendrites and Axons—Previous studies on KIF1A were mainly focused on its transport in the axonal compartment. However, several lines of evidence in our study indicate that KIF1A exists in dendrites in addition to axons: 1) localization of KIF1A in dendrites and axons of brain and cultured neurons revealed by immunofluorescence staining (Fig. 2); 2) localization of KIF1A in both pre- and postsynaptic sites revealed by immunogold EM analysis (Fig. 4); 3) biochemical association of KIF1A with both axonal (synaptotagmin) and dendritic (AMPA receptors) proteins (Fig. 6). In addition, movement of enhanced green fluorescent protein-tagged KIF1A particles has been detected in proximal thick neurites (probably dendrites) and axons of living cultured neurons (30). This is consistent with the movement of enhanced green fluorescent protein-tagged Unc104 particles observed in both dendrites and axons of living C. elegans neurons (29). Collectively, these results suggest that KIF1A/Unc104 proteins are involved in the transport of neuronal proteins in both dendrites and axons.

Liprin-α as a KIF1A Receptor—Recent studies have begun to uncover the motor-binding "receptors" in cargoes (47, 48), which include coat proteins, scaffolding proteins, small GTPases, transmembrane proteins, and other motor proteins. Examples of motor receptors similar to our results are the scaffolding proteins LIN-2·LIN-7·LIN-10 and JIP-1·JIP-2·JIP-3 proteins, which link KIF17 (49) and conventional kinesin (50), respectively, to their specific cargoes. We propose that liprin-α functions as a cargo receptor for KIF1A, since liprin-α directly interacts with KIF1A and also associates with a variety of membrane proteins such as LAR and AMPA receptors, thereby potentially linking KIF1A to cargo vesicles.

It has been recently shown that Unc104 can exhibit a highly processive movement through the formation of dimers at high motor concentrations (34), which may occur *in vivo* through clustering of motor proteins in phosphatidylinositol 4,5-bisphosphate-containing rafts on the surface of cargo vesicles (45, 46). Similar to Unc104, KIF1A also moves processively along the microtubule in the single molecule motility assay, but some KIF1A molecules occasionally exhibit slow movement (34) that is similar to the previously reported biased diffusional movement (32). This suggests that KIF1A may form a relatively unstable dimer, perhaps due to the weakness of the predicted neck coiled-coil probability, and raises the possibility that KIF1A dimers may be stabilized by additional mechanisms (34). Intriguingly, liprin-α forms multimers (1), suggest-

ing the possibility that liprin-α may contribute to the processive movement of KIF1A through the stabilization of KIF1A dimers. This would suggest a dual role for liprin-α, that of both KIF1A receptor and a stabilizer of KIF1A dimers.

GRIP-associated Proteins as KIF1A Cargoes—GRIP and GRIP-associated AMPA receptors comprise an important set of potential KIF1A cargoes (Fig. 6, *B–F*). Several lines of evidence indicate that GRIP is involved in neuronal transport. A significant amount of GRIP immuno-EM labeling associates with vesicles that are often very close to microtubules (10, 14). Biochemically, GRIP distributes to small membrane- and vesicle-enriched fractions (11, 14), similar to the subcellular distribution of liprin-α (Fig. 6*A*). It was reported that synaptic targeting of AMPA receptors is eliminated by disrupting the liprin-α-GRIP interaction by various dominant negative constructs (21). A possible explanation for such results is that the disruption may prevent the AMPA receptor-GRIP complex from associating with KIF1A through liprin-α. Taken together, these results suggest that KIF1A, via liprin-α, may transport GRIP, AMPA receptors, and possibly other GRIP-associated membrane and signaling proteins including ephrin ligands, ephrin receptors, and GRASP-1 (15–18).

It has been reported that conventional kinesin heavy chain interacts with GRIP1 and transports the AMPA receptor-GRIP complex (51). This finding in conjunction with our results indicates that the AMPA receptor-GRIP complex could be transported by more than one type of kinesin motor, KIF1A and conventional kinesin. A similarly redundant transport mechanism, which may exist for physiologically important cargoes, has been identified for *N*-methyl-D-aspartate glutamate receptors, which associate with KIF17 through the LIN-2·LIN-7·LIN-10 complex (49) and with KIF1Bα through PSD-95 or S-SCAM (42). Similarly, liprin-α could also be transported by both KIF1A and conventional kinesin. The minimal effects of *unc-104* mutations in C. elegans on the presynaptic targeting of SYD-2 (19) may support this idea of a redundant mechanism for liprin-α/SYD-2 transport.

Intriguingly, GRIP1 steers conventional kinesin to dendrites (51), which raises the question of whether KIF1A is also steered to dendrites by association with liprin-α or GRIP. Our data indicate that postsynaptic GluR2/3 coimmunoprecipitates with GRIP, liprin-α, and KIF1A, but not with RIM, a presynaptic active zone protein (Fig. 6*F*), suggesting that pre- and postsynaptic cargo proteins partition into different KIF1A cargo vesicles. This suggests that further work is needed to identify the molecular determinants that direct the polarized targeting of KIF1A cargo vesicles with pre- and postsynaptic contents.

KIF1A, Liprin-α, and Presynaptic Differentiation—Genetic deletion of *syd-2* in *C. elegans* and *Dliprin-α* in *Drosophila* leads to abnormal differentiation of the presynaptic active zone (19, 20). One explanation for these results is that liprin-α functions as a structural component of the active zone (52). An equally plausible hypothesis based on our results is that defective liprin-α may limit KIF1A-mediated axonal transport of various liprin-α-associated proteins involved in presynaptic development. We demonstrated that KIF1A associates with liprin-α and liprin-α-associated proteins including RIM, GIT1, and βPIX (Fig. 6). RIM is a multimodular scaffolding protein of the active zone that is involved in the regulation of neurotransmitter release (8, 9). GIT1 distributes to both pre- and postsynaptic sites at the EM level (2) and associates with Piccolo/aczonin (53), a core component of active zones (54, 55). Mutation in the dPix gene, a *Drosophila* homolog of mammalian βPIX, has been shown to modify synaptic structure and targeting of various synaptic proteins (56). Taken together, these results suggest that liprin-α may mediate the transport of these proteins to the nerve terminal for presynaptic differentiation.

In conclusion, we have shown the first evidence for a protein interaction of KIF1A with the multimodular protein liprin-α. Our results suggest that liprin-α, as a KIF1A receptor, may link KIF1A to various liprin-α-associated membrane, signaling, and cytoskeletal proteins during their transport. It will be of use in the near future to perform genetic analysis of the identified protein interactions and determine the functional association of liprin-α with the dimerization and polarized targeting of KIF1A.

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