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

Received for publication, November 21, 2002
Published, JBC Papers in Press, January 8, 2003, DOI 10.1074/jbc.M211874200

Hyewon Shin‡, Michael Wyszynski§§, Kyung-Hye Huh‡, Juli G. Valtschanoff, Jae-Ran Lee†, Jaewon Ko‡, Michel Streuli**‡‡, Richard J. Weinberg*, Morgan Sheng§ §§, and Eunjoon Kim***

From the ‡Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea, the §Center for Learning and Memory, RIKEN-MIT Neuroscience Research Center and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, the ¶Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, and the **Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Liprin-α/SYD-2 is a multimodular scaffolding protein important for presynaptic differentiation and postsynaptic targeting of α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic 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 coimmunoprecipitates with liprin-α and various liprin-α-associated membrane, signaling, and scaffolding proteins including α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic 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/Catp/95-APP/PRL (2), a family of multidomain proteins with GTPase-activating protein activity for ADP-riboseylation 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 AMPA1 glutamate receptors (GlRas) (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 multimodal 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 KIF1A, 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/Uncon104 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 progressive 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 cargo other than synaptic vesicle precursors and about the manner in which KIF1A interacts with specific cargoes.

* This work was supported by grants from the Korean Ministry of Science and Technology, the Korea Research Foundation (to E. K.) and National Institutes of Health Grant NS-35527 (to R. J. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
†† Present address: Angen Inc., Thousand Oaks, CA 91320.
‡‡ Present address: ImmunoGen, Inc., 128 Sidney St., Cambridge, MA 02139.
§§ Associate investigator of the Howard Hughes Medical Institute.
¶¶ To whom correspondence should be addressed: Dept. of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea. Tel.: 42-869-2333; Fax: 42-869-2610; E-mail: kime@mail.kaist.ac.kr.

This paper is available on line at http://www.jbc.org

11393
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-α (aa 351–1105) and liprin-α4 (aa 1–863) were amplified by PCR and subcloned to pBHAvira biesector (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: a 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 HindIII and EcoRI, and subcloned into GW1 (British Biotechnology). HER293T cells transfected with GW1-KIF1A and pMT2-HA-liprin-α were extracted with 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 657–957 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). Affinity purification of specific antibodies was performed using immunoisolated on polyvinylidene difluoride membrane (Amersham Bio- technology, Inc., Lake Placid, NY), GluR2/3 (Chemicon), synaptotagmin (1:500), and synaptotagmin (1:500). Immunofluorescence images were captured using an LSM510 confocal laser-scanning microscope (Zeiss).

**Nerve Ligation Assay—** Sciatic nerves of anesthetized adult rats were ligated for 60 min followed by perfusion with 4% paraformaldehyde. After a brief perfusion 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 Immunoinasting—** 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-α, 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. 1A). 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. 1B).

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. 1A). 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. 1C).

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

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: ++ (+80%), ++ (+60–80%), + (10–50%), − (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-α 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%.

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 1127 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. 2A). The two liprin-α (1120 and 1127) antibodies reacted equally with HA-liprin-α1 and HA-liprin-α2 (Fig. 2B). HA-liprin-α3 and HA-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. 2E) and hippocampus (Fig. 2F). 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. 2G) and in axon bundles of spinal cord (Fig. 2H). In
cultured neurons, KIF1A was found in MAP2-positive dendrites as well as MAP2-negative axons (Fig. 2M). Preincubation of KIF1A antibodies with immunogen eliminated the KIF1A staining (Fig. 2K, 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. 2f, the CA1 region of hippocampus) and NF-H (Fig. 2f, 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. 2L, 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. 3A, an example from the CA1 dendrites of hippocampus) and GRIP (Fig. 3B, hippocampal CA1 dendrites). Triple labeling of KIF1A, liprin-α, or GRIP and NF-H (axons) revealed that KIF1A colocalizes with liprin-α (Fig. 3C) and
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. 5A). Syntaxin also accumulated proximally but did not colocalize with KIF1A (Fig. 5C), 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. 6A). 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. 6B). 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. 6B, left panel). Detergent treatment of the samples prior to centrifugation eliminated the floating (Fig. 6B, 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. 6C). The liprin-α (1120) antibody recognizes both liprin-α1 and liprin-α2 (Fig. 2B), and the GRIP (C8399) antibody recognizes both GRIP1 and GRIP2(ABP) (10, 11). The lack of coimmunoprecipitation of syntaxin that floated together with KIF1A in the flotation assay (Fig. 6B) indicates the specific association of KIF1A 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. 6B) 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. 6B). 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 proteins.
proteins, RIM (a scaffolding protein at active zones) and GIT1 (a multimodular scaffolding protein with an ADP-ribosylation factor GTPase-activating protein activity) (Fig. 6D). In addition, KIF1A antibodies pulled down the /H9252PIX/Cool-1 (Fig. 6D), a Rho-type guanine nucleotide exchange factor that directly interacts with GIT1 (5, 6, 41).

In further coimmunoprecipitation experiments in a reverse orientation, liprin-/H9251antibodies immunoprecipitated liprin-/H9251and coprecipitated KIF1A and other liprin-/H9251-associated proteins including GRIP and RIM (Fig. 6E). In addition, GluR2/3 antibodies brought down GluR2/3 and coprecipitated GRIP, liprin-/H9251, and KIF1A (Fig. 6F), strongly suggesting that KIF1A and GluR2/3 are biochemically associated in the floated membranes. Importantly, GluR2/3 antibodies did not bring down RIM (Fig. 6F), 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-/H9251and various liprin-/H9251-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-/H9251(Fig. 1). The closely related KIF1B/ beta (1770 aa long) requires its tail region (aa 885–1770) for association with vesicles containing synaptophysin, synaptotagmin, and SV2 (40). The C terminus of KIF1B, 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-/H9251, 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-/H9251 may associate with
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 (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 proce-
sive 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-associating 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 signif-
icant 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 ves-
icle-enriched fractions (11, 14), similar to the subcellular dis-
tribution of liprin-α (Fig. 6A). It was reported that synaptic
targeting of AMPA receptors is eliminated by disrupting the
liprin-α-GRIP interaction by various dominant negative con-
structs (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 trans-
ported by more than one type of kinesin motor, KIF1A and
conventional kinesin. A similarly redundant transport mecha-
nism, which may exist for physiologically important cargoes,
hase been identified for N-methyl-D-aspartate glutamate rece-
tors, 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 presyn-
aptic active zone protein (Fig. 6F), 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 Diliprin-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 defec-
tive liprin-α may limit KIF1A-mediated axonal transport of
various liprin-α-associated proteins involved in presynaptic
development. We demonstrated that KIF1A associates with lip-
rin-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 neurotrans-
mittor release (8, 9). GIT1 distributes to both pre- and postsyn-
aptic sites at the EM level (2) and associates with Piccolo/
acaclin (53), a core component of active zones (54, 55).
Mutation in the dPix gene, a *Drosophila* homolog of mamma-
lian βPIX, has been shown to modify synaptic structure and
targeting of various synaptic proteins (56). Taken together,
Association of KIF1A with Liprin-α

V. H., and Coleman, M. P. (1999) Mamm. Genome 10, 617–622
38. Gong, T. W., Winnicki, R. S., Kohrman, D. C., and Lomax, M. I. (1999) Gene (Amst.) 239, 117–127
39. Nangaku, M., Sato-Yoshitake, R., Okada, Y., Noda, Y., Takemura, R., Yamauchi, H., and Hirokawa, N. (1994) Cell 79, 1209–1220
40. Zhao, C., Takita, J., Tanaka, Y., Setou, M., Nakagawa, T., Takeda, S., Yang, H. W., Terada, S., Nakata, T., Takei, Y., Saito, M., Tsuji, S., Hayashi, Y., and Hirokawa, N. (2001) Cell 105, 587–597
41. Zhao, Z. S., Manser, E., Lee, T. H., and Lim, L. (2000) Mol. Cell. Biol. 20, 6354–6363
42. Mok, H., Shin, H., Kim, S., Lee, J. R., Yoon, J., and Kim, E. (2002) J. Neurosci. 22, 5253–5258
43. Dorner, C., Ciossek, T., Muller, S., Muller, P. H., Ullrich, A., and Lammers, R. (1998) J. Biol. Chem. 273, 20267–20275
44. Dorner, C., Ullrich, A., Harrig, H. U., and Lammers, R. (1999) J. Biol. Chem. 274, 33654–33660
45. Klopfenstein, D. R., Tomishige, M., Stuurman, N., and Vale, R. D. (2002) Cell 109, 587–597
46. Scholey, J. M. (2002) Dev. Cell 2, 515–516
47. Goldstein, L. S. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6999–7003
48. Klopfenstein, D. R., Vale, R. D., and Rogers, S. L. (2000) Cell 103, 537–540
49. Setou, M., Nakagawa, T., Seog, D. H., and Hirokawa, N. (2000) Science 288, 1796–1802
50. Verhey, K. J., Meyer, D., Deehan, R., Ilenis, J., Schnapp, B. J., Rapoport, T. A., and Margolis, B. (2001) J. Cell Biol. 152, 959–970
51. Setou, M., Seog, D. H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M., and Hirokawa, N. (2002) Nature 417, 83–87
52. Baran, R., and Jin, Y. (2002) Neuron 34, 1–7
53. Kim, S., Ko, J., Shin, H., Lee, J. R., Lim, C., Han, J. H., Allrock, W. D., Garner, C. C., Gundelfinger, E. D., Fremont, R. T., Kaang, B. K., and Kim, E. (2003) J. Biol. Chem. 278, 6291–6300
54. Fenster, S. D., Chung, W. J., Zhai, R., Cases-Langhoff, C., Voss, B., Garner, A. M., Kaempf, U., Kindler, S., Gundelfinger, E. D., and Garner, C. C. (2000) Neuron 25, 203–214
55. Wang, X., Kitschull, M., Laue, M. M., Lichte, B., Petrasch-Parwez, E., and Kilimann, M. W. (1999) J. Cell Biol. 147, 151–162
56. Parnas, D., Haghihgi, A. P., Fetter, R. D., Kim, S. W., and Goodman, C. S. (2001) Neuron 32, 415–424