Liprins, a Family of LAR Transmembrane Protein-tyrosine Phosphatase-interacting Proteins*

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LAR family transmembrane protein-tyrosine phosphatases function in axon guidance and mammary gland development. In cultured cells, LAR bends to the intracellular, coiled coil LAR-interacting protein at discrete ends of focal adhesions, implicating these proteins in the regulation of cell-matrix interactions. We describe seven LAR-interacting protein-like genes in humans and Caenorhabditis elegans that form the liprin gene family. Based on sequence similarities and binding characteristics, liprins are subdivided into α-type and β-type liprins. The C-terminal, non-coiled coil regions of α-liprins bind to the membrane-distal phosphatase domains of LAR family members, as well as to the C-terminal, non-coiled coil region of β-liprins. Both α- and β-liprins homodimerize via their N-terminal, coiled coil regions. Liprins are thus multivalent proteins that potentially form complex structures. Some liprins have broad mRNA tissue distributions, whereas others are predominately expressed in the brain. Co-expression studies indicate that liprin-α2 alters LAR cellular localization and induces LAR clustering. We propose that liprins function to localize LAR family tyrosine phosphatases at specific sites on the plasma membrane, possibly regulating their interaction with the extracellular environment and their association with substrates.

The highly organized and coordinated response of cells to diverse extracellular stimuli is partially mediated by tyrosine phosphorylation of proteins, some of which relay information from the cell surface to the nucleus and others of which control cytoskeletal organization. The degree of tyrosine phosphorylation of such signaling proteins, including enzymes, adapter proteins, and structural proteins, is regulated by the concerted activities of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPases). Both protein-tyrosine kinases and PTPases comprise large gene families encoding transmembrane-type and intracellular-type enzymes (1, 2). The physiological role of many protein-tyrosine kinases, and some PTPases, is well documented (3–6). Recent genetic analysis of LAR-like transmembrane PTPases indicates that members of this subfamily play a role in Drosophila axon guidance (7, 8) and murine mammary gland development and function (9). About half of the embryos with Drosophila LAR (DLAR)-inactivating mutations die as late instar larvae, and the other half die before or at eclosion (7). Examination of the nervous systems of Dlar−/− mice revealed specific defects in motor axon guidance and, to a lesser degree, in the formation of certain CNS axon pathways. Female mice with a targeted disruption of the Lar gene are incapable of delivering milk due to impaired terminal differentiation of alveoli at late pregnancy (9). Consequently, the glands fail to switch to a lactational state and rapidly involute postpartum. The molecular basis for the axon guidance defect in the Dlar−/− mutants and the impaired development of mammary alveoli in the Lar−/− mutant mice is unknown, but given that axon guidance, as well as mammary epithelial differentiation and lactation, is regulated by soluble factors, cell-cell interactions, and cell-matrix interactions, it is likely that DLAR and LAR function in one or more of these signaling pathways (10, 11).

LAR and DLAR are members of the LAR subfamily of transmembrane PTPases, which consists of the highly related vertebrate LAR (12, 13), PTP8 (14–16), and PTPμ (17–20), and Drosophila DLAR (21, 22). These PTPases contain extracellular regions comprised of three N-terminal Ig-like domains and a variable number of fibronectin type III-like domains connected via a transmembrane segment to an intracellular region containing two PTPase domains. The overall architecture of the LAR family extracellular regions is similar to several cell or matrix adhesion molecules, indicating that these PTPases function as receptors for cell surface molecules and/or extracellular matrix molecules (3, 23). Furthermore, human LAR localizes to focal adhesions (FAs) (24), which are sites of cell-extracellular matrix interactions, and to sites of cell-cell contact (25, 26). Because FAs are assembled by a tyrosine phosphorylation-dependent process following integrin ligation (27), LAR may play a role in FA disassembly.

Two proteins were identified that bind the LAR membrane-distal D2 PTPase domain. One of these, Trio, contains a rac1 guanine nucleotide exchange factor domain, a rhoA guanine nucleotide exchange factor domain, a protein kinase domain, and several auxiliary domains (28). Because rac and rho are regulators of actin reorganization and cell growth (29), a LAR/Trio complex may integrate multiple signals and determine the response of cells to diverse extracellular stimuli. The second protein, LAR-interacting protein 1 (LIP.1), is a coiled coil protein that colocalizes with LAR at FAs (24). LIP.1 may form rod-like dimers or higher order structures similar to other proteins that contain coiled coil α-helical domains, such as the myosin II heavy chain and intermediate filaments (30). The

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Fig. 1. Predicted structures of liprins. A, deduced amino acid sequence of human liprin-β1, human liprin-β2, and *C. elegans* celiprin-β. B, deduced amino acid sequence of human liprin-α2, human liprin-α3, human liprin-α4, and *C. elegans* celiprin-α. Sequences are aligned with the liprin-α1 (LIP.1) sequence; residues conserved in three out of the four sequences are shown with a black background. Numbers followed by a prime symbol (') indicate provisional amino acid numbering because the structures are incomplete. Underlined sequences are the LH domain sequences. C, the overall structures of α- and β-liprins, with their N-terminal, coiled coil regions and their C-terminal LH domains are schematically depicted.
APC colorectal tumor suppressor gene product also contains coiled coil domains, and APC is believed to mediate the attachment of cadherin/catenin complexes to the cytoskeleton (31). LIP.1 does not appear to be tyrosine-phosphorylated and hence is unlikely to be a PTPase substrate (24). Instead, LIP.1 likely anchors LAR at FAs where LAR may dephosphorylate FA-associated protein(s) to alter FA assembly and/or signaling. Thus, the LAR/LIP.1 complex may represent a matrix/cytoskeletal linkage that augments the actin/integrin and cadherin/catenin linkages by its intrinsic PTPase activity.

Herein we describe the identification and characterization of human and Caenorhabditis elegans LIP.1-related genes, which we have designated the liprin (derived from “LIP-related protein”) gene family. Based on sequence homology and binding properties, liprins are divided into α-liprins and β-liprins. α-Liprins, including LIP.1 (renamed liprin-α1) bind to the membrane-distal D2 PTPase domains of the LAR family PTPases, LAR, PTPδ, and PTPζ, whereas β-liprins bind to α-liprins but not to LAR family PTPases. Furthermore, both α- and β-liprins homodimerize via their N-terminal, coiled coil regions. Whereas some of the liprins have a broad mRNA tissue distribution, others are highly restricted, particularly to brain. Co-expression studies indicate that liprin-α2 alters the cellular distribution of LAR, supporting a role for liprins in localizing LAR family members to specific sites within the cell and creating specific linkages between the extracellular environment and the cytoskeleton.

**EXPERIMENTAL PROCEDURES**

**Interaction Trap Assay—** Plasmid DNAs and yeast strains used for the interaction trap assay were provided by Dr. R. Brent and colleagues (32) and used as described (24, 32). The human fibroblast cell WI-38 (ATCC CCL 75) cDNA library used for the interaction trap assay was kindly provided by Dr. C. Sardet. The various liprin and LAR-like PTPase regions fused to LexA or the B42 transcription activation domain are given in Tables I and II.

**cDNA Cloning and Plasmid Constructions—** Liprin cDNA clones were isolated from λgt11 human fetal brain, adult brain, heart, and kidney cDNA libraries (CLONTECH, Palo Alto, CA), as well as from the interaction trap fibroblast cDNA library, essentially as described (33). In the human expression sequence tag data base, two sequences were identified (accession nos. H08934 and H11896) that encode peptides with high sequence identity to liprin-α1 (83 and 73% identity over 77–102 amino acids, respectively). The initial 300-bp liprin-α2 and liprin-α3 cDNA probes used for library screenings were generated by reverse transcription polymerase chain reaction using kidney poly(A)+ mRNA and oligonucleotides derived from the human expression sequence tag sequences H08934 and H11896, respectively. Liprin cDNAs were sequenced by the dideoxy method. For COS cell transient transfections, liprin cDNAs were cloned into pMT.2 or pMT.HAtag, which encodes a hemagglutinin (HA) epitope tag sequence immediately up-
Liprins Potentially Mediate Localization of LAR-like PTPases

stream of the cloning site (24). pMT.Liprin-α1 encodes liprin-α1 amino acids 1–1202 (24); pMT.Liprin-α2 encodes liprin-α2 amino acids 1–1257; pMT.Liprin-β1 encodes liprin-β1 amino acids 1–1005; pMT.HA-Liprin-α1C (HA-Lo1A3C) encodes HA-tagged liprin-α1 amino acids 3–760; pMT.HA-Liprin-α2C (HA-Lo2A3C) encodes HA-tagged liprin-α2 amino acids 3–701; pMT.HA-Liprin-α2N encodes HA-tagged liprin-α2 amino acids 821–1257; pMT.HA-Liprin-β1C (HA-Lo1B3C) encodes HA-tagged liprin-β1 amino acids 1–1227; pMT.HA-Liprin-β1N encodes HA-tagged liprin-β1 amino acids 678–1005; pMT.HA-Liprin-β2A3N encodes HA-tagged liprin-β2 amino acids 257–783; pMT.LAR encodes amino acids 1–1881 (12); pMT.LAR-D1 encodes amino acids 1–1610 and pGST.LAR encodes LAR amino acids 1275–1881 fusing GST (20). The cell-path-α1 and cell-path-β coding sequences were obtained by others using GeneFinder (GenBank™ accession nos. Z50794 and Z78546, respectively). The 5′ promoter regions of celiprin-α and celiprin-β used to construct the green fluorescent protein (GFP) reporter plasmids pPD.celiprin-α-GFP and pPD.celiprin-β-GFP were generated by polymerase chain reaction using C. elegans cosmid DNA (clones F59F5 and T21H8, respectively, obtained from the Sanger Centre, Cambridge, United Kingdom) and inserted into the pDP95.67 plasmid, which encodes GFP and the SV40 nuclear localization signal sequence (kindly provided by Dr. Andrew Fire). The 5′ promoter region isolated for celiprin-α spans bp 29331–24665 of the C. elegans cosmid clone F59F5 (34), with the predicted initiation methionine codon located at bp 24762–24769 (35). The 5′ promoter region isolated for celiprin-β spans bp 11771–6365 of the C. elegans cosmid clone T21H8 (34), with the predicted initiation methionine codon located at bp 8760–8758. Sequence comparisons were done using the Wisconsin Package (version 8) software from Genetics Computer Group (Madison, WI).

The GenBank™ accession numbers for the liprins are as follows: human liprin-α2, AFO34799; human liprin-α3, AFO34800; human liprin-α4, AFO34801; human liprin-β1, AFO34802; and human liprin-β2, AFO34803. The accession numbers for the two liprin-α1 splice variants, liprin-α1a and liprin-α1b (previously designated L1a and L1b) are U22815 and U22816, respectively. The C. elegans celiprin-α and celiprin-β structures were derived from GenBank™ accession numbers Z50794 (F59F5.6) and Z78546 (T21H8.1), respectively. To generate anti-liprin-α1 and anti-liprin-β1 immunized mice were initially selected using enzyme-linked immunosorbent assay (ELISA) (35, 36). At least two independent extrachromosomal lines were established for each construct. GFP was observed in both the cytoplasm and nucleus for both constructs, despite the SV40 nuclear localization signal in the GFP vector. Adobe Photoshop was used to create “negatives” of black and white images originally captured using a Sensys camera and ImagePro Plus software.

C. elegans Expression Analysis—Animals were injected with plasmid pPD.celiprin-α-GFP and pPD.celiprin-β-GFP DNAs at 50 ng/μl using lin-15 as a marker (35, 36). At least two independent extrachromosomal arrays were examined for each construct. GFP was observed in both the cytoplasm and nucleus for both constructs, despite the SV40 nuclear localization signal in the GFP vector. Adobe Photoshop was used to create “negatives” of black and white images originally captured using a Sensys camera and ImagePro Plus software.

Monoclonal Antibodies—The anti-liprin-α1 (LIP.1) mAb anti-LIP.1.77 was described previously (24), as were the anti-LAR mAbs 11A, 75.3A, and 128.4A (12). The anti-α HAs 11A and 12CA5 were from Berkeley Antibody Co. (Richmond, CA) and the Harvard University mAb facility (Cambridge, MA), respectively. To generate anti-liprin-α2 and anti-liprin-β1 Abs, mice were immunized with purified, Schizosaccharomyces polispora-derived GST-Liprin-α2 (α amino acids 3–470) and GST-Liprin-β1 (α amino acids 63–446) fusion proteins (33). Hypoxanthine aminopterin/thymidine-resistant hybridomas derived from GST-Liprin-β1 immunized mice were initially selected using enzyme-linked immunosorbent assay and then by immunoprecipitation studies. The anti-liprin-α1 mAb thus obtained was termed anti-liprin-β1L.68.1 (IgG1). The anti-liprin-α2 polysera was obtained from mice immunized with the GST-Liprin-α2 fusion protein.

Cell Transfection Analysis—Cell proteins were metabolically labeled with [35S]methionine as described previously (24). Following labeling, cells were washed in PBS, lysed in Nonidet P-40 lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10 mM sodium fluoride. Insoluble material was removed from the lysates by centrifugation in a microcentrifuge. Cell lysates were then preclarified once with 25 μl of protein A-Sepharose slurry (Amersham Pharmacia Biotech) for 1–2 h. For immunoprecipitations, ~2 μg of anti-LIP.1.77 mAb (anti-liprin-α1), 3 μg of mouse anti-liprin-α2 serum, 100 μl of anti-liprin-β1L.68.1 hybridoma supernatant, 2 μg of control isotype-matched mAb, or 1 μl of ascites fluid of anti-HA mAb 11A and 25 μl of protein A-Sepharose slurry were added per ml of preclarified lysate for 2 h. Immunoprecipitates were then washed with buffer containing 0.1% Nonidet P-40, 0.05% SDS, 150 mM NaCl, and 50 mM Tris-HCl (pH 8.0). Immunoprecipitated proteins were analyzed using SDS-PAGE with reducing conditions followed by autoradiography (16–40 h).

Cell Transfections—COS-7 cell transient transfections were done by the DEAE-dextran/Me2SO method using 2 μg of plasmid DNA per 2 × 106 cells per 9-cm2 dish, and cells were harvested ~20 h after transfection (33). Proteins were metabolically labeled with [35S]methionine during the final 4 h prior to harvesting of cells.

Immunofluorescence—COS-7 cells were plated on glass coverslips 5 h following transfection with pMT.2-based expression plasmids and grown for ~20 h prior to staining. Cells were rinsed in PBS, fixed in 2% paraformaldehyde/PBS for 15 min, and then permeabilized for 10 min in 0.1% Triton X-100/PBS-containing 2% horse serum. Nonspecific antibody binding sites were blocked by a 30-min incubation in blocking buffer (10% normal goat serum in PBS). To detect liprin-α1 and liprin-β1, permeabilized cells were exposed to the anti-liprin-α1 mAb anti-LIP.1.77 mAb and the anti-liprin-β1L.68.1 mAb at a concentration of 2 μg of αLIP.1.77/ml blocking buffer and a 1:3 dilution of anti-liprin-β1L.68.1 hybridoma supernatant in blocking buffer for 1 h and washed, and the primary antibody was detected with a 30-min treatment of 1:1000 goat anti-mouse IgG2a-Texas red (Southern Biotechnology, Birmingham, AL) and 1:1000 goat anti-mouse IgG1-fluorescein isothiocyanate (Southern Biotechnology). To detect LAR and HA-tagged liprin-α2, permeabilized COS cells were exposed to a 1:11 mixture of the anti-LAR mAbs, 75.3A, 11.1A, and 128.4A at 2 μg/ml blocking buffer, and/or the anti-HA mAb 12CA5 at 0.5 μg/ml blocking buffer for 1 h and washed, and the primary antibody detected as above with a 30-min treatment of 1:1000 goat anti-mouse IgG2b-Texas red and 1:1000 goat anti-mouse IgG1-fluorescein isothiocyanate. Slides were mounted in a polyvinyl alcohol medium and viewed on a Olympus BX60 microscope equipped for epifluorescence. Photographs were taken on Kodak Ektachrome film.
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N-terminal, coiled coil regions and their C-terminal LH domains are schematically depicted for liprin-α1 and liprin-β1 in Fig. 1C.

The relatively high degree of sequence conservation between the mammalian α-liprins and C. elegans celiprin-α (50–58% identity), as well as the high degree of conservation between the mammalian β-liprins and C. elegans liprin-β (29–31% identity), indicates that liprins are evolutionarily conserved. Based on the predicted phylogenetic tree (Fig. 1D), it is likely that α- and β-liprins were derived from an ancestral gene by gene duplication prior to the divergence of chordates and nematodes.

Tissue-specific Expression of Human Liprins—The human LAR, PTPδ, and PTPσ PTPases have distinct, but overlapping, mRNA tissue distributions (20). For instance, all three mRNAs are expressed in brain, whereas only LAR and PTPσ mRNA are expressed in kidney and pancreas. To determine the tissue distribution of α- and β-liprin mRNAs, Northern blot analysis was performed using poly(A)+ RNA isolated from various human tissues and gene-specific cDNA probes (Fig. 2). The 5.3-kb liprin-α1 mRNA and the less abundant 6.8- and 4.0-kb liprin-α1 mRNAs are expressed in all eight samples (heart, lung, placenta, lung, liver, skeletal muscle, kidney, and pancreas), whereas the 6.5-kb liprin-α2 mRNA and the 5.0-kb liprin-α3 mRNA are present only in the brain sample. The 7.5-kb liprin-α4 mRNA is present only in the heart, brain, and skeletal muscle samples. Both the 7.0-kb liprin-β1 and the 3.9-kb liprin-β2 are broadly expressed, being present either in all eight samples (liprin-β2) or in seven of the eight samples (liprin-β1 is absent in the liver sample). In addition to the 3.9-kb liprin-β2 mRNA species, there is also a 5.1-kb species present in the heart and skeletal muscle samples. These results indicate that liprin mRNAs, like LAR family PTPase mRNAs, have distinct, but overlapping, expression patterns. The tissue restriction of liprin-α2 and liprin-α3 mRNA to brain suggests that these liprins may play specific roles in brain, perhaps in localizing PTPδ and PTPσ, which are both predominately expressed in the brain.

Expression of C. elegans Liprins—The expression of the C. elegans liprins celiprin-α and celiprin-β was examined using celiprin promoter driven expression of the GFP in transgenic nematodes (35). Two extrachromosomal array lines were characterized for each GFP reporter construct (Fig. 3). Celiprin-α-GFP expression is detected in vulval muscle and other cells near the vulva; in neurons located in the lateral ganglion, posterior ganglion, ventral cord, and lateral body; and in pharyngeal and body wall muscle cells (Fig. 3, A and B). Celiprin-β1-GFP expression is seen in pharyngeal muscle, particularly posterior bulb, adjacent to the dorsal and ventral cord (but not in ventral cord neurons), and in body wall muscles (Fig. 3, C and D). Overall, celiprin-α and celiprin-β expression appears predominately in neurons and muscle cells, with celiprin-α cells and celiprin-β being co-expressed in pharyngeal and body wall muscle but not in any other obvious regions.

![Fig. 4. Homo- and heterodimerization of liprins via their N-terminal, coiled coil regions.](http://www.jbc.org/)

**TABLE I**

| Bait          | Interactor      | Liprin-α1 (794–1202) | Liprin-α2 (821–1257) | Liprin-α3 (3–443) | Liprin-β1 (678–1005) | Liprin-β2 (257–783) | Control |
|---------------|-----------------|----------------------|----------------------|------------------|----------------------|----------------------|---------|
| Liprin-α1     | Liprin-α2       | 0                    | 0                    | 0                | 222                  | 667                  | 0       |
| Liprin-α2     | Liprin-α1       | 1                    | 5                    | 8                | 150                  | 667                  | 1       |
| Liprin-α3     | Liprin-β1       | 2                    | 0                    | 0                | 122                  | 521                  | 0       |
| Liprin-β2     | Liprin-β1       | 108                  | 442                  | 44               | 0                    | 0                    | 3       |

Protein-protein interaction between the C-terminal, non-coiled coil regions of α-liprins and β-liprins using the interaction-trap assay

Shown are β-galactosidase levels in liquid cultures for various pairs of liprin interaction-trap baits and interactors. Numbers in parentheses are shown. For each transfection five clones were analyzed for growth on Leu-deficient medium, as well as β-galactosidase activity on X-galactosidase-containing medium. Measurements of β-galactosidase levels in liquid cultures were done in duplicate, and the average values of β-galactosidase units are shown.
Liprins Homodimerize and Heterodimerize via Their N-terminal, Coiled Coil Regions—Previously, co-immunoprecipitation studies demonstrated that the N-terminal region of liprin-\(\alpha1\) (LIP1) dimerizes, consistent with the predicted coiled coil structure of this region (24). To determine whether the N-terminal regions of liprin-\(\alpha2\) and \(\beta1\) homodimerize or heterodimerize, full-length liprin-\(\alpha1\), \(-\alpha2\), or \(-\beta2\) was co-transfected into COS cells together with HA-tagged liprin C-terminal truncation (\(\Delta C\)) constructs encoding only the N-terminal, coiled coil region, but not HA-liprin-\(\beta1\Delta C\) (aa 1–227) co-precipitated full-length liprin-\(\alpha1\) (aa 1–1202) (Fig. 4, lanes 1–3). Similarly, HA-liprin-\(\alpha2\Delta C\) co-precipitated full-length liprin-\(\alpha1\) and \(-\alpha2\) but not \(-\beta1\) (Fig. 4, lanes 4–6).

Thus, the N-terminal regions of liprin-\(\alpha1\) and \(-\alpha2\) interact to form liprin-\(\alpha1\)/liprin-\(\alpha1\) and liprin-\(\alpha2\)/liprin-\(\alpha2\) homodimers, as well as liprin-\(\alpha1\)/liprin-\(\alpha2\) heterodimers, although it appears that \(\alpha\)-liprins preferentially homodimerize (Fig. 4; homodimerization is seen in lanes 1 and 5, whereas liprin-\(\alpha1\)/liprin-\(\alpha2\) heterodimerization is seen in lanes 2 and 4). HA-liprin-\(\beta1\Delta C\) co-precipitated full-length liprin-\(\beta1\) but not liprin-\(\alpha1\) or \(-\alpha2\), demonstrating that the liprin-\(\beta1\) N-terminal region homodimerizes (Fig. 4, lanes 7–9). These binding studies indicate that the liprin-\(\alpha2\) and liprin-\(\beta1\) N-terminal regions, like liprin-\(\alpha1\), form coiled coil structures. Furthermore, the ability of the coiled coil region of liprin-\(\alpha1\) to heterodimerize with liprin-\(\alpha2\) but not with liprin-\(\beta1\) indicates that liprin coiled coil regions within a subfamily may form homodimers, heterodimers, and/or higher order structures.

Interaction between the C-terminal Regions of \(\alpha\)-Liprins and \(\beta\)-Liprins—Liprin-\(\beta1\) and liprin-\(\beta2\) were identified in the interaction trap screen for liprin-\(\alpha1\)-binding proteins. Both the liprin-\(\alpha1\) bait and the original liprin-\(\beta1\) and liprin-\(\beta2\) interactors isolated contain only the C-terminal, non-coiled coil regions, demonstrating that liprin-\(\alpha1\)/liprin-\(\beta1\) and liprin-\(\alpha1\)/liprin-\(\beta2\) binding occurs via the C-terminal, non-coiled coil regions. To determine whether the C-terminal regions of liprin-\(\alpha2\) and \(-\alpha3\) also bind \(\beta\)-liprins, interaction trap assays were performed. Both the C-terminal, non-coiled region of liprin-\(\alpha2\) (aa 821–1257) and liprin-\(\alpha3\) (aa 3’–443’) bound the C-terminal region of liprin-\(\beta1\) and \(-\beta2\), indicating that a general property of \(\alpha\)-liprins is the ability to bind \(\beta\)-liprins through their C-terminal regions, which contain the LH domains (Table 1). The binding of \(\alpha\) and \(\beta\)-liprins was not observed in co-precipitation experiments using mammalian cells (data not shown). The reason for this lack of binding is unknown, but possibly the \(\alpha\) and \(\beta\)-liprin interaction is sensitive to the detergents present in the lysis buffer. Interaction of liprins via their C-terminal regions was restricted to \(\alpha/\beta\) interactions because the C-terminal regions of \(\alpha\)-liprin subfamily members did not bind other \(\alpha\)-liprins, and the C-terminal region of liprin-\(\beta1\) did not bind liprin-\(\beta2\) (Table 1). Taken together, these data indicate that the C-terminal, non-coiled coil regions of \(\alpha\)-liprins bind to the C-terminal regions of \(\beta\)-liprins.

To determine whether \(\alpha\) and \(\beta\)-liprins interact in vivo, COS cells were co-transfected with liprin-\(\alpha1\) and \(-\beta1\) expression vectors, and co-localization was assessed by immunofluorescence (Fig. 5). Liprin-\(\alpha1\) (Fig. 5A, green) and liprin-\(\beta1\) (Fig. 5A, red) co-localize predominantly to the plasma membrane of COS cells (Fig. 5C, double exposure of co-localized green and red results in yellow/orange). The localization of either liprin-\(\alpha1\) or \(-\beta1\) in singly transfected COS cells is similar to their localization in co-transfected cells (data not shown), indicating that localization of liprin-\(\alpha1\) or \(-\beta1\) to the membrane in COS
TABLE II
Protein-protein interaction between LAR family PTPases and α-liprins using the interaction trap assay

| Bait       | Liprin-α1 (784–1292) | Liprin-α2 (821–1257) | Liprin-α3 (3–443) | Liprin-β1 (678–1005) | Liprin-β2 (257–1531) | Control |
|------------|----------------------|----------------------|------------------|----------------------|----------------------|---------|
| LAR (1275–1881) | 140                  | 296                  | 282              | 0                    | 0                    | 0       |
| PTPδ (1624–1892) | 297                  | 804                  | 511              | 0                    | 0                    | 2       |
| PTPη (1586–1919)* | 165                 | 89                   | 102              | NT                   | 0                    | 0       |
| PTPμ (1189–1452) | 0                    | 0                    | 5                | 6                    | 0                    | 0       |
| CD45 (584–1281)    | 8                    | 0                    | 0                | 0                    | 0                    | 4       |

* For the PTPo-liprin interactions, PTPη (aa 1586–1919) was fused to the B42 transcription activation domain, and the liprin were fused to the LexA DNA binding domain. For each transfection, five clones were analyzed for growth on Leu-deficient medium as well as β-galactosidase activity on X-galactosidase-containing medium. Measurements of β-galactosidase levels in liquid cultures were done in duplicate, and the average values of β-galactosidase units are shown.

lysates prepared from [35S]methionine-labeled COS cells transiently expressing the liprin-α1, -α2, -β1, or -β2 (Table II). These results indicate that the C-terminal, non-coiled coil region of α-liprins but not β-liprins binds the C-terminal region of LAR family PTPases (Table II). The binding of liprin-α1 and -α2 to LAR, as well as the lack of binding of liprin-β1 or -β2 to LAR, was also observed in co-precipitation experiments using cell extracts prepared from [35S]methionine-labeled COS cells transiently expressing the liprin-α1, -α2, -β1, or -β2 C-terminal regions and GST-LAR fusion protein or control GST protein (Fig. 6). These results demonstrate that in addition to binding the C-terminal, non-coiled coil regions of β-liprins, the α-liprin C-terminal regions also bind the three mammalian LAR family PTPases (Table II). Furthermore, because β-liprins do not bind LAR family PTPases, the binding of LAR family PTPases by liprins is restricted to members of the α-liprin subfamily.

Liprin-α2 Expression Affects LAR Cellular Localization—To determine whether liprin-α2, like -α1, colocalizes with LAR in cells, transiently transfected COS cells were analyzed by immunofluorescence (Fig. 7). COS cells were transfected with various combinations of expression vectors encoding LAR, a LAR truncation mutant (LAR-D1) that lacks the α-liprin interacting D2 PTPase domain, and HA-liprin-α2. In cells transfected with LAR only, LAR (green) was uniformly distributed throughout the plasma membrane and Golgi (Fig. 7A), whereas in liprin-α2-only transfected cells, liprin-α2 (red) was observed in large plaque-like structures at the cell surface (Fig. 7B). In cells co-expressing LAR (green) and liprin-α2 (red), both proteins co-localized at the cell surface in plaque-like structures and to a lesser extent at ruffling edges (single exposures of the same field for LAR (Fig. 7C) and liprin-α2 (Fig. 7D)). The specificity of the LAR-liprin-α2 association is supported by the lack of significant co-localization of LAR-D1 with liprin-α2 (Fig. 7, single exposures of the same field for LAR-D1 (Fig. 7E) and liprin-α2 (Fig. 7F)). In contrast to the punctate expression pattern of LAR in the LAR/liprin-α2 co-expressing cells (Fig. 7C), the LAR-D1 expression pattern in the LAR-D1/liprin-α2 co-expressing cells (Fig. 7E) is similar to the expression pattern of LAR in the LAR-only transfected cells (Fig. 7A). Furthermore, the liprin-α2 expression pattern is similar in the liprin-α2-only transfected cells and in the LAR/liprin-α2 or LAR-D1/liprin-α2 doubly transfected cells (Fig. 7, B, D, and F), indicating that LAR expression does not alter liprin-α2 localization. Taken together, these results demonstrate that LAR and liprin-α2 co-localize in COS cells, and that the LAR membrane-distal D2 PTPase domain is required for co-localization and LAR clustering. Thus, liprin expression modifies LAR distribution.

DISCUSSION

We describe the liprins, a novel gene family that contains at least six mammalian and two C. elegans members. The overall predicted structure of α-liprins and β-liprins is an N-terminal, coiled coil region and a C-terminal, non-coiled coil region. This structure suggests that the liprin N-terminal regions interwine to form rod-like structures, similar to those seen in intermediate filaments and myosin II heavy chains (30). The prototype member of this family, LIP.1 (renamed liprin-α1), was previously identified as a LARPase-binding protein and is thought to function in anchoring LAR at FAs (24). Based on
sequence homology, as well as their binding to LAR family PTPases or to themselves, liprins are subdivided into α-liprins and β-liprins.

The characterization of the liprin genes suggests several general properties of liprins: 1) the N-terminal, coiled coil region of α-liprins mediates homodimerization, as well as heterodimerization with other α-liprin subfamily members. The coiled coil region of β-liprins also allows for homodimerization and possibly for heterodimerization with other β-liprins. The coiled coil regions of α- and β-liprins do not heterodimerize. 2) α-Liprins and β-liprins interact via their C-terminal LH domains. However, α-LH domains do not bind other α-LH domains, and β-LH domains do not bind other β-LH domains. 3) Only α-LH domains, not β-LH domains, bind to the membrane-distal D2 domain of LAR family PTPases.

The ability of the N-terminal, coiled coil regions to form α/α or β/β dimers and the C-terminal LH domains to form α/β dimers suggests that liprins are multivalent proteins that form complex structures. Such structures could function as scaffolds for the recruitment and anchoring of LAR family PTPases. For instance, in tissues such as brain, in which all the known α- and β-liprins are expressed, as well as LAR, PTPβR, and PTPε, the potential complexities of the interactions between liprins and PTPases are substantial. Distinct combinations of α- and β-liprins may determine where in the plasma membrane particular PTPases are located and determine the protein composition of the liprin/PTPase complexes. It is unknown whether α-liprins can simultaneously bind LAR family PTPases and β-liprins or whether the PTPases and β-liprins compete for α-liprin binding. LAR family PTPases bound to α-liprins may also be brought together with other liprin-binding proteins by liprin dimerization.

C. elegans liprins may function in a manner similar to mammalian liprins in LAR family PTPase signaling. Indeed, there exists a LAR-like gene in C. elegans (CECO9D8–1/2; GenBankTM accession number Z46811) that is predicted to contain an extracellular region composed of Ig-like and fibronectin III-like domains, connected via a transmembrane peptide to an intracellular region with two PTPase domains. However, because the expression pattern of celiprin-α and -β is only partially overlapping, it is unclear whether celiprin-α and -β interaction is essential for liprin function in C. elegans. If liprin function, at least in part, depends on the interaction of α- and β-liprins, then one would assume that there are additional C. elegans liprins or proteins that functionally substitute for liprins. Alternatively, the function of C. elegans liprins does not require α/β association.

Based on the binding properties of liprins, we postulate that liprins recruit LAR family PTPases to specific areas within the plasma membrane, as well as facilitating the recruitment/anchoring of other signaling proteins. A role for α-liprin in localizing LAR family PTPases within the cell was initially indicated by the co-localization of LAR and liprin-α1 to discrete ends of FAs (24). Such a role for other liprins is supported by the altered cellular distribution of LAR in LAR/liprin-α2 coexpressing COS cells. In singly transfected cells, LAR is expressed homogeneously throughout the plasma membrane, whereas liprin-α2 is distributed into large plaque-like structures and to the cell edges. In doubly transfected cells, LAR is redistributed to the liprin-α2 aggregates, indicating that lip-
Liprins Potentially Mediate Localization of LAR-like PTPases

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