Proteome of Acidic Phospholipid-binding Proteins

SPATIAL AND TEMPORAL REGULATION OF CORONIN 1A BY PHOSPHOINOSITIDES

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Reversible interactions between acidic phospholipids in the cellular membrane and proteins in the cytosol play fundamental roles in a wide variety of physiological events. Here, we present a novel approach to the identification of acidic phospholipid-binding proteins using nano-liquid chromatography-tandem mass spectrometry. We found more than 400 proteins, including proteins with previously known acidic phospholipid-binding properties, and confirmed that several candidates, such as Coronin 1A, mDia1 (Diaphanous-related formin-1), PIR121/CYFIP2, EB2 (end plus binding protein-2), KIF21A (kinase family member 21A), eEF1A1 (translation elongation factor 1α1), and TRIM2, directly bind to acidic phospholipids. Among such novel proteins, we provide evidence that Coronin 1A activity, which disassembles Arp2/3-containing actin filament branches, is spatially and temporally regulated by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂). Whereas Coronin 1A co-localizes with PI(4,5)P₂ at the plasma membrane in resting cells, it is dissociated from the plasma membrane during lamellipodia formation where the PI(4,5)P₂ signal is significantly reduced. Our in vitro experiments show that Coronin 1A preferentially binds to PI(4,5)P₂-containing liposomes and that PI(4,5)P₂ antagonizes the ability of Coronin 1A to disassemble actin filament branches, indicating a spatiotemporal regulation of Coronin 1A via a direct interaction with the plasma membrane lipid. Collectively, our proteomics data provide a list of potential acidic phospholipid-binding protein candidates ranging from the actin regulatory proteins to translocation regulators.

Cytosolic proteins that directly bind to acidic phospholipids, such as phosphatidylserine (PS)² and poly-phosphorylated phoshoinositides, play important roles in fundamental membrane-associated processes such as signal transduction, membrane trafficking, and actin cytoskeletal regulation (1, 2). These proteins can specifically recognize acidic phospholipids via their lipid-binding modules exemplified by the following: conserved region-2 (C2); pleckstrin homology (PH); Fab1, YOTB, Vac1, EEA1; epsin N-terminal homology; Phox homology; and Bin/amphiphysin/Rvs domains, or via relatively short amino acid sequences rich in basic residues as seen in actin-binding proteins (ABPs) (3–5). In particular, there is evidence that the activities of ABPs, such as Gelsolin, Profilin, Capping protein, and Cofilin, are regulated by phosphoinositides, especially phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) (5–8). In vitro and structural studies showed that actin-binding sites of these ABPs are overlapped with PI(4,5)P₂-binding sites and that the activity of ABPs is inhibited by PI(4,5)P₂ (6–8). Therefore, the activity of ABPs have thought to be inhibited by PI(4,5)P₂ binding that occludes F-actin-binding sites and to be activated by PI(4,5)P₂ hydrolysis when phospholipase C (PLC) is activated by receptor stimulation (5). Indeed, recent in vivo study have reported that Cofilin, an actin-severing protein, is inactivated at the plasma membrane in complex with PI(4,5)P₂ instead of F-actin in resting cells (9).

Although most of lipid-binding domains and proteins have been mainly discovered by the functional analyses, a comprehensive identification of lipid-binding proteins using proteomic analysis may be an ideal approach to identify not only novel but unexpected lipid-binding proteins. Here, we have performed a proteomics approach using nano-liquid chromatography-tandem mass spectrometry combined with a liposome co-sedimentation method to identify potential acidic phospholipid-binding protein candidates from rat brain extracts. We identify more than 400 proteins, including several important proteins, such as Coronin 1A, mDia1 (Diaphanous-related formin-1), PIR121/CYFIP2, EB2 (end plus binding protein-2), KIF21A (kinase family member 21A), eEF1A1 (translation elongation factor 1α1), and TRIM2 (tripartite RING finger protein), that bind directly to acidic phospholipids.

Among such novel proteins, we investigate the role of lipid interaction by Coronin 1A. Coronins are actin-binding proteins involved in phagocytosis, chemotaxis, immune function, and

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Sf The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3, Videos 1–7, and Tables S1–S4.

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2 The abbreviations used are: PS, phosphatidylserine; PI(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; ABPs, actin-binding protein; AG, Azami-Green; PH, pleckstrin homology; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TIRF, total internal reflection fluorescence; PLC, phospholipase C; GST, glutathione S-transferase.

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lamellipodia formation (10–17). Coronin is believed to be a key regulator that contributes to actin disassembly (11, 17). Together, it has been proposed that Coronin 1B promotes actin disassembly via coordination between the activities of Arp2/3 complex and Cofilin for lamellipodia formation (13). Moreover, Coronin 1B disassembles the Arp2/3 complex from actin filament branches and promotes the turnover of actin networks in lamellipodia (14). However, the mechanisms of how the actin disassembly by Coronins is regulated are poorly understood. In this study, we provide evidence that Coronin 1A activity is spatially and temporally regulated by PI(4,5)P2.

EXPERIMENTAL PROCEDURES

Reagents—Phospholipids were purchased as follows: PE, PC, cholesterol, and PS were obtained from Avanti Polar Lipids; phosphatidic acid and acidic lipids (Folch fraction 1) were obtained from Sigma; phosphoinositides were from Cell Signaling Technology; ionomycin, U73122, U73343, and rapamycin were from Sigma; mouse anti-hemagglutinin antibody was from Cell Signaling; Alexa Fluor 568-conjugated sec- ondary antibody and Alexa Fluor 647-conjugated phalloidin were from Invitrogen; and rhodamine-conjugated actin was from Cytoskeleton.

Preparation of Acidic Phospholipid-binding Proteins—Rat brain extracts were obtained by homogenization using buffer A (50 mM Hepes-NaOH, pH 7.4, 100 mM NaCl). After centrifugation at 600 × g to remove intact cells and nuclei, the extracts were centrifuged at 200,000 × g for 1 h at 4 °C in a TL100 rotor (Beckman) to obtain supernatants (cytosol fraction). The resulting pellets were resuspended with a high salt buffer (50 mM Hepes-NaOH, pH 7.4, 1 M NaCl) and centrifuged at 200,000 × g for 1 h. Obtained supernatants (membrane fraction) were dialyzed with buffer A overnight.

Mass Spectrometry and Protein Identification—The proteins in each gel slice were digested into peptides and extracted from the gel piece as described previously (18). After application of the peptide mixture to a C-18 column (800 mm long), reversed phase separation of the captured peptides was done on a column (150 mm inner diameter × 3 mm long), reversed phase separation of the captured peptides was done on a column (150 μm inner diameter × 75 mm long) filled with HiQ sil C18 (3-μm particles, 120-Å pores, KYA Technologies) using a direct nanoflow liquid chromatography system (Dina, KYA Technologies). The peptides were eluted with a linear gradient of acetonitrile containing 0.1% formic acid over 110 min at a flow rate of 200 nl/min and sprayed into a quadropole time-of-flight tandem mass spectrometer (Q-Tof-2, Micromass), according to the previous report (19). The tandem mass spectrometry signals were converted to text files using MassLynx (version 3.5, Micromass) and processed against the RefSeq (NCBI) rat protein database (35,936 sequences; as of April 2, 2007) using the Mascot algorithm (version 2.2.04, Matrix Science) with the following parameters: variable modifications, oxidation (Met), N-acetylation, pyroglutamination (Gln); maximum missed cleavages, 1; peptide mass tolerance, 500 ppm; tandem mass spectrometry tolerance, 0.5 Da. Protein identification was based on the criteria of at least one tandem mass spectrometry data with Mascot scores that exceeded the thresholds (p < 0.05).

DNA Constructs and Protein Expression—Human Coronin 1A, PIR121 (1–200, 1–400, and 1–600 amino acids), the tail domain of KIF21A (1325–1674 amino acids), EB2, eEF1A1, TRIM2, and mouse mDia1 (66–570 and 744–1255 amino acids) were obtained by reverse transcription-PCR. These sequences were verified and subcloned into pGEX 6P-1 and -3 (GE Healthcare). Coronin 1A were also subcloned into humanized monomeric Azami Green vector (AG; MBL). pCMV-HA 2×PLC81 PH domain was prepared as described previously (20) and was subcloned into monomeric tag red fluorescent protein vector (Evrogen) and pGFP C-1 vector (Clontech). pGEX-VCA of N-WASP was described previously (21). PM-FRB-CFP and mRFP-FKBP-5-pase domain were provided by T. Balla (NICHD, Bethesda, MD). Mutagenesis was performed by PCR with mutated primers. GST fusion proteins were expressed in BL21 pLys strain and were purified using standard protocols. The GST tag of Coronin 1A was removed by PreScission Protease (GE Healthcare). Other GST fusion proteins were eluted by glutathione and dialyzed.

Liposome and F-actin Co-sedimentation Assay—Liposome co-sedimentation assay was performed as described previously (22). Acidic lipids (total bovine brain lipids (Folch fraction 1; Sigma)) were suspended at 1 mg/ml in a binding buffer (25 mM Hepes, pH 7.4, 100 mM NaCl, and 1 mM dithiothreitol), and liposomes were formed by vortex or sonication followed by hydration. Purified proteins (5 μg) were incubated with liposomes for 15 min at room temperature and were centrifuged at 60,000 rpm for 20 min at 25 °C in the TL100 rotor. Obtained supernatants and pellets were subjected to SDS-PAGE, and the gels were stained with Coomassie Brilliant Blue. Liposomes with a defined maximum diameter were made by extrusion of liposomes through polycarbonate filter membranes (Avanti Polar Lipids). The F-actin co-sedimentation assay was performed as described previously (21).

Transfection and Microscopy—COS-1 cells and B16 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Transfection was performed using Lipofectamine LTX (Invitrogen) according to the manufacturer's protocol. For live cell imaging, cells were grown on poly-L-lysine-coated 35-μm glass bottom dishes and imaged at room temperature. Fluorescent images were taken through a microscope with a confocal microscopy system (FV1000, Olympus).

In Vitro Actin Branch Formation Assay with Total Internal Reflection Fluorescence Microscopy—Actin branching assay with total internal reflection fluorescence (TIRF) microscopy was performed as described previously (23). Briefly, glass cover slips were incubated with 0.2 mg/ml N-ethylmaleimide-inactivated myosin to allow actin filament binding. Formation of actin branches was initiated in the presence of 0.8 μM actin (40% rhodamine-labeled), 25 nM Arp2/3 complex (21), and 25 μM GST-VCA in freshly prepared TIRF buffer (20 mM imidazole, 20 mM Hepes-NaOH, pH 7.0, 50 mM KCl, 2 mM MgCl2, 2 mM EGTA, 10 mM dithiothreitol, 30 mM glucose, 400 μM ATP, 40 μg/ml catalase, and 200 μg/ml glucose oxidase). The images were acquired for 20 min at 30-s intervals with a TIRF microscopy system (Olympus) and analyzed using the MetaMorph software. PI(4,5)P2 liposome-bound Coronin 1A was prepared by incubating purified Coronin 1A with 100 μM liposomes con-
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RESULTS

Identification of Potential Acidic Phospholipid-binding Protein Candidates by Nano-liquid Chromatography-Tandem Mass Spectrometry—To better understand the role of acidic phospholipids with respect to the lipid-protein interactions, we conducted a proteomics analysis and comprehensively identified proteins that selectively interact with acidic phospholipids. To isolate acidic phospholipid-binding proteins, rat brain cytosol and membrane fraction were incubated with neutral liposomes (1 mg/ml) composed of PE (50%) and PC (50%) and then sedimented. These fractions were subsequently incubated with acidic liposomes (1 mg/ml) made from Folch fraction I (Folch lipids are rich in acidic phospholipids such as PS (~50% of total lipids) and phosphoinositides (~10% of total lipids)). After centrifugation, pellets were washed with buffer A and suspended with SDS sample buffer and subjected to nano-liquid chromatography-tandem mass spectrometry (Nano-LC-MS/MS). B, fractions of each step performed in A were subjected to SDS-PAGE and Coomassie Brilliant Blue staining. Sup and S, supernatant.

FIGURE 1. Procedure of identification of acidic phospholipid-binding protein candidates. A, schematic procedure of isolation and identification of acidic phospholipid-binding proteins. Rat brain cytosol and membrane fraction were incubated with neutral liposomes (1 mg/ml) composed of PE (50%) and PC (50%) and then sedimented. These fractions were subsequently incubated with acidic liposomes (1 mg/ml) made from Folch fraction I (Folch lipids are rich in acidic phospholipids such as PS (~50% of total lipids) and phosphoinositides (~10% of total lipids)). After centrifugation, pellets were washed with buffer A and suspended with SDS sample buffer and subjected to nano-liquid chromatography-tandem mass spectrometry (Nano-LC-MS/MS). B, fractions of each step performed in A were subjected to SDS-PAGE and Coomassie Brilliant Blue staining. Sup and S, supernatant.

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many previously identified proteins included those containing already established phospholipid-binding domains, such as C2, PH, Fab1, YOTB, Vac1, EEA1, Phox homology, epsin N-terminal homology, Bin/amphiphysin/Rvs, and F-BAR/EFC, and some proteins such as dynamins and EHD (Eps15 homology domain)-containing proteins that are known to oligomerize on acidic membranes (supplemental Table S3), supporting the validity of our approach. The newly found potential acidic phospholipid-binding protein candidates were summarized according to their suggested functions (supplemental Table S4). We subsequently selected several proteins and checked their ability to bind acidic phospholipids (Fig. 2B). It was revealed that Coronin 1A, PIR121/CYFIP2, and mDia1 (Diaphanous-related formin-1), all of which play important roles in dynamic actin-remodeling events, directly bound to acidic liposomes but not to neutral liposomes (Fig. 2C). Deletion experiments narrowed down the lipid-binding site to the Rac-binding domain in PIR121 and a region covering both the formin homology-2 domain and the donor-acceptor-donor motif in mDia1 (supplemental Fig. S1, A and B). In addition, it was found that two microtubule-associated proteins, end plus binding protein-2 (EB2) and KIF21A (kinesin family member 21A), have significant affinities for acidic liposomes (Fig. 2C). Interestingly, translation eEF1A1 (elongation factor 1α1) exhibited significant binding affinities to acidic liposomes (Fig. 2C). TRIM2, a ring finger-containing ubiquitin ligase (24), also bound specifically to acidic liposomes (Fig. 2C).

Coronin 1A Binds to PI(4,5)P₂—The strong interaction of Coronin 1A with acidic phospholipids (Fig. 2C) prompted us to investigate the role of this actin regulatory protein with respect to its phospholipid-binding function. First, we tested whether Coronin 1A shows curvature-dependent binding because it was found that Coronin 1A bound to large multilamellar vesicles

FIGURE 2. Identification of acidic phospholipid-binding proteins. A, classification of identified proteins (total number of proteins: cytosol fraction = 408; membrane fraction = 331). B, domain structures of new acidic phospholipid-binding proteins. C, liposome co-sedimentation assay. Proteins (5 μg) were incubated with synthetic PE/PC liposomes or acidic liposomes. Concentrations of lipids used for each proteins were as follows: Coronin 1A (0.2 mg/ml); PIR121 (0.5 mg/ml); mDia1 (0.5 mg/ml); KIF21A (0.5 mg/ml); EB2 (0.8 mg/ml); eEF1A (0.8 mg/ml); TRIM2 (0.4 mg/ml). After centrifugation, the supernatant (S) and the pellet (P) were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue. aa, amino acids.
(vortexed liposomes) but not small unilamellar vesicles (sonicated liposomes) (data not shown). Indeed, Coronin 1A exhibited high affinity for low curvature liposomes with diameters larger than 200 nm (Fig. 3, A and B), consistent with the fact that Coronin 1A is localized at the plasma membrane (25). Next, on phospholipid specificity analysis, it was revealed that Coronin 1A preferentially bound to PI(4,5)P₂ but not to other acidic phospholipids such as phosphatidic acid, PS, and phosphatidylinositol (PI) (Fig. 3, C and D). Among the poly-phosphorylated phosphoinositides, Coronin 1A exhibited the highest affinity

![Figure 3](https://example.com/figure3.png)
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for PI(4,5)P₂, whereas other D5-phosphorylated poly-phosphoinositides such as PI(3,5)P₂ and PI(3,4,5)P₃ were also preferred (supplemental Fig. S2A). It is well known that both PI(4,5)P₂ and PI(3,4,5)P₃ can target their binding proteins to the plasma membrane; however, the concentration of PI(3,4,5)P₃ is considerably lower than that of PI(4,5)P₂ in resting cells. Thus, we examined in COS-1 cells whether the plasma membrane localization of Coronin 1A is mediated by an interaction with PI(4,5)P₂. The elimination of PI(4,5)P₂ by the treatment with ionomycin, a Ca²⁺ ionophore and a potent activator of PLCs (26), resulted in the dissociation of AG-fused Coronin 1A from the plasma membrane (supplemental Fig. S3A and supplemental Video 1). Interestingly, the elimination of PI(4,5)P₂ by activation of PLC resulted in membrane protrusion, in which Coronin 1A was subsequently localized (supplemental Fig. S3A). By contrast, inhibition of PI(3,4,5)P₃ production by treatment with LY294002, a phosphatidylinositol 3-kinase inhibitor, did not alter AG-Coronin 1A localization (data not shown). In addition, AG-Coronin 1A showed a marked co-localization with PI(4,5)P₂, suggesting the possibility of plasma membrane interactions (Fig. 3E). Moreover, treatment with a PLC inhibitor, U73122, enhanced localization of AG-Coronin 1A at the plasma membrane, whereas a control reagent, U73343, did not (supplemental Fig. S3B). These data demonstrate that Coronin 1A localizes to the plasma membrane via PI(4,5)P₂ binding. We next predicted that like Cofilin (7), the lipid-binding site of Coronin 1A overlaps with the F-actin-binding site. To test this possibility, we mutated Arg-29 of Coronin 1A on the basis of a previously established finding that Arg-30 in Coronin 1B, which is a closely related isoform of Coronin 1A, is necessary for F-actin binding (27). As expected, it was found that R29E mutation of Coronin 1A led to a simultaneous reduction of both F-actin- and lipid-binding abilities (Fig. 3F and supplemental Fig. S2B). Another mutant of Coronin 1A (Coronin 1A ΔCC), which lacks the coiled-coil region and has been predicted to lose its F-actin binding (27), similarly showed reduced lipid-binding abilities (supplemental Fig. S2C). In this case, the coiled-coil region alone showed no affinity for liposomes (data not shown), indicating that the association of Coronin 1A with the membrane is enhanced by oligomerization.

PL(4,5)P₂ Reduction Induces a Translocation of Coronin 1A to the Extending Lamellipodia—A more recent study has demonstrated that Coronin 1B retains the ability to disassemble Arp2/3-containing actin filament branches and thus promotes their turnover for efficient lamellipodia formation (14). Although it is highly probable that this activity is strictly regulated in vivo, the precise mechanism remains poorly understood. We hypothesized that, like other ABPs such as Cofilin and Profilin (5), binding to PI(4,5)P₂ regulated the activity of Coronin 1A. To address this hypothesis, we first examined the localizations of Coronin 1A and PI(4,5)P₂ at the plasma membrane. We used a rapamycin-inducible system to reduce the PI(4,5)P₂ level at the plasma membrane, which utilizes rapamycin-induced heterodimerization of the membrane-targeted FRB domain and the FKBP12-tagged-5-ptide domain (28, 29). Upon addition of rapamycin, rapid reduction of PI(4,5)P₂ and concurrent formation of membrane protrusion were observed (Fig. 4A and supplemental Video 2), consistent with a recent finding that PLC-dependent PI(4,5)P₂ hydrolysis induced lamellipodia formation (9). This treatment again induced a translocation of Coronin 1A to the extending lamellipodia (Fig. 4B and supplemental Video 3). These observations suggest that Coronin 1A may be activated on PI(4,5)P₂ reduction and may subsequently promote lamellipodia formation to which it will localize.

Ability of Coronin 1A to Disassemble Arp2/3-containing Actin Filament Branches Is Inhibited by PI(4,5)P₂ Liposomes—We further investigated whether the actin branch disassembling activity of Coronin 1A, which is important for rapid turnover of actin network thus efficient lamellipodia formation, is
modulated by PI(4,5)P$_2$. TIRF microscopy was used to visualize the branch formation of rhodamine-labeled actin in live observations (14, 23). As expected, in the presence of Arp2/3 and its activator, the VCA region of N-WASP, extensive actin branch formation was observed (Fig. 5, A and B, and supplemental Video 4). Addition of Coronin 1A resulted in a smaller number of branches, indicating a strong disassembling activity (Fig. 5, A and B, and supplemental Video 5). Importantly, further addition of PI(4,5)P$_2$ to the system effectively reversed the branch disassembly, resulting in similar branch numbers as the control (Fig. 5, A and B, and supplemental Video 6). PI(4,5)P$_2$ by itself did not promote or inhibit actin branching (Fig. 5, A and B, and supplemental Video 6).
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and supplemental Video 7). These data suggest that the actin branch disassembling activity of Coronin 1A is regulated by a competitive mechanism, in which the F-actin-binding ability of Coronin 1A is hampered by PI(4,5)P₂, because these binding sites are overlapped (Fig. 3F and supplemental Fig. S2B).

DISCUSSION

Our powerful proteomics approach reveals that Coronin 1A, the critical regulator of branched actin network, is a novel phosphoinositide-binding protein. Although we cannot exclude the possibility that the both lipid and protein binding may be required for the plasma membrane localization of Coronin 1A, it was suggested that Coronin 1A binds directly to PI(4,5)P₂ at the plasma membrane as observed in vivo (Fig. 3C). Furthermore, the reduction of PI(4,5)P₂ caused the release of Coronin 1A from the plasma membrane (Fig. S3). In addition, time lapse observation with TIRF microscopy reveals that the ability of Coronin 1A, which inhibits F-actin disassembling activity, is antagonized by PI(4,5)P₂ liposomes. These data indicate that the spatiotemporal regulation of Coronin 1A is achieved by PI(4,5)P₂. A recent study has reported that Coronin 1B coordinates the activities of Arp2/3 complex and cofilin by recruiting Slingshot, which dephosphorylates and thus activates cofilin (13). In addition, Cofilin is inactivated at the plasma membrane by binding to PI(4,5)P₂ (9). In light of these findings, we propose a plausible mechanism to explain how the cooperated activities of these two actin-depolymerizing factors are spatially and temporally regulated by PI(4,5)P₂ (Fig. 5C). In this model, Coronin 1A localizes to the plasma membrane in resting cells together with Cofilin where they bind to PI(4,5)P₂ and are therefore inactivated. This model is also supported by a recent study suggesting that a constitutive targeting of Coronin 1B to the plasma membrane strongly enhances its phosphorylation (14), which negatively regulates Coronin activity by reducing its affinity for the Arp2/3 complex (12, 30). Upon growth factor stimulation, activated PLC hydrolyzes PI(4,5)P₂, thus inducing Coronin 1A and Cofilin translocations from the plasma membrane to the F-actin compartment of lamellipodia, where these molecules will be dephosphorylated by Slingshot. Consequently, this would enable them to promote actin branches disassembly spatially and temporally in a coordinated fashion between Arp2/3 complex dissociation and F-actin severance (Fig. 5C). In addition, Coronin 1A has been reported to form a complex with PLCγ1 and was implicated in Ca²⁺ mobilization (15). Our data suggest that Coronin 1A might locally modulate inositol 1,4,5-trisphosphate generation by connecting PLC to its substrate, PI(4,5)P₂.

Our proteomics identified more than 400 proteins that were precipitated with acidic phospholipids. Unexpectedly, we also noted that some typical acidic phospholipid-binding proteins like PLCδ1 were not found in the co-precipitated fractions. About 90 of the identified proteins are known acidic phospholipid-binding proteins or those with known acidic phospholipid-binding domains, such as PH, confirming the validity of our method. However, it should be noted that some proteins could be identified as results of nonspecific interactions with acidic phospholipids via merely their positively charged surfaces. In addition, it is difficult to discriminate between proteins that directly bind to acidic phospholipids and those in complex with such lipid-binding proteins. However, we believe that our proteomics data will be valuable tools as a useful and comprehensive list of potential acidic phospholipid-binding protein candidates for further validation of their lipid-binding abilities and biological significance.

Our proteomics approach revealed important cytoskeletal regulators such as mDia1, PIR121/CYFIP2, and EB2 to be new acidic phospholipid-binding proteins (Fig. 2C). In addition, KIF21A may recognize its cargo by interacting with acidic phospholipids in a manner that is similar to the mechanism reported for Unc104 (31). Moreover, interaction of eEF1A1 with acidic phospholipids is intriguing given its proposed function in local translation (32). In this regard, PIR121/CYFIP2 is thought to be probably involved in not only the WAVE complex actin nucleation machinery (33) but also in the translation machinery, because Sra1/CYFIP1, an isofrom of PIR121 (identified by our analysis [Table S4]), has been implicated in this process (34). Our data suggest that some translational regulators might be regulated by acidic phospholipids to spatiotemporally regulate translation (35).

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