Conserved Residues in the N Terminus of Lipin-1 Are Required for Binding to Protein Phosphatase-1c, Nuclear Translocation, and Phosphatidate Phosphatase Activity*

Bernard P. C. Kok, Tamara D. Skene-Arnold, Ji Ling, Matthew G. K. Benesch, Jay Dewald, Thurl E. Harris, Charles F. B. Holmes, and David N. Brindley

From the Signal Transduction Research Group, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2S2, Canada and the Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Background: Lipin-1 functions as a phosphatidate phosphatase in glycerolipid synthesis and as a co-transcriptional regulator. Lipin-1 contains conserved N-terminal motifs, which when mutated decrease phosphatase activity, nuclear localization, and binding to protein phosphatase-1cγ.

Results: Lipin-1 binds to protein phosphatase-1cγ through its N-terminal domain, and this potentially regulates lipin-1 localization and function.

Conclusion: The lipin-1 N-terminal domain is important in regulating its activities.

Significance: Lipin-1 binds to protein phosphatase-1cγ through its N-terminal domain, and this potentially regulates lipin-1 localization and function.

Lipin-1 is a phosphatidate phosphatase in glycerolipid biosynthesis and signal transduction. It also serves as a transcriptional co-regulator to control lipid metabolism and adipogenesis. These functions are controlled partly by its subcellular distribution. Hyperphosphorylated lipin-1 remains sequestered in the cytosol, whereas hypophosphorylated lipin-1 translocates to the endoplasmic reticulum and nucleus. The serine/threonine protein phosphatase-1 catalytic subunit (PP-1c) is a major protein dephosphorylation enzyme. Its activity is controlled by interactions with different regulatory proteins, many of which contain conserved RVXF binding motifs. We found that lipin-1 binds to PP-1cγ through a similar HVRF binding motif. This interaction depends on Mg^{2+} or Mn^{2+} and is competitively inhibited by (R/H)VXF-containing peptides. Mutating the HVRF motif in the highly conserved N terminus of lipin-1 greatly decreases PP-1cγ interaction. Moreover, mutations of other residues in the N terminus of lipin-1 also modulate PP-1cγ binding. PP-1cγ binds poorly to a phosphomimetic mutant of lipin-1 and binds well to the non-phosphorylatable lipin-1 mutant. This indicates that lipin-1 is dephosphorylated before PP-1cγ binds to its HVRF motif. Importantly, mutating the HVRF motif also abrogates the nuclear translocation and phosphatidate phosphatase activity of lipin-1. In conclusion, we provide novel evidence of the importance of the lipin-1 N-terminal domain for its catalytic activity, nuclear localization, and binding to PP-1cγ.

Lipins comprise a multifunctional, three-membered protein family involved in regulating glycerolipid synthesis, fatty acid metabolism, adipogenesis, and inflammatory signaling (1–4). Lipin-1 is the best characterized member of the mammalian family, followed by lipin-2. Lipins are predominantly cytosolic proteins that translocate to their sites of action in the endoplasmic reticulum and nucleus (5–13). These changes are dictated by a polybasic nuclear localization motif (6, 9, 14), which also promotes an electrostatic interaction with negatively charged phosphatidate, fatty acids, and acyl-CoA esters on the membrane surface (9, 14–17). Conversely, positively charged amphiphilic compounds, such as chlorpromazine and sphingosine, reverse this translocation (16, 18). Importantly, increasing the negative charge on the lipins through phosphorylation decreases their interactions with negative charges on the surfaces of membranes to control subcellular distribution and function (5, 6, 10, 19). This is demonstrated by the cytosolic localization of hyperphosphorylated forms of lipins, whereas hypophosphorylated lipins translocate to the nucleus and endoplasmic reticulum (5, 6, 11, 19). Additionally, 14-3-3 proteins bind to hyperphosphorylated lipin-1 to promote cytosolic sequestration (6).

Phosphorylation of lipin-1 is promoted by mTOR (mammalian target of rapamycin) complex 1, downstream of insulin signaling (5, 6, 20). Lipin-1 is also phosphorylated and inhibited by cyclin-dependent kinases during mitosis (7). On the other hand, less is known about the phosphatases responsible for dephosphorylating lipin-1. This is partly achieved by CTDNEP1 (C-terminal domain nuclear envelope phosphatase 1; previously known as Dullard phosphatase) and its regulatory partner NEP1-R1 (nuclear envelope phosphatase 1-regulatory subunit 1; previously known as TMEM188) (10, 21, 22). Han et al. (10) demonstrated that overexpressing both components of this phosphatase complex in cultured cells increases the dephosphorylation of a proportion of overexpressed lipin-1 proteins as well as increasing the nuclear accumulation of lipin-1. However, this phosphatase complex...
localizes at the nuclear envelope and perinuclear region. At present, it is unclear whether the majority of cytosolic lipin-1 has to be targeted to the perinuclear region for dephosphorylation by the CTDNSPE1-NPEP1-R1 complex or if lipin-1 can also be dephosphorylated in the cytosol. Whereas the human genome encodes for over 500 Ser/Thr protein kinases, there are only a small number of Ser/Thr protein phosphatases to counteract the actions of these kinases (23). Of these phosphatases, protein phosphatase-1 catalytic subunit (PP-1c) is one of the major enzymes involved in the dephosphorylation of cellular proteins. PP-1c does not exist alone as a monomer within cells; instead, it binds to more than 180 identified regulatory subunits, forming hundreds of mutually exclusive holoenzyme complexes that regulate enzyme activity and subcellular localization (24–26). These regulatory subunits act as regulators of PP-1c function but can still be substrates for PP-1c-mediated dephosphorylation (23). The majority of PP-1c regulatory proteins interact with PP-1c via a conserved RVXF binding motif ((K/R){X}{V/I/L}X(F/W), where X can be any amino acid except proline) (23). Mutation of the hydrophobic valine and phenylalanine positions within the RVXF motif abolishes the binding of PP-1c regulatory proteins to PP-1c (27–29).

Lipin-1 contains an RVXF-like motif, and we, therefore, hypothesized that lipin-1 should bind to PP-1c, and this could possibly regulate the subcellular localization of lipin-1 through controlling its level of dephosphorylation. This study provides novel evidence that lipin-1 interacts with the catalytic subunit of protein phosphatase-1γ (PP-1cγ) and that this interaction depends on the well-conserved HVRF motif present in the N terminus domain of lipins (NLIP) (Fig. 1). Mutation of the HVRF motif in the non-phosphorylatable lipin-1 mutant also completely prevents lipin-1 nuclear localization, which is surprising because the non-phosphorylatable mutant is well known to localize easily to the nucleus. Even more surprisingly, interaction of PP-1cγ with lipin-1 is stronger when lipin-1 is in its dephosphorylated state. Our results show that lipin-1 can be dephosphorylated by PP-1cγ and/or other cellular phosphatases (e.g. CTDNSPE1) before consequently interacting with PP-1cγ through the HVRF motif region. The conserved N-terminal domain of lipin-1 is also important for the actions of lipin-1 as a phosphatidate phosphatase and for its nuclear localization.

**EXPERIMENTAL PROCEDURES**

Materials—Microcystin-LR was obtained from Enzo Life Sciences (Farmingdale, NY). Microcystin-Sepharose (MC-Sepharose) resin was prepared by China Peptides Co. (Shanghai, China). Lipase from Rhizopus nigricans (EC 3.1.1.1) was purchased from Sigma-Aldrich. Microcystin-Sepharose, affinity-purified bovine serum albumin (BSA), and protease inhibitors (EDTA, phenylmethylsulfonyl fluoride) were purchased from Sigma-Aldrich. Polyjet™ transfection reagent (SignaGen Laboratories, Gaithersburg, MD) was used according to the manufacturer’s instructions unless HEK 293 cells were transfected for confocal microscopy studies. In this case, 67% of the normal amount of plasmid was used to lower the transfection efficiency. HEK 293 cells overexpressing recombinant lipin-1 proteins were sonicated in 25 mM HEPES, pH 7.4, containing 250 μM MgCl₂.
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mm sucrose, 2 mm DTT, protease inhibitor mixture (Sigma-Aldrich), 1 mm MnCl₂, 30 mm microcystin-LR, and 0.1% (w/v) Tween 20. Expression of lipin-1 mutant proteins was determined by spotting different amounts of the cell lysates (0.1–1 µg) onto a nitrocellulose membrane. After drying, the membranes were blocked with Odyssey®-PBS (1:1, v/v) for 1 h at room temperature, washed with PBS containing 0.1% (w/v) Tween 20 (PBST), and incubated for 1 h at room temperature with mouse anti-FLAG or mouse anti-HA (diluted 1:2,500 and 1:2,000, respectively) in Odyssey® blocking buffer-PBS (1:1, v/v) containing 0.1% (w/v) Tween 20. After washing with PBST, the blot was incubated for 1 h at room temperature with Alexa Fluor® 680-conjugated goat anti-mouse IgG (Invitrogen) diluted 1:10,000 in Odyssey® blocking buffer-PBS (1:1, v/v) containing 0.1% (w/v) Tween 20 and 0.01% (v/v) SDS. The blot was washed with PBST and once with PBS before scanning using the LI-COR Odyssey® imaging system (LI-COR Biosciences, Lincoln, NE).

Expression and Purification of PP-1cα and FLAG Lipin-1 Wild Type and HARA Mutant Proteins—Recombinant PP-1cα was expressed and purified as described previously (32). FLAG lipin-1 and mutant were expressed and purified using anti-FLAG affinity resin and FLAG displacement peptide (14) but without pretreating with any phosphatase. The eluted fractions of pooled lipins in 25 mM Tris-HCl, pH 7.4, 138 mM NaCl, and 1 mM MnCl₂ were concentrated using Amicon Ultra 0.5-ml centrifugal filter units (10,000 NMWL) (EMD Millipore, Bilherica MA) in a benchtop centrifuge at 15,000 × g for 20 min. Glycerol and DTT were added at final concentrations of 10% (v/v) and 2 mM, respectively. DTT was excluded in the circular dichroism experiments.

Microcystin-Sepharose Binding—Briefly, PP-1cα was bound to 25 µl of microcystin-Sepharose for 1 h at 4 °C (32). The resin was washed and incubated with HEK 293 cell lysate overexpressing FLAG lipin-1 wild type overnight at 4 °C. After washing resin with buffer containing 50 mM NaCl, bound protein was eluted with 2× SDS-PAGE sample buffer (65 mM Tris-HCl, pH 6.8, 26% glycerol (v/v), 2% (w/v) SDS, and 0.1% (w/v) bromphenol blue) and by boiling at 100 °C for 5 min. Eluted proteins were analyzed by Western blotting.

Solid Phase Protein-Protein Binding Assays—Purified recombinant PP-1cα (3 µg, 81 pmol) was incubated in 150 µl of phosphate-buffered saline (PBS; 8.1 mM Na₂HPO₄, 1.1 mM KH₂PO₄, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) containing 2 mM DTT and 0.5 mM MnCl₂ using a 96-well µClear® black-coated, tissue culture-treated plate (Greiner Bio-One, Kremsmuenster, Austria). As controls, equivalent molar amounts (81 pmol) of bovine serum albumin (BSA) or potato acid phosphatase (Sigma-Aldrich) were bound to the wells instead of PP-1cα. Incubations were performed in the presence of microcystin-LR at a 3:1 molar ratio to recombinant PP-1cα. The plate was swirled at 40 rpm overnight at 4 °C. After washing with PBST, the wells were blocked with Odyssey® blocking buffer (LI-COR Biosciences) for 1 h at room temperature. Cell lysates overexpressing equal amounts of recombinant lipin-1 or mutants were incubated overnight at 4 °C. For the cation dependence experiments, MnCl₂ was excluded, and other cations were added. The wells were then treated with Odyssey® blocking buffer for 1 h at room temperature and washed with PBST, followed by overnight incubation with mouse anti-FLAG tag antibody or mouse anti-HA diluted 1:2,500 and 1:2,000, respectively, in Odyssey® blocking buffer-PBS (1:1, v/v) containing 0.1% (w/v) Tween 20. After washing with PBST, wells were incubated for 90 min at room temperature while swirling with Alexa Fluor® 680-conjugated goat anti-mouse IgG (Invitrogen) diluted 1:10,000 in Odyssey® blocking buffer-PBS (1:1, v/v) containing 0.1% (w/v) Tween 20 and 0.01% (v/v) SDS and scanned using the LI-COR Odyssey® imaging system.

Confocal Microscopy—Cultured HEK 293 cells were plated onto fibronectin-coated coverslips and transfected for 24 h. Cells were fixed using 4% (w/v) paraformaldehyde in 60 mM PIPES, 27 mM HEPES, 13 mM EGTA, 8.2 mM MgSO₄, pH 7.0, followed by methanol fixation at −20 °C. After washing with PBS, the cells were permeabilized using 0.3% (w/v) Triton X-100 in PBS and blocked in 1% (v/v) donkey serum in PBS containing 0.1% (w/v) Tween 20. Coverslips were incubated with primary antibodies diluted in blocking buffer (1:100 rabbit FLAG, 1:50 mouse pan-PP-1c, 1:100 rabbit HA) for 1 h at room temperature. Donkey anti-rabbit Alexa Fluor 488 and donkey anti-mouse Alexa Fluor 555 (Invitrogen) were used as the secondary antibodies. Transfected cells incubated with control rabbit IgG and mouse IgG₂b showed almost no background staining. Hoechst 33342 (Sigma-Aldrich) was used to stain nuclei. Coverslips were mounted onto microscope slides using ProLong Antifade mounting medium (Invitrogen), and confocal images were taken with a Leica TCS SP5 laser-scanning microscope. Lipin-1 subcellular localization was classified as described previously (6) (i.e. predominantly nuclear, predominantly cytoplasmic, or both nuclear and cytoplasmic). For quantification, three independent experiments were performed, and at least six representative fields of each lipin-1 protein were taken per experiment.

SDS-PAGE and Western Blots—SDS-PAGE and Western blotting were performed as described previously using 8% gels (33), and lipin-1 was detected with a C terminus antibody (5). Alexa Fluor® 680-conjugated goat anti-mouse IgG (Invitrogen) and IRDye® 800-conjugated goat anti-rabbit IgG (LI-COR Biosciences) were used as the secondary antibodies. Western blots were quantified using the LI-COR Odyssey® infrared system.

Assays of Phosphatidate Phosphatase (PAP) Activity—PAP assays were performed essentially as described previously (33).

Dephosphorylation Assay of 32P-Labeled Lipin-1 Wild Type and HARA Protein Using PP-1cα—HEK 293 cells in 15-cm dishes were transfected with FLAG lipin-1 wild type or HARA plasmids for 24 h and then incubated in phosphate-free DMEM (Invitrogen) containing 10% (v/v) FBS that was dialyzed for 6 h to deplete inorganic phosphate. The cells were then incubated in 20 ml of phosphate-free DMEM containing 10% dialyzed FBS, 100 nM insulin (to promote phosphorylation), and 2.5 µCi/ml [32P]orthophosphate (PerkinElmer Life Sciences). 32P-Labeled recombinant lipin-1 wild type and the HARA mutant were purified from the cell lysates as described above. Purified, recombinant PP-1cα was added at a 1:1 protein ratio, and the assay was conducted over 45 min in 25 mM Tris buffer, pH 7.4, containing 138 mM NaCl, 1 mM MnCl₂, 10% (w/v) glycerol, and 2 mM DTT. At each time point, a sample of the reaction was collected, and the reaction was quenched by boiling in 3× sam-

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ple loading buffer for 5 min. After gel electrophoresis and Western blotting, radioactivity was detected by autoradiography using a Biomax high energy transcreen for enhancement (Eastman Kodak Co.). Radioactivity was quantified by isolating the lipin-1 bands and measuring $^{32}$P with Cytoscint (Fisher) and scintillation counting.

**Far-UV Circular Dichroism Studies**—The J-720 spectropolarimeter (Jasco Inc., Easton, MD) was used for far-UV circular dichroism (CD) studies on equal amounts of purified FLAG-lipin-1 wild type and HARA mutant at 20 °C. Results were collected at 0.1 nm resolution with a scan speed of 50 nm/min from 250 to 190 nm.

**Statistics**—Results are expressed as means ± S.E. Statistical significance ($p < 0.05$) was calculated using the two-tailed Student’s $t$ test when two groups were analyzed. GraphPad Prism® 4 (La Jolla, CA) was used to calculate one-way analysis of variance, followed by the Newman-Keuls post hoc test or the Bonferroni test after two-way analysis of variance.

**RESULTS**

**Lipin-1 Interacts with Protein Phosphatase-1c through Its HVRF Motif**—Lipin-1 has two highly conserved domains: the NLIP and C-terminal lipin (CLIP) domains (34). The CLIP domain contains the PAP catalytic motif (DIDGT) and the transcriptional co-activator motif (LGHIL) (1–3). The function of the NLIP domain is less clear, although a point mutation (G84R) in mice produces the same phenotype as lipin-1-deficient fld mice (34); this mutation also abrogates PAP activity (5) and blocks lipin-1 nuclear localization (34). We noticed that lipin-1, -2, and -3 contain a potential PP-1c binding motif (His-Val-Arg-Phe, HVRF) in the NLIP domain, which is also conserved in the yeast lipin, Pah1p (Fig. 1). The NLIP domains are predicted by Jpred algorithms (35) to have an $\alpha$-helix at the beginning of the N terminus (Fig. 1). This matches the findings by Karanasios et al. (8), who showed that this $\alpha$-helix facilitates the binding of yeast Pah1p to phosphatidate on nuclear/endo-plasmic reticulum membranes. The $\alpha$-helix is followed by a predicted array of $\beta$-strand (Fig. 1).

Second, we expressed a series of lipin-1 mutants in HEK 293 cells to investigate how lipin-1 might interact with PP1c. We then quantified the expression of each of the mutants by dot blot analysis (Fig. 2). The recombinant lipin-1 wild type and mutant proteins were then normalized such that recombinant lipin-1 expression per mg of cell lysate was equal in all samples. To investigate whether lipin-1 can bind to PP-1c, we immobilized purified recombinant PP-1c on microcystin-Sepharose and showed that recombinant lipin-1, which was overexpressed in HEK 293 cell lysates, selectively attached to PP-1c (Fig. 3A).

To investigate whether lipin-1 can bind to PP-1c, we immobilized purified recombinant PP-1c on microcystin-Sepharose and showed that recombinant lipin-1, which was overexpressed in HEK 293 cell lysates, selectively attached to PP-1c (Fig. 3A). Second, we bound PP-1c to a 96-well plate in the presence of microcystin-LR and studied the attachment of lipin-1 while using bound albumin or acid phosphatase as controls. There was again a specific binding of lipin-1 to PP-1c (Fig. 3B and C). Lipin-1 binding to PP-1c depended on Mg$^{2+}$ ($K_d = 2.56 ± 0.73$ mm) (Fig. 3D). Mn$^{2+}$ ($K_d = 0.80 ± 0.23$ mm) could substitute for Mg$^{2+}$ (Fig. 3E). Ca$^{2+}$ was less effective (Fig. 3E), and Co$^{2+}$, Zn$^{2+}$, and Na$^+$ had no significant effect on binding (results not shown).

Short peptides derived from RVXF sequences can be used to determine the binding specificity of PP-1c to RVXF-containing
regulatory proteins (28, 29, 32, 36). These peptides include the ZAP wild type peptide (derived from the ZAP3 protein, also called YLP motif-containing protein 1) and its non-binding control RARA (RVRW mutated to RARA) (Fig. 4A). Preincubation of PP-1c with the wild type ZAP peptide blocked the interaction of lipin-1 to PP-1c in a dose-dependent manner.

FIGURE 2. Protein dot blots and Western blot of HEK 293 cell lysates overexpressing lipin-1 proteins. A, representative protein dot blots using 0.1–1 μg of protein from the HEK 293 cell lysates. The results show the expression of different FLAG-tagged lipin-1 proteins such as the catalytically inactive (D712E, D714E), non-phosphorylatable (215/T to A), and N-terminal point mutants of lipin-1. B, linear regression analysis of the different concentrations of lysates overexpressing lipin-1 proteins usually produce R² values between 0.97 and 0.99, and the slopes of each line were used to calculate relative lipin-1 expression. The expression of the different lipins was then equalized by diluting higher expressing lipin-1 proteins with control cell lysate. C, Western blot of the same proteins in Fig. 2A after normalization. The numbers denote the different lipin-1 proteins, as identified in A.

FIGURE 3. Lipin-1 binds to protein phosphatase-1. A, human embryonic kidney 293 (HEK293) cell lysate overexpressing FLAG-tagged recombinant lipin-1 wild type was incubated with recombinant PP-1c bound to microcystin-LR-linked Sepharose beads (MC-LR; second lane) or with microcystin-LR-linked Sepharose beads alone (first lane) in the presence of 1 mM MnCl₂. B, the interaction of HEK 293-overexpressed FLAG-tagged recombinant lipin-1 wild type with purified recombinant PP-1c, BSA, and potato acid phosphatase (81 pmol each) bound to 96-well black-walled, clear-bottomed plates. C, quantification of the interaction between overexpressed lipin-1 with purified PP-1c in Fig. 2B (n = 4). The background integrated intensity from the nonspecific binding of overexpressed lipin-1 to BSA was subtracted from the integrated intensity of lipin-1 wild type-PP-1c binding. D and E, quantification of the effect of increasing the Mg²⁺ (D) or Mn²⁺ or Ca²⁺ (E) concentration on the interaction of lipin-1 and PP-1c (n = 3). Other cations tested, such as Co²⁺, Zn²⁺, and Na⁺, did not promote the binding of lipin-1 to PP-1c. Background integrated intensity from the nonspecific binding of overexpressed lipin-1 to BSA was subtracted after quantification. Error bars, S.E.
Hypothetically, this interaction of PP-1c with lipin-1 might facilitate its dephosphorylation. To test this, the non-phosphorylatable mutant, in which 21 serine/threonine residues were mutated to alanine (21S/T to A) and the phosphomimetic (21S/T to E) mutant of lipin-1 were used in binding assays. Surprisingly, the 21S/T to E lipin-1 mutant bound poorly to PP-1c (Fig. 5A), whereas the 21S/T to A mutant had good binding affinity to PP-1c, similar to wild type lipin-1 (B_{max} = 210.9 \pm 35.5 \text{ versus } 137.6 \pm 6.0) (Fig. 5, A and B). It is important to note that the catalytically inactive lipin-1 mutant (D712E,D714E) mutant bound to the same extent as wild type protein (Fig. 5B). To determine the dependence of the lipin-1 HVRF motif on PP-1c binding, we also mutated other well conserved residues in the NLIP domain of the non-phosphorylatable lipin-1 mutant (Fig. 1) and tested the extent of their interactions with PP-1c. Several lipin-1 point mutations (21S/T to A V57A, L58A, and V64A) had no effect on PP-1c binding (Fig. 5C). However, double point mutations of lle-67 and lle-69 to alanines (DAEA mutant) caused a decrease in PP-1c binding similar to the HARA mutation. Two other lipin-1 point mutants (21S/T to A F87A and L80A) showed an intermediate binding phenotype. We also tested whether lipin-2 could bind to PP-1c and found that there was significant interaction although to a lesser extent compared with lipin-1 (Fig. 5D).

Importantly, the rate of dephosphorylation of lipin-1 by PP-1c is not altered, although binding through HVRF is decreased by mutation to HARA (Fig. 5E). Furthermore, about 40% of {sup}{sup}32P-labeled residues on both lipin-1 proteins were not readily dephosphorylated by PP-1c (Fig. 5F).

**Mutation of the HVRF Motif of Lipin-1 to HARA Blocks Nuclear Localization and Phosphatidate Phosphatase Activity—** We also investigated the effects of introducing the HARA mutation into the wild type lipin-1 protein on subcellular localization and function. The lipin-1 HARA mutant was predominantly cytosolic, whereas wild type lipin-1 was present in both cytoplasm and nucleus (Fig. 6, A and B). Significantly, the catalytic activity of lipin-1 does not dictate nuclear localization because inactivating the catalytic motif (by mutating Asp-712 and Asp-714 to Glu) in lipin-1 did not prevent nuclear localization (Fig. 6, A and B). Deletion of the NLIP domain containing the HVRF motif (321–2775 mutant) also resulted in nuclear exclusion and cytoplasmic localization (Fig. 6B). We also determined the effect of expressing the non-phosphorylatable 21S/T to A lipin-1 mutant or the 21S/T to E phosphomimetic mutant in HEK 293 cells (Fig. 6B), which were predominantly localized to the nucleus and cytosol, respectively, as expected (11). Significantly, there was no increase in nuclear PP-1c when the 21S/T to A lipin-1 construct was overexpressed (Fig. 7, A and B). It is also important to note that no lipin-1 nuclear localization was observed when the HARA mutation was introduced into the non-phosphorylatable 21S/T to A mutant of lipin-1 (Fig. 7, A and B). Point mutations (F87A, L80A, and DAEA) in the 21S/T to A form of lipin-1, which bound poorly to PP-1c (Fig. 5C), also had dramatic decreases in their nuclear localization (Fig. 7B). The 21S/T to A point mutants (V57A, L58A, and V64A) that showed no loss of binding to PP-1c had subcellular localization profiles similar to those of the 21S/T to A lipin-1 protein (Fig. 7B).

**FIGURE 4.** Preincubation with PP-1c-interacting peptides prevents the association of lipin-1 with PP-1c. A, the amino acid sequences of synthetic peptides known to compete against PP-1c regulatory subunits (ZAP WT peptide) as well as a short peptide of lipin-1 containing the HVRF motif thought to bind to PP-1c were made together with the non-interacting mutant controls. B, different concentrations of peptides were incubated with PP-1c bound to the 96-well plate for 8 h at 4°C. A constant amount of HEK 293 cell lysate expressing lipin-1 was then added into each well in the presence of 1 mM Mn2+ and found that there was significant interaction although to a lesser extent compared with lipin-1 (Fig. 5D).

Importantly, the rate of dephosphorylation of lipin-1 by PP-1c is not altered, although binding through HVRF is decreased by mutation to HARA (Fig. 5E). Furthermore, about 40% of {sup}{sup}32P-labeled residues on both lipin-1 proteins were not readily dephosphorylated by PP-1c (Fig. 5F).

**Mutation of the HVRF Motif of Lipin-1 to HARA Blocks Nuclear Localization and Phosphatidate Phosphatase Activity—** We also investigated the effects of introducing the HARA mutation into the wild type lipin-1 protein on subcellular localization and function. The lipin-1 HARA mutant was predominantly cytosolic, whereas wild type lipin-1 was present in both cytoplasm and nucleus (Fig. 6, A and B). Significantly, the catalytic activity of lipin-1 does not dictate nuclear localization because inactivating the catalytic motif (by mutating Asp-712 and Asp-714 to Glu) in lipin-1 did not prevent nuclear localization (Fig. 6, A and B). Deletion of the NLIP domain containing the HVRF motif (321–2775 mutant) also resulted in nuclear exclusion and cytoplasmic localization (Fig. 6B). We also determined the effect of expressing the non-phosphorylatable 21S/T to A lipin-1 mutant or the 21S/T to E phosphomimetic mutant in HEK 293 cells (Fig. 6B), which were predominantly localized to the nucleus and cytosol, respectively, as expected (11). Significantly, there was no increase in nuclear PP-1c when the 21S/T to A lipin-1 construct was overexpressed (Fig. 7, A and B). It is also important to note that no lipin-1 nuclear localization was observed when the HARA mutation was introduced into the non-phosphorylatable 21S/T to A mutant of lipin-1 (Fig. 7, A and B). Point mutations (F87A, L80A, and DAEA) in the 21S/T to A form of lipin-1, which bound poorly to PP-1c (Fig. 5C), also had dramatic decreases in their nuclear localization (Fig. 7B). The 21S/T to A point mutants (V57A, L58A, and V64A) that showed no loss of binding to PP-1c had subcellular localization profiles similar to those of the 21S/T to A lipin-1 protein (Fig. 7B).
We also determined the effect of mutating the HVRF motif to HARA on lipin-1 catalytic PAP activity. Unexpectedly, PAP activity was completely abrogated (Fig. 8), although the mutations of valine and phenylalanine to alanine residues are relatively conservative. We also determined the PAP activities of the different lipin-1 point mutants and found that the DAEA double point mutant had minimal PAP activity, similar to the HARA mutation (Fig. 8). This also corresponds with the lack of PP-1c binding (Fig. 5) and loss of nuclear localization (Fig. 7).

Loss of catalytic activity can result from gross protein misfolding. Therefore, we affinity-purified wild type lipin-1 and the HARA mutant using the FLAG tag but could find no significant difference in the far-UV circular dichroism spectra (Fig. 9). We conclude that the HARA mutation did not cause gross misfolding.

DISCUSSION

The subcellular localization of lipins is a key factor in controlling their functions in regulating glycerolipid synthesis, cell signaling, and transcriptional regulation. Our group demonstrated the ability of Mg2+/H11001-dependent PAP activity (now attributed to the lipins) to translocate from the cytosol onto the membranes of the endoplasmic reticulum when stimulated with unsaturated fatty acids due to the increase in negative charge on the membrane surface (15–17, 37). This was essentially corroborated after the lipins were discovered to be responsible for PAP activity (4, 5, 9). Since 2007, several groups have shown that the association of lipins with the endoplasmic reticulum and nucleus is decreased by the introduction of negative charges caused by hyperphosphorylation (5–7, 9, 11, 14). Some of this work and the present study depend on the use of phosphomimetic mutants. We recognize that although this is a valuable approach, the results need to be interpreted with caution (38).
We demonstrate that lipin-1 and lipin-2 interacted with PP-1c in a Mg\(^{2+}\)-dependent manner. Mn\(^{2+}\) was equally effective compared with Mg\(^{2+}\) for the binding of lipin-1 to PP-1c, whereas Ca\(^{2+}\) was less effective. Lipin-1 and PP-1c both require Mg\(^{2+}\) or Mn\(^{2+}\) for their respective catalytic activities (23, 39, 40). Moreover, the interaction of lipin-1 with PP-1c is mediated through a conserved HVRF motif on lipins, which closely resembles the canonical RVXF motif present on all PP-1c regulatory proteins. It is important to note that only a small proportion of endogenous lipin-1 would be likely to interact with PP-1c, given that there are hundreds of PP-1c binding partners in the cell. Also, the binding of any PP-1c regulatory protein would preclude other protein interactions using the same RVXF-like motifs.

We had initially hypothesized that PP-1c would bind to phosphorylated forms of lipin-1 and subsequently dephosphorylate the lipin-1 proteins, thereby facilitating their subcellular localization to the nucleus and endoplasmic reticulum. However, the wild type lipin-1 and the HARA mutant were dephosphorylated at the same rate by PP-1c, and phosphorylated lipin-1 (in the form of the phosphomimetic mutant) appeared to bind more poorly to PP-1c. Although such mutants provide important information about the lipin-1 and PP-1c interaction, there are limitations to the interpretation of the results obtained from phosphomimetic mutant proteins (38). PP-1c is known to efficiently dephosphorylate lipin-1 (e.g. on serine 106) (5), but not all of the 21 phosphorylation sites are necessarily accessible. This conclusion is compatible with the observation that a significant proportion of the phosphorylation sites on lipin-1 remained intact when incubated with only PP-1c. These remaining sites could be the substrates for other phosphatases, including CTDNEP1 (10, 21, 22). It is also significant that this phosphatase and its regulatory subunit can only partially dephosphorylate lipin-1 (10). A further explanation is that another PP-1c binding partner would facilitate complete dephosphorylation of lipin-1 by PP-1c because untargeted PP-1c phosphatase activity does not occur physiologically.
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We also showed that non-phosphorylatable lipin-1 localized to the nucleus and that this localization was impaired when HVRF was mutated to HARA. This result could imply that binding of PP-1c to lipin-1 and lipin-1 dephosphorylation subsequently facilitates entry of lipin-1 into the nucleus. Interestingly, the lipin-1 HARA mutant has a lower electrophoretic mobility on the Western blot than the wild type protein (Fig. 2C). This could be due to the SUMOylation of lipin-1 wild type, which can localize to the nucleus, whereas the lipin-1 HARA cannot. It could also be a result of increased dephosphorylation of lipin-1 HARA proteins in an effort to promote nuclear localization. However, all forms of the lipin-1 HARA mutant remain cytoplasmic; therefore, regulation of lipin-1 HARA proteins by phosphorylation to contain them in the cytoplasm is not required.

We also established that there are secondary sites of interaction in the lipin-1 NLIP domain that mediate PP-1c binding, which is also seen with other PP-1c binding partners (23, 25, 41). The closest phosphorylation site that is modified in the lipin-1 21S/T to A mutant is very close to the edge of the NLIP domain (serine 106). Mutation of this site alone did not affect lipin-1 activity or subcellular localization (results not shown). However, it is possible that there are serine/threonine residues on lipin-1 that can modulate PP-1c binding when phosphorylated. For example, there are three serines in the NLIP domain (serine 10). Mutation of these sites decreases PAP activity, membrane association, and triacylglycerol synthesis (42, 43). This is similar to the deleterious effects found with some of the NLIP mutants. Moreover, cyclin-dependent kinase phosphorylation of lipin-1 and -2 during cell mitosis also decreases PAP activity and membrane association (7). This suggests that phosphorylation of unidentified serine/threonine residues in lipin-1 by protein kinase A or cyclin-dependent kinases would recapitulate the effects seen in yeast Pah1p on PAP activity and subcellular localization. These could also play a role in lipin-1 interaction with PP-1c.

We could not detect a significant change in the translocation of PP-1c from the cytoplasm to the nucleus even when we overexpressed the lipin-1 21S/T to A mutant. This could be expected if lipin-bound PP-1c only contributes a small proportion of the nuclear PP-1c. However, other nuclear-localized PP-1c regulatory proteins, such as Ikaros, do promote nuclear localization of PP-1c when overexpressed (44). Perhaps PP-1c could facilitate lipin-1 nuclear entry but is not itself imported into the nucleus with lipin-1. Alternatively, PP-1c could be shuttled into the nucleus with lipin-1 but be readily exported from the nucleus. This could possibly occur through interactions with other nucleus-localized PP-1c binding partners while lipin-1 remains in the nucleus. However, we cannot rule out the possibility that the mutations of conserved amino acids in the NLIP domain prevent nuclear entry independently of the effects on the binding of lipin-1 to PP-1c.

The HVRF motif of lipin-1 is very important for the functions of lipin-1 because its mutation to HARA abolishes not only nuclear localization but also the PAP activity (Fig. 10). Also, we could not detect any changes in the PAP activity of lipin-1 wild type in the presence of PP-1c (results not shown). Furthermore, PP-1c interaction is not required for lipin-1 PAP activity because recombinant human lipin-1 purified from Escherichia coli retains its PAP activity, and E. coli do not possess a PP-1c orthologue (40). Importantly, nuclear exclusion and the loss of PAP activity cannot be explained by gross conformational changes in lipin-1. However, there were small changes in the predicted secondary structure when the HARA mutation was introduced (Fig. 1), which suggests that these minor structural changes could be an explanation for the loss of PAP activity. Harris et al. (5) had previously demonstrated that the conserved N terminus is essential for providing catalytic activity because the G84R point mutation in mouse lipin-1 abrogates PAP activity by 75%. This mutant has also been shown to be excluded from the nucleus (34), which mirrors the point mutants we have generated in this study. Removing the majority of the lipin-1 NLIP domain also eliminates PAP activity (5).
In summary, we have identified several conserved sites in the NLIP domain (HVRF, DIEI) of lipin-1 that are absolutely required for facilitating binding to PP-1c, maintaining PAP activity, and promoting nuclear localization. Two point mutants of conserved residues (L80A and F87A) are also affected in these three parameters, but their phenotypes are intermediate, whereas other conserved residues (e.g. Val-57 and Val-64) in the NLIP domain do not affect any of the phenotypes tested. Conceptually, our results with the lipin-1 HARA mutant could still bind to PP-1c and was localized to the nucleus (Fig. 10). Previous work demonstrated that PAP catalytic activity does not dictate nuclear lipin-1 localization (9, 11). Therefore, exclusion of the lipin-1 HARA mutant from the nucleus cannot be explained by the loss of catalytic PAP activity.

Overall, we have established a novel physical interaction between lipin-1 and PP-1c. This binding depends on an HVRF motif and several other amino acids that are conserved in the NLIP domain of mammalian lipins and yeast Pah1p. Mutations of these conserved residues also decrease PAP activity and nuclear entry of lipin-1. More extensive studies are required to elucidate how the interaction of lipins with PP-1c controls the subcellular distributions and physiological functions of these two classes of proteins.

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REFERENCES

1. Kok, B. P., Venkatraman, G., Capatos, D., and Brindley, D. N. (2012) Unlike two peas in a pod: lipid phosphate phosphatases and phosphatidate phosphatases. *Chem. Rev.* 112, 5211–5246
2. Harris, T. E., and Finck, B. N. (2011) Dual function lipid proteins and glycerolipid metabolism. *Trends Endocrinol. Metab.* 22, 226–233
3. Csaki, L. S., Dwyer, J. R., Fong, L. G., Tontonoz, P., Young, S. G., and Reue, K. (2013) Lipins, lipinopathies, and the modulation of cellular lipid storage and signaling. *Prog. Lipid Res.* 52, 305–316
4. Han, G. S., Wu, W. L., and Carman, G. M. (2006) The *Saccharomyces cerevisiae* Lipin homolog is a Mg$^{2+}$-dependent phosphatidate phosphatase enzyme. *J. Biol. Chem.* 281, 9210–9218
5. Harris, T. E., Huffman, T. A., Chi, A., Shabanowitz, J., Hunt, D. F., Kumar, A., and Lawrence, J. C., Jr. (2007) Insulin controls subcellular localization and multisite phosphorylation of the phosphatidic acid phosphatase lipin-1. *J. Biol. Chem.* 282, 277–286
6. Pétery, M., Harris, T. E., Fujita, N., and Reue, K. (2010) Insulin-stimulated interaction with 14–3–3 promotes cytoplasmic localization of lipin-1 in adipocytes. *J. Biol. Chem.* 285, 3857–3864
7. Grimsey, N., Han, G. S., O’Hara, L., Rochford, J. J., Carman, G. M., and Siniossoglou, S. (2008) Temporal and spatial regulation of the phosphatidate phosphatases lipin-1 and 2. *J. Biol. Chem.* 283, 29166–29174
8. Karanasios, E., Han, G. S., Xu, Z., Carman, G. M., and Siniossoglou, S. (2010) A phosphorylation–regulated amphipathic helix controls the membrane translocation and function of the yeast phosphatidate phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17539–17544
9. Ren, H., Federico, L., Huang, H., Sunkar, M., Drennan, T., Frohman, M. A., Smyth, S. S., and Morris, A. J. (2010) A phosphatidic acid binding/nuclear localization motif determines lipin-1 function in lipid metabolism and adipogenesis. *Mol. Biol. Cell.* 21, 3171–3181
10. Han, S., Bahmanyar, S., Zhang, P., Grishin, N., Oegema, K., Crooke, R., Graham, M., Reue, K., Dixon, J. E., and Goodman, J. M. (2012) Nuclear envelope phosphatase 1-regulatory subunit 1 (formerly TMEM188) is the metazoan Spo7p ortholog and functions in the lipin activation pathway. *J. Biol. Chem.* 287, 3123–3137
11. Peterson, T. R., Sengupta, S. S., Harris, T. E., Carmack, A. E., Kang, S. A., Balderas, E., Guertin, D. A., Madden, K. L., Carpenter, A. E., Finck, B. N., and Sabatini, D. M. (2011) mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell* 146, 408–420
12. Huang, H., Gao, Q., Peng, X., Choi, S. Y., Sarma, K., Ren, H., Morris, A. J., and Frohman, M. A. (2011) piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. *Dev. Cell* 20, 376–387
13. Pétery, M., Phan, J., and Reue, K. (2005) Alternatively spliced lipin isoforms exhibit distinct expression pattern, subcellular localization, and role in adipogenesis. *J. Biol. Chem.* 280, 32883–32888
14. Eaton, J. M., Mullins, G. R., Brindley, D. N., and Harris, T. E. (2013) Phosphorylation of lipin-1 and charge on the phosphatidic acid head group control its phosphatidic acid phosphatase activity and membrane association. *J. Biol. Chem.* 288, 9933–9945
15. Cascales, C., Mangiapane, E. H., and Brindley, D. N. (1984) Oleic acid promotes the activation and translocation of phosphatidate phosphohydrolase from the cytosol to particulate fractions of isolated rat hepatocytes. *Biochem. J.* 219, 911–916
16. Hopewell, R., Martin-Sanz, P., Martin, A., Saxton, J., and Brindley, D. N. (1985) Regulation of the translocation of phosphatidate phosphohydrolase between the cytosol and the endoplasmic reticulum of rat liver. Effects of unsaturated fatty acids, spermine, nucleotides, albumin and chlorpromazine. *Biochem. J.* 232, 485–491
17. Gomez-Muñoz, A., Hamza, E. H., and Brindley, D. N. (1992) Effects of sphingosine, albumin and unsaturated fatty acids on the activation and translocation of phosphatidate phosphohydrolases in rat hepatocytes. *Biochim. Biophys. Acta* 1127, 49–56
18. Martin, A., Hopewell, R., Martin-Sanz, P., Morgan, J. E., and Brindley, D. N. (1986) Relationship between the displacement of phosphatidate phosphohydrolase from the membrane-associated compartment by chlorpromazine and the inhibition of the synthesis of triacylglycerol and phosphatidylcholine in rat hepatocytes. *Biochim. Biophys. Acta* 876, 581–591
19. Choi, H. S., Su, W. M., Morgan, J. M., Han, G. S., Xu, Z., Karanasios, E., Siniossoglou, S., and Carman, G. M. (2011) Phosphorylation of phosphatidate phosphatase regulates its membrane association and physiological functions in *Saccharomyces cerevisiae*: identification of Ser$^{602}$, Thr$^{231}$, and Ser$^{44}$ as the sites phosphorylated by CDC28 (CDK1)-encoded cyclin-dependent kinase. *J. Biol. Chem.* 286, 1486–1498
20. Huffman, T. A., Mothe-Satney, I., and Lawrence, J. C., Jr. (2002) Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. *Proc. Natl. Acad. Sci. U.S.A.* 99, 1047–1052
21. Santos-Rosa, H., Leung, J., Grimsey, N., Peak-Chew, S., and Siniossoglou, S. (2005) The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. *EMBO J.* 24, 1931–1941
22. Kim, Y., Gentry, M. S., Harris, T. E., Wiley, S. E., Lawrence, J. C., Jr., and Dixon, J. E. (2007) A conserved phosphatase cascade that regulates nuclear membrane biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 104, 6596–6601
23. Ceulemans, H., and Bollen, M. (2006) Functional diversity of protein phosphatase-1, a cellular economizer and reset button. *Physiol. Rev.* 84, 1–39
24. Hendrickx, A., Beulens, M., Ceulemans, H., Den Abt, T., Van Eynde, N., Nicolaescu, E., Lesage, B., and Bollen, M. (2009) Docking motif-guided mapping of the interactome of protein phosphatase-1. *Chem. Biol.* 16, 365–371
25. Peti, W., Nairn, A. C., and Page, R. (2013) Structural basis for protein phosphatase 1 regulation and specificity. *FEBS J.* 280, 596–611
26. Heroes, E., Lesage, B., Gørmenn, J., Beulens, M., Van Meer-veldt, L., and Bollen, M. (2013) The PPI binding code: a molecular-­logo strategy that governs specificity. *FEBS J.* 280, 584–595
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27. Wakula, P., Beullens, M., Ceulemans, H., Stalmaens, W., and Bollen, M. (2003) Degeneracy and function of the ubiquitous RVXF motif that mediates binding to protein phosphatase-1. J. Biol. Chem. 278, 18817–18823
28. Egloff, M. P., Johnson, D. F., Moorhead, G., Cohen, P. T., Cohen, P., and Barford, D. (1997) Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1. EMBO J. 16, 1876–1887
29. Ulke-Lemée, A., Trinkle-Mulcahy, L., Chaulk, S., Bernstein, N. K., Morrice, N., Glover, M., Lamond, A. I., and Moorhead, G. B. (2007) The nuclear PP1 interacting protein ZAP3 (ZAP) is a putative nucleoside kinase that complexes with SAM68, CIA, NSFp10, and HNRNP-G. Biochim. Biophys. Acta 1774, 1339–1350
30. Moorhead, G., MacKintosh, R. W., Morrice, N., Gallagher, T., and MacKintosh, C. (1994) Purification of type 1 protein (serine/threonine) phosphatases by microcystin-Sepharose affinity chromatography. FEBS Lett. 356, 46–50
31. Moorhead, G. B., Haystead, T. A., and MacKintosh, C. (2007) Synthesis and use of the protein phosphatase affinity matrices microcystin-Sepharose and microcystin-biotin-Sepharose. Methods Mol. Biol. 365, 39–45
32. Skene-Arnold, T. D., Luu, H. A., Uhrig, R. G., De Wever, V., Nimick, M., Maynes, J., Fong, A., James, M. N., Trinkle-Mulcahy, L., Moorhead, G. B., and Holmes, C. F. (2013) Molecular mechanisms underlying the interaction of protein phosphatase-1c with ASPP proteins. Biochem. J. 449, 649–659
33. Mannmontri, B., Sariahmetoglu, M., Donkor, J., Bou Khalil, M., Sundaram, M., Yao, Z., Reue, K., Lehner, R., and Brindley, D. N. (2008) Glucocorticoids and cyclic AMP selectively increase hepatic lipin-1 expression, and insulin acts antagonistically. J. Lipid Res. 49, 1056–1067
34. Pétérny, M., Phan, J., Xu, P., and Reue, K. (2001) Lipodystrophy in the fli d mouse results from mutation of a new gene encoding a nuclear protein, lipin. Nat. Genet. 27, 121–124
35. Cole, C., Barber, J. D., and Barton, G. J. (2008) The Ipred 3 secondary structure prediction server. Nucleic Acids Res. 36, W197–W201
36. Moorhead, G. B., Trinkle-Mulcahy, L., Nimick, M., De Wever, V., Campbell, D. G., Gourlay, R., Lam, Y. W., and Lamond, A. I. (2008) Displacement affinity chromatography of protein phosphatase 1 (PP1) complexes. BMC Biochem. 9, 28
37. Martin-Sanz, P., Hopewell, R., and Brindley, D. N. (1984) Long-chain fatty acids and their acyl-CoA esters cause the translocation of phosphatidate phosphohydrolase from the cytosolic to the microsomal fraction of rat liver. FEBS Lett. 177, 284–288
38. Dopheire, N., Gould, K. L., Gygi, S. P., and Kellogg, D. R. (2013) Mapping and analysis of phosphorylation sites: a quick guide for cell biologists. Mol. Biol. Cell 24, 535–542
39. Bowley, M., Cooling, J., Burditt, S. L., and Brindley, D. N. (1977) The effects of amphiphilic cationic drugs and inorganic cations on the activity of phosphatidate phosphohydrolase. Biochem. J. 165, 447–454
40. Han, G. S., and Carman, G. M. (2010) Characterization of the human LPIN1-encoded phosphatidate phosphatase isoforms. J. Biol. Chem. 285, 14628–14638
41. Terrak, M., Kerff, F., Langetmo, K., Tao, T., and Dominguez, R. (2004) Structural basis of protein phosphatase 1 regulation. Nature 429, 780–784
42. Choi, H. S., Su, W. M., Han, G. S., Plote, D., Xu, Z., and Carman, G. M. (2012) Pho85p-Pho80p phosphorylation of yeast Pah1p phosphatidate phosphatase regulates its activity, location, abundance, and function in lipid metabolism. J. Biol. Chem. 287, 11390–11301
43. Su, W. M., Han, G. S., Casciano, J., and Carman, G. M. (2012) Protein kinase A-mediated phosphorylation of Pah1p phosphatidate phosphatase functions in conjunction with the Pho85p-Pho80p and Cdc28p-cyclin B kinases to regulate lipid synthesis in yeast. J. Biol. Chem. 287, 33364–33376
44. Popescu, M., Gurel, Z., Ronni, T., Song, C., Hung, K. Y., Payne, K. J., and Dovat, S. (2009) Ikaros stability and pericentromeric localization are regulated by protein phosphatase 1. J. Biol. Chem. 284, 13869–13880
45. O’Hara, L., Han, G. S., Peak-Chew, S., Grimsey, N., Carman, G. M., and Siniossoglou, S. (2006) Control of phospholipid synthesis by phosphorylation of the yeast lipin Pah1p/Smp2p Mg2+-dependent phosphatidate phosphatase. J. Biol. Chem. 281, 34537–34548