The protein p130 was originally isolated from rat brain as an inositol 1,4,5-trisphosphate-binding protein with a domain organization similar to that of phospholipase C-δ1 but which lacks phospholipase C activity. Yeast two-hybrid screening of a human brain cDNA library for clones that encode proteins that interact with p130 has now led to the identification of the catalytic subunit of protein phosphatase 1α (PP1c)α as a p130-binding protein. The association between p130 and PP1cα was also confirmed in vitro by an overlay assay, a “pull-down” assay, and surface plasmon resonance analysis. The interaction of p130 with PP1cα resulted in inhibition of the catalytic activity of the latter in a p130 concentration-dependent manner. Immunoprecipitation and immunoblot analysis of COS-1 cells that stably express p130 and of mouse brain extract with antibodies to p130 and to PP1cα also detected the presence of a complex of p130 and PP1cα. The activity of glycogen phosphorylase, which is negatively regulated by dephosphorylation by PP1cα, was higher in COS-1 cells that stably express p130 than in control COS-1 cells. These results suggest that, in addition to its role in inositol 1,4,5-trisphosphate and Ca2⁺ signaling, p130 might also contribute to regulation of protein dephosphorylation through its interaction with PP1cα.
proteins that interact with p130. With the unique NH$_2$-terminal region of p130 as the bait for screening a human brain cDNA library, we isolated two positive clones, one of which was required for the interaction between p130 and PP1c, and the other was amino acids 24 to 222 of p130. Phosphatase activity was determined as described (20). Yeast Two-hybrid Screening and β-Galactosidase Assay—Yeast two-hybrid screening of a human brain cDNA library cloned in the pACT2 vector was performed in yeast strain HFR7 with the bait plasmids pGBT9-p130PH or pGBT9-p130D. Transformants (total of 2 $\times$ 10$^6$) were plated and selected with a combination of tryptophan, leucine, and histidine. The positive clones identified by two-hybrid screening were sequenced with an ABI 373A automated DNA sequencer. The domains required for the interaction between p130 and PP1c were investigated by expression of various combinations of bait and target plasmids in yeast SFY526 containing pEGEX-3X. GST Fusion Protein Precipitation and Protein Overlay Analyses—The recombinant GST-PP1c fusion protein was purified from E. coli by affinity chromatography, and recombinant full-length p130 (amino acids 24 to 1069) and the PH domain of p130 (p130PH; amino acids 95 to 232) were prepared as described previously (8, 10). For “pull-down” assays, GST-PP1c was incubated for 1 h at 4°C with glutathione-Sepharose 4B beads in binding buffer (50 mM Tris-HCl (pH 7.5), 5 mM MgCl$_2$, 100 mM NaCl, 10% glycerol, 0.5 mg/ml bovine serum albumin (BSA), 5 mM 2-mercaptoethanol). The beads were washed with 50 volumes of binding buffer and then incubated (6 $\mu$g of GST-PP1c) for 1 h at 4°C, with gentle rotation, in a total volume of 150 $\mu$l with recombinant full-length p130 or p130PH. After washing of the beads at least five times with 500 $\mu$l of binding buffer, bound proteins were eluted with 50 $\mu$l of a solution containing 50 mM Tris-HCl (pH 8.0) and 10 mM reduced glutathione and were then subjected to SDS polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis with antibodies to p130 (2F9) or to p130PH (3, 8).

For overlay analysis, samples were fractionated by SDS-PAGE, and the separated proteins were transferred electrophoretically to a PVDF membrane. After the blocking of nonspecific sites with 5% dried skim milk, the membrane was incubated for 1 h at room temperature with a protein probe (10 $\mu$g/ml). The membrane was then washed and incubated with antibodies to the probe protein followed by alkaline phosphatase-conjugated secondary antibodies, after which immune complexes were detected by enzymatic reaction.

Experimental Procedures

Materials—Cloning vectors pGBK9 and pACT2, a human brain cDNA library, and yeast strains HFR7 and SFY526 were obtained from CLONTECH (Palo Alto, CA). All restriction endonucleases and DNA-modifying enzymes were from Toyobo (Tokyo, Japan). Dropout yeast selection medium and dropout base medium were from BIO101 (Vista, CA). YPD medium for yeast and bacterial medium were obtained from Amresco (Solon, OH). Tris, Cl-$\text{HCl}$, MgCl$_2$, Ins(1,4,5)P$_3$, N-acetyl-L-lysine, NAD$^+$, BSA, and other reagents used were of the highest grade available.

Phosphatase activity was determined as described (20). For overlay analysis, samples were fractionated by SDS-PAGE, and the separated proteins were transferred electrophoretically to a PVDF membrane. After the blocking of nonspecific sites with 5% dried skim milk, the membrane was incubated for 1 h at room temperature with a protein probe (10 $\mu$g/ml). The membrane was then washed and incubated with antibodies to the probe protein followed by alkaline phosphatase-conjugated secondary antibodies, after which immune complexes were detected by enzymatic reaction.

Immunoprecipitation—COS-1 cells, COS-1-p130 cells (COS-1 cells stably expressing p130) (18), and mouse brain extract were subjected to immunoprecipitation with a specific monoclonal antibody to p130 (2F9) or polyclonal antibodies to PP1c (Santa Cruz Biotechnology, Santa Cruz, CA). Cells (5 $\times$ 10$^6$) or mouse brain (weight, 0.2 g) were homogenized in 0.5 ml of a solution containing 20 mM HEPES-NaOH (pH 7.4), 130 mM NaCl, 5 mM EDTA, and a mixture of protease inhibitors. The homogenate was centrifuged (14,000 $\times$ g, 20 min, 4°C), and the resulting supernatant was incubated, with gentle rotation, for 1 h at 4°C with 30 $\mu$g of antibodies to p130 or to PP1c that had been premixed with 10 $\mu$l of a 50% slurry of protein G-Sepharose in phosphate-buffered saline containing 0.1% BSA. The beads were then washed twice with a homogenizing solution (described above) containing 0.2% Triton X-100, boiled in SDS sample buffer, and subjected to SDS-PAGE and immunoblot analysis with antibodies to PP1c or to p130.

Analysis of Protein-Protein Interaction in Real Time—Protein-protein interaction was examined in real time with a BIACORE 2000 surface plasmon resonance analyzer (Biacore International, Uppsala, Sweden). Recombinant GST-PP1c was immobilized on the CM5 sensor chip that had been activated with N-hydroxysuccinimide and N-ethyl-N′-(3-diethylaminopropyl) carbodiimide. Recombinant full-length p130 (0.23, 2.3, 23, 230, or 2300 nM) was injected over the chip surface at a rate of 10 $\mu$l/min in a solution containing 10 mM HEPES-NaOH (pH 7.4), 0.15 mM NaCl, 3.4 mM EDTA, and 0.005% Tween 20. Assay of Glycogen Phosphorylase Activity—COS-1 or COS-1-p130 cells (2 $\times$ 10$^6$) were lysed in a buffer containing 50 mM NaCl, 10 mM MES-NaOH (pH 6.0), 1 mM EDTA, and 10 mM 2-mercaptoethanol. The lysate was subjected to centrifugation at 15,000 $\times$ g for 30 min, and the resulting supernatant was assayed for glycogen phosphorylase activity as described (20). Assay of Phosphatase Activity—Phosphatase activity was determined in a reaction mixture (40 $\mu$l) containing 139.2 mM KCl, 20 mM 4-morpholinoethanesulfonic acid-KOH (pH 7.0), 0.1 mM MnCl$_2$, 0.5 mM dithiothreitol, BSA (0.5 mg/ml), 2 $\mu$m phosphorylated myosin light chain (from bovine stomach), 3.4 nM recombinant rabbit skeletal muscle PP1c, and various concentrations of recombinant full-length p130 or p130PH, in the absence or presence of 10 $\mu$m Ins(1,4,5)P$_3$. The mixture minus the substrate was incubated for 10 min at 25°C, and the reaction was started by the addition of phosphorylated myosin light chain and stopped after 20 min by the addition of 0.2 ml of ice-cold 10% trichloroacetic acid. The unphosphorylated and phosphorylated myosin light chains were separated by two-dimensional electrophoresis, and the density of each spot was determined as described (21).

Results

Two-hybrid Screening for Proteins That Interact with p130—Screening of a human brain cDNA library with a bait plasmid (2 encoding the unique NH$_2$-terminal region of rat p130 (amino acids 24 to 298), including the PH domain and a portion of the EF-hand motif (Fig. 1A), yielded 51 positive clones of a total of 2 million clones examined. A total of 51 clones were sequenced by an automated sequencer encoding the COOH-terminal region (amino acids 548 to 1096) of p130. 10 of the 51 clones identified proved to be false positives, and the remaining 41 clones were divided into two groups on the basis of analysis of their inserts by polymerase chain reaction amplification and restriction enzyme digestion. Sequencing revealed that one of these 41 clones...
Fig. 1. Yeast two-hybrid analysis of the interaction between p130 and PP1c. A, schematic representation of plasmids encoding various regions of p130 (left) and PP1c (right) that were used for analysis of the domains required for binding. The initial screening of the human brain cDNA library for p130-binding proteins was performed with p130 bait plasmid 1 (pGBT9-p130PH[24–298]). Bait plasmids 2 (XhoI-KpnI fragment of p130 cDNA), 4 (XhoI-BamHI), and 5 (KpnI-Smal) encode amino acids 24 to 222, 24 to 82, and 222 to 298 of p130, respectively. These constructs were introduced into yeast strain SFY526, together with pACT2-PP1cα or pACT2-PP1cΔ(30–162), the latter of which was prepared from the former by digestion with PsvI and self-ligation. B, β-Galactosidase assay of protein-protein interaction. Two-hybrid analysis was performed with SFY256 cells transformed with the indicated p130 (1 to 6) and PP1cα plasmids. The activity of β-galactosidase was determined with a filter assay.

Association of p130 with PP1cα

Association of p130 with PP1cα in Vitro—We next examined the interaction of p130 and PP1cα in vitro by several methods. The association was first analyzed with an overlay assay (Fig. 2A). Extracts of nontransformed E. coli and of bacteria expressing a GST-PP1cα fusion protein, as well as recombinant GST-PP1cα purified from such a latter extract, were fractionated by SDS-PAGE, and the separated proteins were transferred to a PVDF membrane and probed with antibodies to PP1cα to confirm the prominent band that migrated at a position corresponding to a molecular size of 37 kDa was indeed PP1cα. Duplicate membranes were incubated in the presence of a recombinant p130 fragment containing the PH domain (p130PH; amino acids 95 to 232), recombinant full-length p130 (residues 24 to 1096), or BSA (negative control). After washing, the membranes were exposed to the corresponding antibodies (residues 24 to 1096), or BSA (negative control). After washing, membranes were incubated in the presence of a GST-PP1cα fusion protein and of bacteria expressing a GST-PP1cα fusion protein. A positive signal was obtained with full-length p130. Neither a bait plasmid (3) encoding amino acids 24 to 82 nor one (5) encoding residues 222 to 298 yielded a positive signal (Fig. 1B). A plasmid encoding a PP1cα mutant lacking amino acids 30 to 162 did not yield a positive signal with any of the p130 bait plasmids examined. These results thus suggested that the region of p130 composed of residues 83 to 222 interacts with that of PP1cα comprising residues 30 to 162.

Association of p130 with PP1cα in Vitro—We next examined the interaction of p130 and PP1cα in vitro by several methods. The association was first analyzed with an overlay assay (Fig. 2A). Extracts of nontransformed E. coli and of bacteria expressing a GST-PP1cα fusion protein, as well as recombinant GST-PP1cα purified from such a latter extract, were fractionated by SDS-PAGE, and the separated proteins were transferred to a PVDF membrane and probed with antibodies to PP1cα to confirm the prominent band that migrated at a position corresponding to a molecular size of 37 kDa was indeed PP1cα. Duplicate membranes were incubated in the presence of a recombinant p130 fragment containing the PH domain (p130PH; amino acids 95 to 232), recombinant full-length p130 (residues 24 to 1096), or BSA (negative control). After washing, the membranes were exposed to the corresponding antibodies (residues 24 to 1096), or BSA (negative control). After washing, membranes were exposed to the corresponding antibodies to p130PH or to p130. Both the recombinant GST-PP1cα present in the bacterial extract and the purified protein interacted with both full-length p130 and p130PH. Together with the results from the yeast two-hybrid analysis, these data indicate that residues 95 to 222 of p130 (which include the entire PH domain and the 20 residues preceding it) mediate the interaction of this protein with PP1cα.

The GST-PP1cα fusion protein was also subjected to a pull-down assay with recombinant p130 or p130PH (Fig. 2B). Incubation of a GST-PP1cα resin with p130PH and subsequent immunoblot analysis of bead-bound proteins with antibodies to p130PH revealed that p130PH was precipitated by GST-PP1cα and that this interaction was sensitive to the presence of low concentrations of full-length p130 but not to PLC-δ1 (Fig. 2B, panel a). In contrast, although full-length p130 also bound to GST-PP1cα (but not to GST alone), this interaction was not sensitive to the presence of p130PH (Fig. 2B, panel b). These results indicate that, although the PH domain of p130 is primarily responsible for the binding of this protein to PP1cα, other regions of p130 also contribute to the interaction between these two proteins.

Analysis of the interaction of various regulatory subunits with PP1cα has led to the identification of a consensus sequence for binding, (K/R)(K/R)(V/I)G (22). The sequence VSF (residues 95 to 97) is present in the region of p130 shown to bind to PP1cα. To determine whether this sequence participates in the interaction of p130 with PP1cα, we examined the effect of a peptide (GMP peptide, GRRVSFADNFGFN) that has been shown to inhibit the association between PP1cα and several regulatory subunits (22). This peptide inhibited the interaction of PP1cα with either full-length p130 or p130PH, whereas a random peptide with the same amino acid composition had no such effect (Fig. 2B, panel c). To confirm the role of the VSF sequence of p130 in the interaction of this protein with PP1cα, we expressed in and purified from E. coli p130 fragments comprising amino acids 82 to 232. Whereas the wild-type fragment bound to PP1cα, fragments containing either V85L or P97A mutations bound to the lesser extent (Fig. 2B, panel d).

Given that p130 contains four consensus motifs for phosphorylation by PKA (74Arg Arg Thr Ser77, 90Arg Lys Lys Thr93, 104Lys Ile Ser107, and 567Arg Arg Val Ser570 [underlining refers to phosphorylatable residues] one of which (104Lys Lys Ile Ser107) is present in p130PH, it was possible that p130 associates with PP1cα because it is a substrate for phosphatase activity of this enzyme. Indeed, p130 was phosphorylated by PKA (Fig. 2C, lane 2), although the precise site (or sites) phos-
Association of p130 with PP1α

Fig. 2. Association between p130 and PP1α in vitro. A, overlay assay. Extracts (10 μg of protein) of either nontransformed E. coli (lane 3) or E. coli expressing recombinant GST-PP1α (lane 1), or 1 μg of purified recombinant GST-PP1α (lane 2), were subjected to SDS-PAGE. The separated proteins were transferred to a PVDF membrane, which was subsequently subjected to immunoblot analysis with antibodies to PP1α (panel a). Alternatively, the membranes were first incubated in the presence of p130PH (panel b), full-length p130 (panel c), or BSA (panel d), each at a concentration of 10 μg/ml, and were then subjected to immunoblot analysis with antibodies to p130PH or to p130 as indicated. B, pull-down assay. Recombinant GST-PP1α (or GST) attached to glutathione-Sepharose 4B beads was incubated with various protein samples, after which proteins that had bound to the beads were eluted with reduced glutathione and subjected to SDS-PAGE and immunoblot analysis with appropriate antibodies. Panel a, beads were incubated with 1 μM p130PH in the absence or presence of 0.01 or 0.1 μM full-length p130 or of 0.1 or 1 μM PLC-81 (prepared as described in Ref. 7); immunoblot analysis was performed with antibodies to p130PH. Panel b, beads were incubated with 0.1 μM full-length p130 in the absence or presence of 0.1, 1, or 10 μM p130PH; immunoblot analysis was performed with antibodies to full-length p130. Panel c, beads were incubated in the presence of 1 μM p130PH or full-length p130, in the absence or presence of 50 μM GTPγS or a peptide of the same amino acid composition but of random sequence (GRVRFADNFGFN) or a peptide of the same amino acid composition but of random sequence (GRVRFADNFGFN) or a peptide of the same amino acid composition but of random sequence (GRVRFADNFGFN) or a peptide of the same amino acid composition but of random sequence (GRVRFADNFGFN). C, phosphorylation of p130 by PKA and its effect on association with PP1α. Panel a, recombinant full-length p130 (150 pmol) was incubated in a volume of 50 μl with 100 μM [γ-32P]ATP in the absence (lane 1) or presence (lane 2) of 0.1 μg of the catalytic subunit of PKA, after which the reaction mixtures were subjected to SDS-PAGE and autoradiography. Panels b and c, recombinant p130 phosphorylated by PKA (lane 2) or treated with ATP alone (lane 1) was incubated with recombinant GST-PP1α immobilized on glutathione-Sepharose 4B beads, after which bead-bound protein was eluted with reduced glutathione and subjected to SDS-PAGE and immunoblot analysis with antibodies to p130 (b) or to PP1α (c). D, surface plasmon resonance analysis. GST-PP1α was immobilized on a sensor chip and exposed to various concentrations of full-length p130 (0.23, 2.3, 23, 230, and 2300 nM) for 360 s before the application of buffer alone; data are expressed in relative units (RU).

Phosphorylated remains to be determined. However, this explanation for the interaction between p130 and PP1α is unlikely, because phosphorylated p130 did not associate with PP1α, whereas p130 treated with ATP alone (without PKA) bound to PP1α (Fig. 2C, lane 1).

The association between p130 and PP1α was further confirmed by surface plasmon resonance analysis. Full-length p130 was introduced into the analysis chamber after immobilization of GST-PP1α onto the sensor chip. Positive signals indicative of protein-protein interaction were generated in a p130 concentration-dependent manner and were abolished by washing away of the applied p130 (Fig. 2D). The dissociation constant was calculated to be 1.2 ± 0.1 nM (mean ± S.E. of values from five independent determinations). Replacement of the full-length p130 molecule with p130PH yielded a dissociation constant in the micromolar range, consistent with the results obtained with pull-down assays (Fig. 2B, panels a and b).

We next investigated whether the activity of PP1α is affected by the association with p130. The dephosphorylation of phosphorylated smooth muscle myosin light chain (21) by recombinant rabbit skeletal muscle PP1α was inhibited by full-length p130 in a concentration-dependent manner (Fig. 3). Recombinant p130PH also inhibited the activity of PP1α, although higher concentrations of p130PH than of full-length p130 were required for this effect.

The effects of Ins(1,4,5)P3 and water-soluble (short-chain) PtdIns(4,5)P2 on the association of p130 with PP1α, as well as on the inhibition of PP1α activity by p130, were also examined, given that the site of p130 responsible for the association with PP1α was shown to be located immediately upstream of the PH domain and that PH domains mediate binding to Ins(1,4,5)P3 or PtdIns(4,5)P2. The presence of Ins(1,4,5)P3 or short-chain PtdIns(4,5)P2 at a concentration of 10 μM in the reaction mixture for the pull-down assay had no effect on the interaction of p130 with PP1α (data not shown), and 10 μM Ins(1,4,5)P3 had no effect on p130-induced inhibition of PP1α activity (Fig. 3).

Association between p130 and PP1α in Intact Cells—To determine whether p130 and PP1α interact in living cells, we first examined COS-1 cells that stably express recombinant p130 (COS-1p130 cells) (18). Immunoblot analysis of extracts of both control COS-1 cells (which lack endogenous p130) and COS-1p130 cells with antibodies to PP1α revealed that both cell lines express similar amounts of PP1α (Fig. 4A, a). Cell extracts were then subjected to immunoprecipitation with antibodies to either p130 (Fig. 4A, b) or PP1α (Fig. 4A, c), and the
resulting precipitates were subjected to immunoblot analysis with the same two types of antibodies. Stable association of p130 with PP1α was apparent in COS-1p130 cells but not in control COS-1 cells (Fig. 4A). We also examined whether these two proteins interact in mouse brain, which contains both molecules (Fig. 4B). PP1α was detected in p130 immunoprecipitates (Fig. 4B, b), and p130 was detected in PP1α immunoprecipitates (Fig. 4B, c) prepared from mouse brain.

PP1α is thought to catalyze protein dephosphorylation reactions that underlie many aspects of cell function (23, 24). Glycogen phosphorylase, which catalyzes the conversion of glycogen to glucose 1-phosphate, is a substrate for PP1α in a wide variety of cell types (25); its dephosphorylation by this phosphatase results in inhibition of phosphorylase activity. Measurement of glycogen phosphorylase activity in extracts of various cell types (25); its dephosphorylation by this phosphatase indicates that glycogen phosphorylase is phosphorylated to a greater extent in COS-1p130 cells than in COS-1 cells.

**DISCUSSION**

With the use of its specific NH₂-terminal region as a bait, we applied the yeast two-hybrid screen to identify human brain proteins that bind to p130, designated henceforth as PRIP-1. This approach identified PP1α as one such protein. PP1 is a widely expressed serine-threonine protein phosphatase that exists in several isoforms, including α, β, γ1, γ2, and δ (23, 24). Various regulatory subunits have been shown to associate with PP1α and thereby to influence its catalytic activity (26). For example, G₉ and G₁ subunits function to target PP1α to glycogen granules; phosphorylation of these subunits by PKA induces their dissociation from PP1α, whereas that triggered by insulin promotes their association with and activation of PP1α, resulting in inhibition of glycogen breakdown. The association of I-1 (inhibitor 1) or DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) with PP1α appears not to affect phosphatase activity, whereas phosphorylation of I-1 or DARPP-32 by PKA induces marked inhibition of such activity. Our results now suggest that PRIP-1 also functions as a regulatory subunit of PP1α that inhibits phosphatase activity. The binding of Ins(1,4,5)P₃ or PtdIns(4,5)P₂ to PRIP-1 had no effect on its association with or inhibition of PP1α. Our previous observations suggested that Ins(1,4,5)P₃ may be a physiological ligand for PRIP-1 and that this protein is localized predominantly to the cytosol (18). PRIP-1 may therefore serve not only to inhibit the activity of PP1α but also to target this enzyme to the cytosol.

Amino acid residues 95 to 97 of PRIP-1, located upstream of the PH domain, appear to contribute to the binding site for PP1α. The fragment of PRIP-1 comprising residues 24 to 222 interacted with PP1α in the yeast two-hybrid assay, and p130PH (PRIP-1PH) (residues 95 to 222) as well as the full-length molecule, associated with PP1α in vitro, as demonstrated with a variety of binding assays. A G₉ peptide that disrupts the interaction of PP1α with several regulatory subunits and that contains the VSF (residues 95 to 97) sequence of PRIP-1 also inhibited the association of PRIP-1 with PP1α. Furthermore, mutation of residues 95 or 97 of PRIP-1 prevented the association of this protein with PP1α. Other regions of the PRIP-1 molecule may also interact with PP1α, as suggested by the observations that the full-length molecule bound to PP1α was not displaced by an excess amount of PRIP-1PH and that the dissociation constant obtained by surface plasmon resonance analysis for the interaction with PP1α was smaller for the full-length molecule than for PRIP-1PH. However, the observation that the G₉ peptide was similarly
effective in inhibiting the association of PP1cα with full-length PRIP-1 and with PRIP-1PH suggests rather that other regions of PRIP-1 promote the interaction of the region containing residues 95 to 97 with PP1cα.

Phosphorylation of PRIP-1 by PKA resulted in inhibition of the association between PRIP-1 and PP1cα. Although the phosphorylated residues of PRIP-1 that underlie this effect remain to be identified, T93, which is located immediately upstream of the putative binding site for PP1cα, is a likely candidate.

PP1cα contributes to the regulation of many aspects of cellular metabolism, including glycogen metabolism (through dephosphorylation of phosphorylase, glycogen phosphorylase, and glycogen synthase) and lipid metabolism (through dephosphorylation of acetyl-CoA carboxylase, hormone-dependent lipase, and hydroxymethylglutaryl-CoA reductase). Furthermore, it participates in the regulation of Ca2+ transport (through dephosphorylation of phospholamban and Ca2+ channel proteins), smooth muscle contraction (through dephosphorylation of myosin light chain), DNA replication (through dephosphorylation of histones H2B and H1), and protein synthesis (through dephosphorylation of initiation factor eIF-2, RNA-dependent protein kinase, heat shock protein, S6 protein, and S6 kinase) (24, 25). It remains to be determined which of these cellular activities are physiologically regulated by PRIP-1 through its interaction with PP1cα. Our data do suggest, however, that the association between PRIP-1 and PP1cα occurs in living cells, and we have shown that the activity of glycogen phosphorylase, which is regulated exclusively by phosphorylation, was increased in COS-1 cells by the expression of PRIP-1, probably as a result of the interaction of PRIP-1 with, and the consequent inhibition of, PP1cα. Glycogen phosphorylase may therefore be a physiological target for regulation by the interaction of PRIP-1 with PP1cα.

In summary, we have shown that (i) p130, which belongs to the PRIP family of proteins and is here renamed PRIP-1, associates with PP1cα through a Gα peptide-like region located upstream of the PH domain; (ii) association with PRIP-1 results in inhibition of the catalytic activity of PP1cα as measured in vitro with phosphorylated myosin light chain as substrate; and (iii) glycogen phosphorylase activity was increased by expression of PRIP-1 in intact cells, likely as a result of inhibition of PP1cα and accumulation of the phosphorylated, active form of glycogen phosphorylase. In addition to its role in Ins(1,4,5)P3 and Ca2+ signaling (18), PRIP-1 might therefore also contribute to the regulation of protein dephosphorylation. Given that the binding of Ins(1,4,5)P3 to the PH domain of PRIP-1 had no effect on the association of PRIP-1 with PP1cα or on its inhibition of PP1cα activity, PRIP-1 may contribute to both Ca2+ signaling and regulation of protein dephosphorylation simultaneously and, in some instances, cooperatively.

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