Phactrs 1–4: A family of protein phosphatase 1 and actin regulatory proteins

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Protein phosphatase 1 (PP1) is a multifunctional enzyme with diverse roles in the nervous system, including regulation of synaptic activity and dendritic morphology. PP1 activity is controlled via association with a family of regulatory subunits that govern subcellular localization and substrate specificity. A previously undescribed class of PP1-binding proteins was detected by interaction cloning. Family members were also found to bind to cytoplasmic actin via Arg, Pro, Glu, and Leu repeat-containing sequences. The prototypical member of this family, phosphatase and actin regulator (phactr) 1 was a potent modulator of PP1 activity *in vitro*. Phactr-1 protein is selectively expressed in brain, where high levels were found in cortex, hippocampus, and striatum, with enrichment of the protein at synapses. Additional family members displayed highly distinct mRNA transcript expression patterns within rat brain. The current findings present a mechanism by which PP1 may be directed toward neuronal substrates associated with the actin cytoskeleton.

A notable observation arising from the sequencing of mammalian genomes is that the number of predicted Ser/Thr kinases greatly exceeds that of the predicted Ser/Thr phosphatases (1, 2). This discrepancy may be accounted for in part by the relatively elaborate regulatory apparatus that exists to promote multifunctional diversity in the activity of major Ser/Thr phosphatases. Thus, the catalytic cores of these enzymes can be adapted to multiple needs by means of association with large families of regulatory subunits (3–5).

Protein phosphatase 1 (PP1) is a ubiquitously expressed enzyme involved in a wide array of physiological processes, including gene expression, muscle contraction, and glycogen metabolism. The role of PP1 in these diverse aspects of cellular physiology has been difficult to dissect by using available inhibitors because of a lack of pharmacological specificity. One approach to identifying specific targets for the enzyme has been to elaborate on the control mechanisms that direct PP1 activity toward specific substrates. This strategy has led to characterization of multiple regulatory subunits, and many of these proteins have been shown to either promote, or to inhibit, PP1 activity toward substrates in specific biochemical settings (6).

In the nervous system, PP1 plays a role in the regulation of synaptic plasticity by controlling activity of a broad range of ion channels and signal transduction enzymes (7, 8). As in other tissues, the orchestration of PP1 activity toward its various targets in brain is likely to depend on association with diverse regulatory subunits. Some of the neuronal regulatory subunits have been well characterized. Within the basal ganglia and cortical regions, multiple neurotransmitters act through convergent biochemical pathways to reversibly control PP1 activity by means of the regulatory molecule, dopamine- and cAMP-regulated phosphoprotein-32 kDa (DARPP-32) (7, 9). A protein structurally related to DARPP-32, inhibitor-1, has been implicated in the PP1 inhibition required for the induction of long-term potentiation at hippocampal synapses (10, 11). Conversely, spinophilin serves to functionally promote PP1 in regulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor responses (12) and plays a role in the induction of long-term depression (12, 13).

Still, a simple correlation between altered PP1 activity and the direction of change in synaptic efficacy is not so easy to predict. For example, at parallel fiber/Purkinje cell synapses in cerebellum, PP1 inhibition appears to be required for the induction of long-term depression (14, 15). In contrast, at Schaffer collateral/cornu ammonis (CA)1 synapses, PP1 activity is required for the induction of long-term depression (16, 17). Thus, not all synapses are likely to share the same biochemical mechanisms for control. A desire to better understand the processes that modulate synaptic function has motivated continued characterization of unique proteins that associate with PP1. We describe a family of such proteins, abundantly expressed in the nervous system, which may also play a role in regulation of the actin cytoskeleton. We refer to members of this family of proteins as phosphatase and actin regulators (phactr) 1–4 based on their putative functional roles.

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Materials and Methods

Yeast Two-Hybrid Screening, cDNA Expression, and Coimmunoprecipitation. A rat brain cDNA library was constructed and screened with the α isoform of the PP1 catalytic subunit as described (18).

Phactr-1 cDNA was ligated into mammalian expression vector pcDNA3 (Invitrogen). HEK 293T cells were transfected with plasmid DNA by the calcium phosphate precipitation method. Transfected cells and rat brain were lysed in 0.5% Nonidet P-40/1 mM EDTA/50 mM Tris, pH 8.0/120 mM NaCl/1 mM PMSF/20 μg/ml leupeptin–antipain/5 μg/ml pepstatin–chymostatin. Total protein (1 mg) was used for immunoprecipitation (IP) with 2 μg of Ab and 10 μl of protein A-Sepharose beads. After three washes in lysis buffer and one wash in 50 mM Tris (pH 7.0), proteins were separated by SDS/PAGE and immunoblotted. Enhanced chemiluminescence was used for detection.

Immunocytochemistry and Immunohistochemistry. N2a cells were grown in 45% DMEM/45% opti-MEM/10% FCS (GIBCO/BRL) at 37°C in 5% CO_2. Cells were washed with PBS, fixed with 4% sucrose/4% paraformaldehyde, permeabilized with 0.3% Triton X-100, blocked in 1% normal goat serum, and stained overnight at 4°C with 0.5 μg/ml anti-hemagglutinin Ab (Babco, Richmond, CA) and 0.2 μg/ml anti-PP1α Ab (19). After rinsing in PBS, secondary Abs were applied. Anti-rabbit/Cy-5 (Amersham Biosciences), anti-mouse/Alexa-488, and Alexa 568-phalloidin (Molecular Probes) were incubated with cells for 1 h at room temperature.

After transcardial perfusion of one adult male rat (with 4%

Abbreviations: PP1, protein phosphatase 1; RPEL, amino acid consensus sequence containing Arg, Pro, Glu, and Leu; phactr, phosphatase and actin regulator; PSD, postsynaptic density; IP, immunoprecipitation; DG, dentate gyrus; CA, cornu ammonis.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY494977, AY500157, and AY500158).

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paraformaldehyde/0.4% picric acid in sodium phosphate buffer), the brain was removed and cryoprotected in 10% sucrose. Sections were cut on a cryostat, permeabilized with 0.3% Triton X-100, and blocked in 1% normal goat serum. Anti-phactr mouse 1C10 mAb (400 ng/ml) was applied overnight at 4°C, followed by secondary Ab (Alexa-488).

Hippocampal neurons were cultured from E17 rat embryos. Brain tissue was digested in 0.25% trypsin/1 mM EDTA (GIBCO), triturated, and resuspended in DMEM plus 10% FBS. Cells were plated onto laminin/poly(D) lysine-coated coverslips (Becton Dickinson). Adherent cells were incubated in neurobasal medium and 2% B27/1% N2 (GIBCO). Neurons were transfected by application of a GFP-phactr expression plasmid in calcium phosphate precipitate for 20 min, followed by replacement of cell medium. Fixation was as for N2a cells. Images were acquired on a LSM 510 laser-scanning confocal microscope (Zeiss).

Ab Production and Purification. The predicted amino acid sequence of phactr-1 was used to design peptides (synthesized at the W. M. Keck Facility at Yale University) for immunization. Peptides were conjugated to thyroglobulin with glutaraldehyde. Peptide MEPVPMPRDPCSYEVLQASDIMDC was used for generation of rabbit Ab RU142 (Cocalico Biologicals, Reamstown, PA). DDNKENVPHEPDYEDSSC was used to generate 1C10 mAb (Maine Biotechnology Services, Portland, ME). Abs were affinity purified over immunization peptide coupled to sulfolink resin (Pierce).

Preparation of Bacterial GST Fusion Protein. The phactr-1 cDNA was modified by PCR primer-based mutagenesis to incorporate a *Sal*I site immediately upstream of the Thr-501 codon. This PCR fragment, encoding phactr-1 amino acids 501–580, was cloned into bacterial expression vector pGEX-4T-2 (Amersham Biosciences) in frame with coding sequence for GST. Bacterial protein was expressed and purified as described (21).

Protein Phosphatase Activity Assay. Phosphorylase *a* was kindly provided by Thomas McAvoy. Assays were performed as described (18). In brief, reactions were assembled with equal volumes of substrate, varying concentrations of phactr, and ≈150 pM of rabbit skeletal muscle PP1 or PP2A (Upstate Biotechnology, Lake Placid, NY); incubated at 30°C for 10 min; and stopped by trichloroacetic acid precipitation. After centrifugation, the radiolabeled phosphate remaining in supernatants was measured by Cherenkov counting.

Northern Blotting and *in Situ* Hybridization. A multiple-tissue Northern blot (Clontech) was probed according to the manufacturer's instructions by using phactr-1 probe prepared from cDNA AY494977 nucleotides 541–1,061.

We prepared ^{35}S-labeled antisense and sense cRNA probes as described (20) by *in vitro* transcription from cDNA clones corresponding to the following fragments: phactr-1, nucleotides 541–1,088 of cDNA AY494977; phactr-2, nucleotides 1,461–1,742 of cDNA AY500158; and phactr-3, nucleotides 13–1,563 of cDNA AY500157. Phactr-4 primers were designed based on sequence homology between human phactr-4 (AK023233) and two mouse EST sequences (AA673772 and BE569419). ACTGCCTACCCACATACCTCC and TCATGGGCGATGGTAGCG were used to PCR amplify an ≈1-kb fragment from rat cDNA. Sequence analysis of cloned product confirmed similarity to human phactr-4.

Results

Isolation of a Previously Uncharacterized PP1-Binding Protein. We have described (18, 21) use of the yeast two-hybrid system to isolate protein species that interact with the catalytic subunit of PP1α. Further analyses of isolates from this screen led to characterization of a cDNA encoding a predicted protein product of unknown function. This library cDNA clone (subsequently shown to encode N-terminal truncated phactr) was transferred to a mammalian expression vector, in frame with Flag-tag epitope for Ab recognition. Tagged phactr cDNA was transiently expressed in 293T cells, and protein product immunoprecipitated from cell lysates with anti-Flag Ab. Precipitated proteins were solubilized, subjected to SDS/PAGE and examined by Western blotting with anti-Flag and anti-PP1 Abs. Endogenous PP1 was found to be present in a complex with the ectopically expressed phactr clone (Fig. 1*A*).

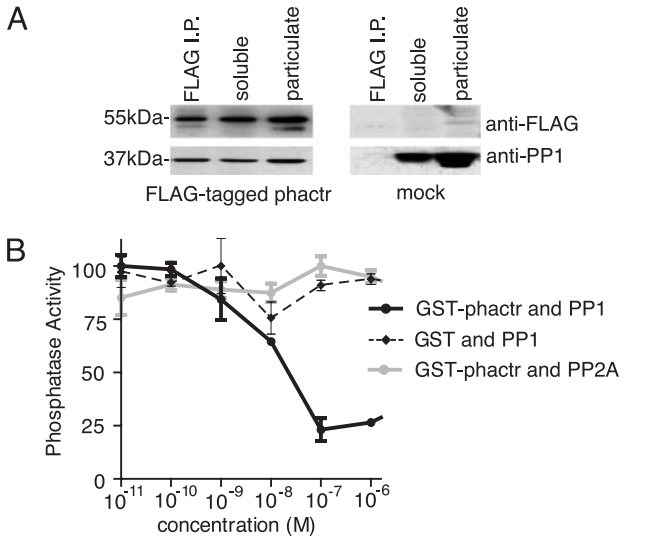

Fig. 1. Identification of a PP1-binding protein. (*A*) Western blots prepared from 293T cell lysate fractions and immunoprecipitates demonstrating (*Upper Left*) expression of phactr yeast two-hybrid clone cDNA tagged with Flag epitope and (*Lower Left*) associated endogenous PP1. (*A Right*) Control fractions and immunoprecipitates prepared from cells transfected with empty expression vector (mock). (*B*) Modulation of purified phosphatase activity toward phosphorylase *a* after coincubation with increasing concentrations of either GST–phactr fusion protein or GST alone. Data are expressed as percentage activity in the absence of added recombinant protein.

Phactr was expressed as a bacterial GST fusion protein, and purified recombinant protein was used to assess its influence on the *in vitro* catalytic activity of PP1. Using phosphorylase *a* as substrate, the purified bacterial protein inhibited PP1 enzymatic activity in a concentration-dependent manner, with IC_{50} near 10 nM, whereas control bacterial GST protein produced no apparent PP1 inhibition, even at micromolar concentrations (Fig. 1*B*). GST-phactr had no discernable effect on catalytic activity of PP2A, a phosphatase closely related to PP1. These results indicate a specific functional interaction between phactr and PP1.

The yeast two-hybrid cDNA clone was used to generate probes to screen a rat hippocampal λ phage library. Five overlapping cDNA clones were isolated, the largest of which contained a long ORF encoding a protein of 580 residues with predicted molecular mass of ≈66 kDa (GenBank accession no. AY494977). This cDNA was transferred to a mammalian expression vector and expressed transiently in 293T cells. Abs were prepared against synthetic peptides conforming to predicted phactr amino acid sequence. After ectopic expression of the phactr cDNA in 293T cells, these Abs recognized a protein with apparent molecular mass of ≈75 kDa. An endogenous rat brain protein migrated at the same molecular mass (Fig. 2*A*). The identity of the initiating ATG was confirmed by its mutagenesis and subsequent expression of a truncated protein (not shown). The ≈10-kDa discrep-

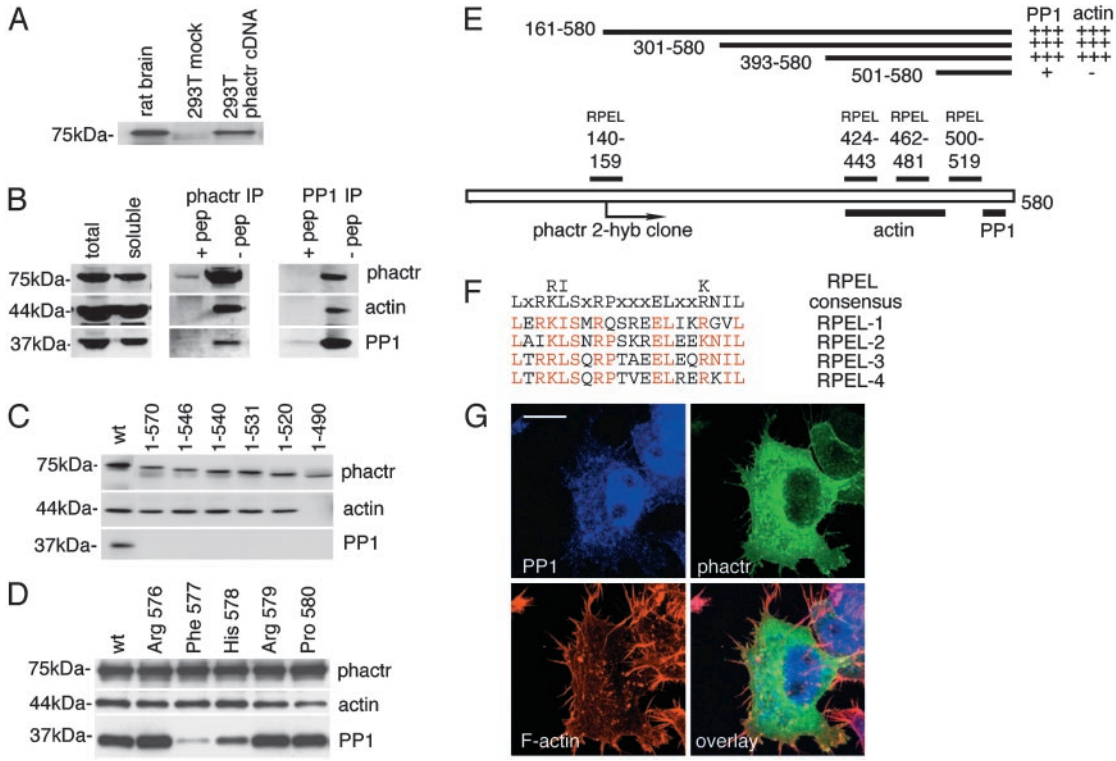

Fig. 2. Phactr interactions with PP1 and actin. (*A*) Abs raised against phactr detected a protein in rat brain lysate migrating with apparent molecular mass of ≈75 kDa. A protein of similar size was detected upon expression of phactr cDNA isolate in 293T cells. (*B*) Coimmunoprecipitation of phactr with PP1 and actin. Western blot analysis of protein levels in cell fractions (*Left*), phactr $IP^{+/-}$ Ab-blocking peptide (*Center*), and PP1 $IP^{+/-}$ Ab-blocking peptide (*Right*). Blot was reprobed sequentially with anti-phactr (*Top*), anti-actin (*Middle*), and anti-PP1 (*Bottom*) Abs. (*C* and *D*) Western blot analysis of coimmunoprecipitation reactions prepared by using cell lysates from 293T cells transfected with a series of phactr C-terminal truncation mutants (*C*) and a series of Ala-substitution mutants (*D*). IP was performed with anti-hemagglutinin Ab directed against N-terminal epitope tag. (*E Upper*) N-terminal mutants of phactr expressed in the yeast two-hybrid system. Interactions with PP1 and actin prey proteins were assessed by β-galactosidase activity in HF7c. (*E Lower*) Depiction of location of structural elements and protein binding sites within phactr. (*F*) Alignment of phactr RPEL repeats with consensus sequence for the motif (conserved domain smart00707.7). Conforming residues are shown in red. (*G*) Fluorescence image of N2a neuroblastoma cell transfected with full-length hemagglutinin epitope-tagged phactr. Red, rhodamine-phalloidin (F-actin); green, anti-hemagglutinin (phactr); blue, anti-PP1α. (*Right Lower*) Triple staining. (Scale bar, 10 μm.)

ancy between the predicted and apparent molecular mass of phactr may involve posttranslational modification or low SDS-binding capacity.

Amino acid sequence analysis of phactr revealed several RPEL conserved domains (smart00707.7) but no other clear structural signatures. To gain further insight into the biochemical context in which this protein might function, we returned to the two-hybrid system with phactr amino acids 161–580 as bait. This screen identified multiple independent isolates of the cDNA for cytoplasmic β-actin.

IP reactions from rat brain lysate with Abs raised against phactr, followed by Western blot analysis, demonstrated that both PP1 and actin could be found in a complex with the endogenous full-length protein (Fig. 2*B*).

A series of C-terminal-truncated phactr mutants was prepared, and coimmunoprecipitation reactions were used to map structural determinants for PP1 and β-actin binding. Sequences near the phactr C terminus were required for efficient binding (Fig. 2*C*) with deletion of as few as the last 10 aa sufficient to abolish detection of PP1 interaction. Phactr does not contain a clear PP1-binding consensus sequence (Arg/Lys-Arg/Lys-hydrophobic-Xaa-Phe/Trp) (22, 23). However, results of a scanning site-directed mutagenesis experiment suggested the presence of a cryptic consensus sequence surrounding a hydrophobic Phe residue. The last five amino acids of phactr were independently mutated to Ala; mutant phactr proteins were expressed in 293T cells and coimmunoprecipitation with endogenous PP1 was evaluated. Mutation of Phe-577 (and to a lesser extent His-578) resulted in greatly reduced PP1 binding (Fig. 2*D*). N-terminal deletions were examined in the yeast two-hybrid system. PP1 binding was partially maintained in a GAL-4 fusion protein incorporating the last 80 aa of phactr, whereas actin binding to this fragment was abolished (Fig. 2*E*). Thus, PP1 binds to phactr near its C terminus, and actin binds in a region containing RPEL repeats 2 and 3 (Fig. 2 *E* and *F*).

To examine phactr cellular localization, full-length protein was expressed in the neuroblastoma cell line, N2a. Fixed cells were permeabilized and stained with Abs against phactr and PP1α, plus the F-actin stabilizing toxin, phalloidin (Fig. 2*G*). Results indicated that there exists a partial overlap among phactr, PP1α, and F-actin distribution in these cells. Phactr was partially colocalized with PP1α in the cytoplasm but essentially absent from the cell nucleus where PP1α was enriched. F-actin was most abundant in filopodia, where staining for phactr was also weakly apparent. No change in cellular morphology was detected upon phactr overexpression in these cells.

Phactr Expression Pattern. Northern blot analysis performed with a phactr cDNA-derived probe revealed a transcript of ≈5 kb which, although most abundant in brain, is present at lower levels in lung, heart, kidney, and testis (Fig. 3*A*). Anti-phactr Abs were used to examine protein expression pattern in different rat tissues. In contrast to the widely expressed mRNA, the ≈75-kDa protein detected in brain extracts was not detectable in other

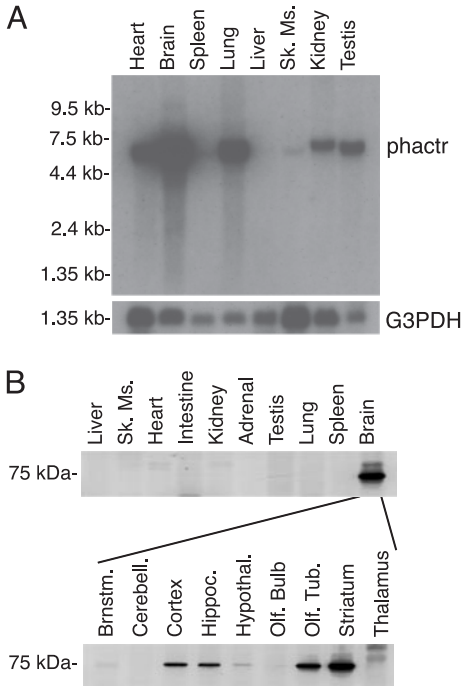

Fig. 3. Expression pattern of phactr. (*A*) Northern blot analysis. DNA probe prepared from phactr cDNA (residues 541–1,061) was used to analyze rat poly(A)$^+$ mRNA samples (*Upper*). Relative loading indicated by signal detected upon reprobing for glyceraldehyde-3 phosphate dehydrogenase (G3PDH) (*Lower*). (*B*) Western blot analysis of phactr protein levels detected in various tissues (*Upper*) and microdissected brain regions (*Lower*). Total protein, 50 μg per lane.

tissues examined (Fig. 3*B*). Brain region-specific Western blots revealed discontinuous distribution of phactr with enrichment in cortex, hippocampus, and, particularly, striatum (Fig. 3*B*). Antisera raised against two separate phactr-based peptides gave equivalent results (data not shown).

Data obtained by immunoblotting were complemented by immunocytochemical determination of phactr distribution within rat brain. Phactr is highly enriched in striatum and olfactory tubercle, with lower levels detected in cortex and septum (Fig. 4*A*). Within striatum and hippocampus, phactr was abundant in cell bodies and dendritic fields, but staining was absent from cell nuclei (Fig. 4 *B* and *C*).

In cultured hippocampal neurons transfected with an N-terminal GFP-tagged phactr construct, subcellular distribution was throughout cell bodies and dendrites, with apparent enrichment in dendritic spines. Phactr was found in the perinuclear region but was absent from cell nuclei (Fig. 5*A*). Subcellular fractionation of rat brain homogenate supported the observation that phactr may be localized at synapses because the protein was found to be present in postsynaptic density (PSD) fractions (Fig. 5*B*).

A Family of Phactr-Related Proteins. Database analysis revealed the presence of several human and mouse gene products similar to the rat phactr protein. Sequence similarity was highest in the N termini, surrounding one of the RPEL repeats, and in the C termini, surrounding the additional RPEL motifs and the actin- and PP1-binding domains (Fig. 6*A*). Family members were arbitrarily designated phactrs 1–4. cDNAs for mouse phactrs 2–4 were amplified by RT-PCR. Epitope-tagged cDNAs were expressed in 293T cells, and their interactions with PP1 and actin were assessed by IP. Phactr family members were found to coimmunoprecipitate with actin and PP1 (data not shown).

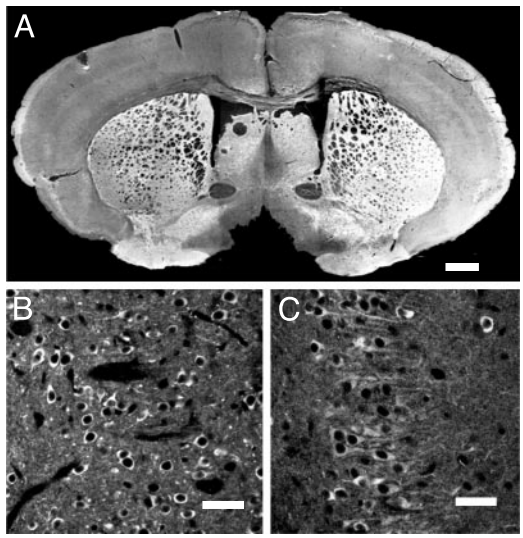

Fig. 4. Immunocytochemical analysis of phactr-1 distribution in rat brain. (*A*) Coronal section revealing intense staining in striatum and olfactory tubercle, with moderate levels in septum and cortex. (Scale bar, 1 mm.) (*B*) Staining in CA1 was apparent in cell bodies and proximal dendrites but was absent from cell nuclei. (*C*) Staining in striatum also shows enrichment in cell bodies. (Scale bars, 25 μm.)

Within rat brain, transcript distributions of various phactr isoforms were found to differ markedly (Fig. 6*B*). Expression of phactr-1 and phactr-2 is remarkably complementary. Phactr-1 is enriched in the olfactory tubercle, nucleus accumbens core and shell, caudate-putamen, cerebral cortex, hippocampus [dentate gyrus (DG) = CA1 = CA3], and piriform cortex. Moderate to high levels are found also in the olfactory bulb, arcuate and

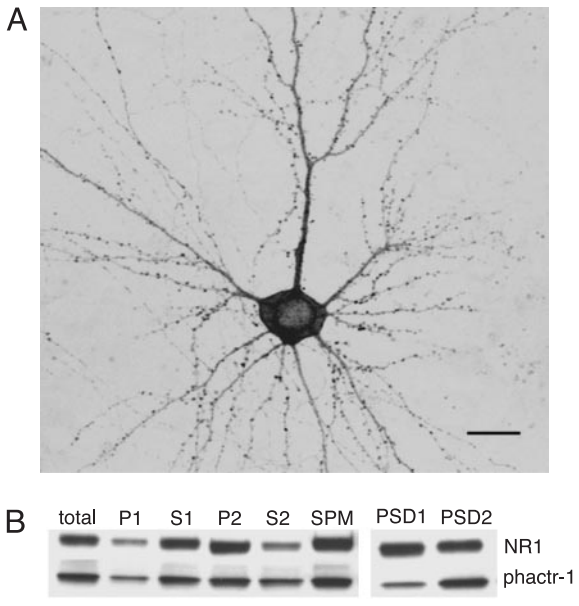

Fig. 5. Phactr distribution within neurons. (*A*) Fluorescence image of a cultured hippocampal neuron transfected with N-terminally GFP-tagged phactr-1. (Scale bar, 20 μm.) (*B*) Western blot analysis for phactr-1 and NR1, indicating relative protein abundance in subcellular fractions. Protein (20 μg) loaded in the first six lanes: totals are 1,400 $\times$ *g* pellet (P1), 1,400 $\times$ *g* supernatant (S1), 13,800 $\times$ *g* pellet (P2), 13,800 $\times$ *g* supernatant (S2), and synaptic plasma membrane. The proteins (2 μg) loaded in the last two lanes were Triton X-100 extracted synaptic plasma membrane (PSD1) and Triton X-100/sarcosyl extracted PSD1 (PSD2).

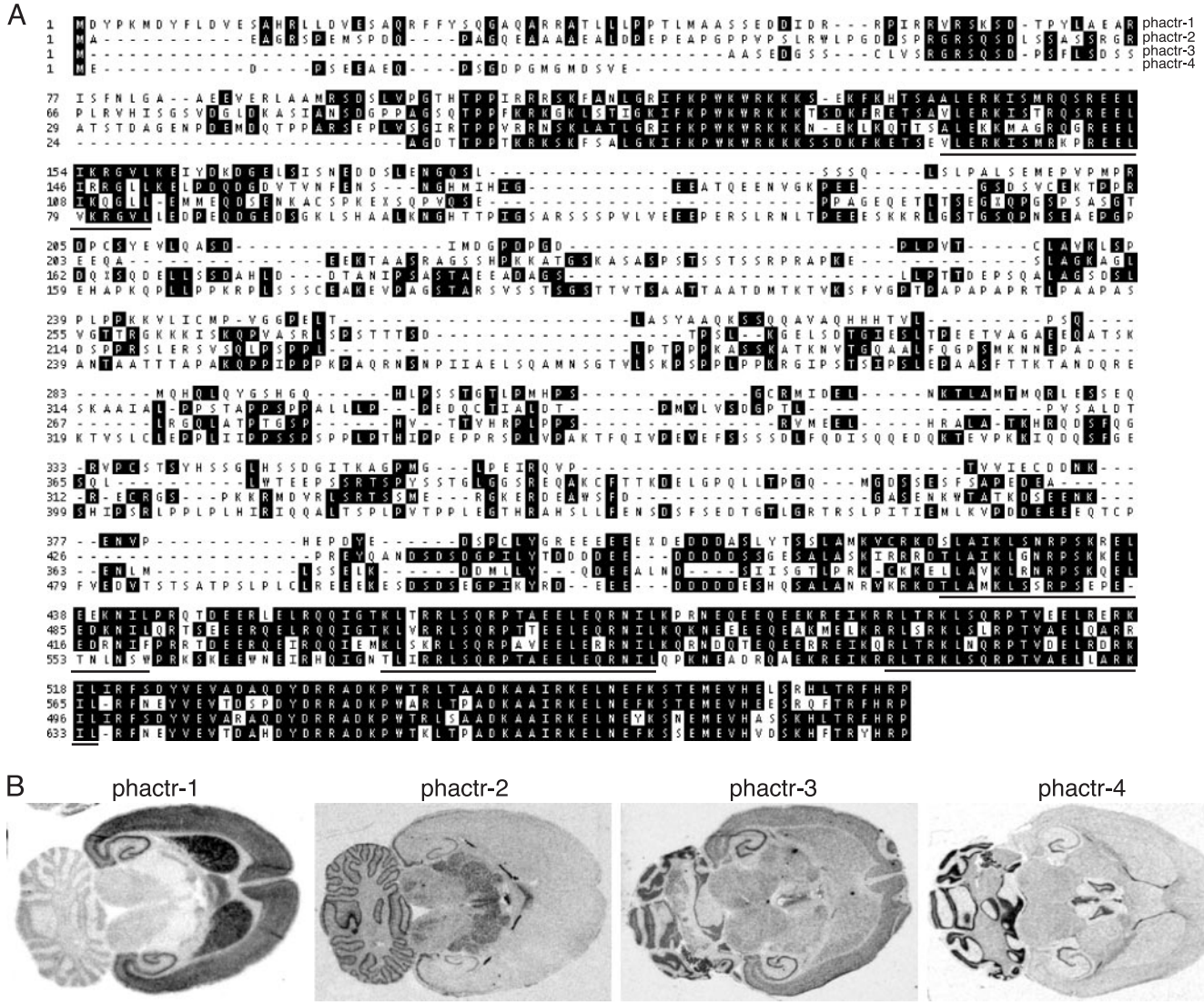

Fig. 6. Phactr family sequence homology and mRNA localization. (*A*) Amino acid similarity among members of the mouse phactr family. Alignment was performed with CLUSTAL IV algorithm. RPEL repeat sequences are underlined. (*B*) *In situ* hybridization of horizontal rat brain sections by using probes prepared for phactrs 1–4.

ventromedial hypothalamus, subthalamic nucleus, amygdala (basolateral > centromedial), lateral septum, habenula, and thalamus (reticular nucleus) (data not shown). Low (if any) expression is detected in substantia nigra pars compacta/pars reticulata and globus pallidus (data not shown). In contrast, phactr-2 is highly expressed in cerebellum (specifically in the Purkinje cell layer), choroid plexus, and thalamus (ventral, rhomboid, and anterior nuclei). Moderate to high expression of phactr-2 is also seen in hippocampus (DG = CA3 > CA1), piriform cortex, olfactory bulb, entorhinal cortex (Fig. 6), and in geniculate bodies, lamboid septal zone, preoptic area, and ventral pallidum (not shown). Phactr-3 is diffusely expressed throughout the brain (cortex = hippocampus > striatum = thalamus), whereas phactr-4 is highly expressed in periventricular regions, cerebellum (granule cell layer), and hippocampus (DG > CA3 > CA1).

Discussion

The family of known PP1 regulatory proteins continues to expand. This burgeoning family reflects the broad range of physiological processes mediated by protein phosphorylation and by PP1 in particular. Phactr-1 was found to inhibit PP1 activity toward a generic PP1 substrate (phosphorylase *a*). This result is indicative of a specific interaction but may be misleading with regard to physiological PP1 control. Phactr proteins are most likely members of a family of PP1 regulatory proteins referred to as "targeting subunits" (6). These proteins dictate PP1 subcellular localization and in some cases serve to potentiate the activity of PP1 toward select substrates at those sites. For example, in neurons, the dendritic spine protein, spinophilin, serves to facilitate the role of PP1 in regulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor responses (12, 13). A further example in the nervous system is the targeting protein yotiao, which directs PP1 activity toward the PKA phosphorylated NMDA receptor subunit NR1 (24). Both spinophilin and yotiao are potent *in vitro* inhibitors of PP1 activity toward phosphorylase *a*. This finding is presumably a reflection of inappropriate biochemical context, and it highlights the need to identify possible phosphoprotein substrates regulated specifically by phactr/PP1 holoenzyme complexes. PP1 is highly enriched in spine heads and in PSD fractions prepared from brain homogenate (6, 22, 23, 25–27). Phactr-1 colocalized with

PP1 at synapses and thus may play a role in regulation of substrates controlling synaptic activity.

The phactr proteins display an unusual structure in terms of their PP1-binding domains. A consensus sequence has emerged that is shared by many PP1-binding proteins: R/K-R/K-hydrophobic-X-F/W (6, 22, 23). This signature is lacking in the phactr proteins, although mutation of a single F residue in the PP1-binding region did produce a dramatic reduction in PP1 affinity, suggesting a conserved requirement for hydrophobic interactions. However, as with other characterized PP1 targeting protein interactions, multiple binding sites are likely to play a role. The unusual structure of the phactr proteins raises the possibility that they may bind to PP1 at a site distinct from that of the conventional PP1 targeting subunits, potentially allowing the formation of multiprotein complexes.

During preparation of this manuscript, a report was published describing "scapinin," a component of the nuclear scaffold found to be down-regulated upon induction of differentiation in the HL60 cell line (28). Scapinin (nuclear scaffold-associated PP1-inhibiting protein) is identical to phactr-3, and was shown to interact with PP1. A notable finding from this study was that scapinin exhibited distinct nuclear localization; this observation is in sharp contrast to the distribution displayed by phactr-1 both in neurons and in the N2a cell line, where it is apparently absent from cell nuclei. Thus, in addition to the strikingly differential distribution patterns of phactr transcripts in rat brain, distinct subcellular distributions also appear likely. A qualification: one possibility that will require further attention is the potential for subcellular translocation of the phactr proteins (see below).

The highest sequence similarity among phactr isoforms resides in their C termini, in the neighborhood of the actin- and PP1-binding domains. The interaction of phactr proteins with β-cytoplasmic actin suggests that this protein family may play a role in the regulation of cytoskeletal dynamics. The ability to coimmunoprecipitate actin from the soluble fraction of cell lysates with high efficiency suggests that phactr proteins associate with soluble G-actin. The PP1-targeting subunits, spinophilin and neurabin, are also actin-binding proteins, but they appear to serve the distinct role of cross-linking filamentous actin into bundles (29, 30). In accordance, neither spinophilin nor neurabin coimmunoprecipitate efficiently with G-actin. The interaction between spinophilin and F-actin is regulated by phosphorylation in the spinophilin actin-binding domain, and this mechanism may contribute to regulation of the F-actin lattice that exists in dendritic spines (31). The phactr proteins, by sequestering G-actin, may conceivably inhibit actin polymerization.

Actin binding takes place in the region of phactr-1 containing RPEL repeats. A recent report (32) demonstrated that the RPEL repeat-containing protein, MAL, also binds to G-actin. MAL was found to function as a transcriptional coactivator by means of its interaction with serum-response factor. Serum stimulation results in translocation of MAL from cytosol to nucleus in a manner dependent on Rho activation. Mutation of RPEL repeats in MAL prevents its interaction with G-actin and disrupts nuclear translocation. This raises the possibility that subcellular distribution of the phactr proteins may be regulated in a similar manner by interaction with G-actin by means of RPEL repeats.

Analysis of the primary sequence of the phactr proteins reveals multiple consensus motifs for phosphorylation. Phactr-1 contains eight predicted sites for PKA phosphorylation and seven for PKC. These sites are clustered into two groups near the RPEL repeats found in the N- and C- termini of the molecule. Phosphorylation at these sites, therefore, has the potential to alter functional interactions of phactr with the actin cytoskeleton and thus may regulate PP1–substrate interactions. Further investigation to determine the structural basis and functional consequences of the molecular interactions of phactr proteins may possibly permit the design of intervention strategies with which to test the role of these complexes in the control of neuronal function.

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