Protein phosphatase 1 (PP1) is found in the cell nucleus and has been implicated in several aspects of nuclear function. We report here the cloning and initial characterization of a novel protein named phosphatase 1 nuclear targeting subunit (PNUTS). This protein interacts with PP1 in a yeast two-hybrid assay, is found in a stable complex with PP1 in mammalian cell lysates, and exhibits a potent modulation of PP1 catalytic activity toward exogenous substrate in vitro. PNUTS is a ubiquitously expressed protein that exhibits a discreet nuclear compartmentalization and is colocalized with chromatin at distinct phases during mitosis. The subcellular localization of PP1 and the activity toward substrates involved in many aspects of cell physiology have previously been shown to be regulated by association with noncatalytic targeting subunits. The properties of PNUTS are consistent with its role as a targeting subunit for the regulation of nuclear PP1 function.

Protein phosphatase 1 (PP1) is a serine/threonine phosphatase that exerts control over many aspects of cellular physiology by reversing the actions of protein kinases, which include protein kinase A and protein kinase C. As a consequence, PP1 is critical to the regulation of diverse processes, such as cell division, muscle contraction, gene expression, glycolysis, metabolism, and neurotransmission (1, 2). PP1 is ubiquitously expressed and is distributed into multiple subcellular compartments. Although in vitro the catalytic subunit of PP1 exhibits a broad substrate specificity, the concerted action of the enzyme in the cell is thought to be directed by interaction of the catalytic subunit with a family of regulatory proteins. This family includes proteins, such as inhibitor-1, its neuronal homologue DARPP-32, inhibitor-2, and NIPP-1, which respond to distinct extracellular stimuli to regulate PP1 activity. A separate subfamily of PP1 regulatory proteins, referred to as targeting subunits, are thought to direct PP1 to specific subcellular locations and, in some cases, to modulate the activity of the enzyme toward specific substrates at these sites. For example, the glyogen binding targeting subunits in skeletal muscle and liver mediate the regulation of PP1 in response to insulin and epinephrine. In addition, PP1 associated with distinct targeting subunits bound to the myofibrils of smooth and striated muscle displays an enhanced rate of myosin dephosphorylation and reduced activity toward the enzymes of glycolysis metabolism (3). Recent biochemical evidence suggests that a number of additional targeting proteins remain to be identified (4, 5).

There is evidence that PP1 plays a critical role in regulating nuclear processes. For example, PP1 has been shown to be important for exit from mitosis in yeast, fungi, and mammalian cells (6–11). Furthermore, RNA splicing appears to be regulated by type 1 phosphatase (12, 13), and PP1 has been shown to interact with a splicing factor (14). PP1 has also been shown to exist in high molecular weight complexes in nuclear extracts, and this can be accounted for in part by association of PP1 with the inhibitory polypeptide NIPP-1 (5). However, it seems clear that there are additional nuclear binding partners that are likely to play a role in directing nuclear PP1 function. We have focused our attention on potential PP1 regulatory proteins that are expressed in the nervous system and have used a yeast two-hybrid screen to identify a novel protein that exhibits properties expected of a nuclear PP1 targeting subunit.

**EXPERIMENTAL PROCEDURES**

**Library Construction and Yeast Two-hybrid Screening—**cDNA for library construction was synthesized from 5 μg of oligo(dT)-purified total rat brain mRNA using the adapter 5'-pGACTAGTTCTAGATCGC-GAGGCCGCGCC(T)15 to prime first strand synthesis with SuperScript II reverse transcriptase (Life Technologies, Inc.). Second strand synthesis was achieved using nick translational mRNA replacement. The SacI adapter 5'-TCGACCCAGGGTCCG/5'-pCGAGCCTGTTGG was ligated to double stranded DNA followed by digestion with NotI. cDNAs (~3.5 × 10⁶) were directionally subcloned into the NotISalI-digested GAL4 activation domain expression vector pPC86 (15). The cDNA for PP1α was expressed as a GAL4 fusion in the expression vector pChES (15).

HFC7 yeast cells (16) were transfected by the lithium acetate method: for the library transformation, a 1-liter culture was grown to an A₆₀₀ of 0.5 and resuspended in 20 ml of 0.1 M lithium acetate, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA. 500 μg of library plasmid DNA was supplemented with 10 mg of denatured salmon sperm DNA and mixed with the cell suspension. 140 ml of 40% polyethylene glycol 4000 in lithium acetate buffer was added to the cells, which were then incubated for 30 min at room temperature. 17.6 ml of DMSO was added, followed by heat shock at 42 °C for 6 min. Cells were then pelleted, resuspended, grown in 1 liter ofYPD medium at 30 °C for 1 h, replated, and plated on selective media.

*lecZ expression was detected by filter assay: colonies were scraped onto Whatman no. 1 paper and lyed by freezing in liquid nitrogen and thawing. The filter was developed in 38 mM 2-mercaptoethanol, 0.034% X-Gal, 100 mM NaPO₄ buffer, pH 7.0, 10 mM KCl, 1 mM MgSO₄ at 30 °C. Yeast plasmid DNA was extracted by resuspending cells in 0.2 ml of breaking buffer: 2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, adding 0.3 g of 0.5-mm acid-
Protein Phosphatase 1 Nuclear Targeting Subunit

washed glass beads (Sigma) plus 0.2 ml 25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol and vortexing for 2 min followed by microcentrifugation for 5 min. DNA was ethanol precipitated and used to transform XL1 Blue Escherichia coli.

cDNA Expression and Immunoprecipitation—The expression vector pcDNA3 (Invitrogen) was modified using a synthetic double stranded oligonucleotide (5′-pGG CCG GGT GGG GGC ATG GAC GGC GAC GAT GAC AAA GAT GGG TGG TCG ACC C; 5′-pGG CCG GTG CAC ACC TTT GTC ATC GTC GTC GAT GTA GTC CAT GGC TGC TGG A) that was ligated into the HindIII/NotI-digested plasmid. This oligonucleotide provided a ribosomal RNA binding site, an initiation codon, followed by sequences encoding the GAL4 DNA-binding domain fused to residues 309–691 of the PP1-binding protein cDNA). The probe was immobilized on an amino-terminal FLAG tag. The tagged protein was expressed transiently to a high level in 293T cells and immunoprecipitated with 1 mg of either PP1 isoform specific antibody (17) and 10 μl of protein A-Sepharose (Pharmacia). Following several washes in lysis buffer and one in 50 mM Tris-HCl, pH 7.0, complexes were solubilized by boiling in SDS-PAGE sample buffer. Proteins were separated by SDS-PAGE and immunoblotted as described below. Immunoprecipitation from PC12 cells followed the same procedure except that tissue was dissected without using a probe and lysates were harvested with protein A-Sepharose prior to incubation with a protein A-Sepharose-primary antibody complex with or without preabsorption of the synthetic immunization peptide.

cDNA Cloning, Characterization and Expression Pattern—The 5′ end of the cDNA was characterized using four clones isolated from a rat hippocampal λZAP cDNA library that was kindly provided by Dr. Martin Colmar (Stoffel). Sequencing of both cDNA strands and the 3′ end were performed using 5′-32P end-labeled oligonucleotides (residues 384–407) was provided by Dr. Janet Crawford (W. M. Keck Foundation, Yale University). For immunization, 5 mg of peptide was conjugated to 33 mg of thyroglobulin (Sigma) using 2.5 ml of 0.2% glutaraldehyde added dropwise over 20 min at 4 °C followed by further incubation at 4 °C with mixing for 2 h. The reaction was quenched with 1.8 mg of sodium borohydride in 15 ml of water. The mixture was dialyzed extensively against PBS and used for immunization of two rabbits: RU154 and RU155 (Cocalico Biologicals). Crude sera were affinity-purified on a column of immunizing peptide coupled to SulfoLink (Pierce), which was prepared according to the manufacturer’s recommendation. A second round of affinity purification was against purified GST-110 fusion protein. This protein was first dialyzed extensively against PBS and then immobilized on cyanogen bromide activated Sepharose 4B according to the manufacturer’s recommendation.

Indirect Immunofluorescence—PC12 cells were grown overnight on 30-mm Nunclon tissue culture dishes coated with 0.1 mg/ml poly-D-lysine and fixed for 15 min in 4% paraformaldehyde-PBS. Cells were permeabilized and blocked in PBS containing 1% bovine serum albumin, 5% fetal calf serum, and 0.1% saponin for 1 h. Cells were then incubated with primary antibody RU154 (1:200) for 2 h at room temperature, washed with PBS, and incubated for 45 min with Texas red-conjugated goat anti-rabbit immunoglobulin (Rockland). After washing three times with PBS, cells were stained for DNA with DAPI for 10 min, and the coverslips were mounted with Fluormount (Fluka). We used a rat brain cDNA-GAL4 expression plasmid library and a “baits” hybrid protein consisting of PP1α fused to the GAL 4 DNA binding domain. Out of 60 clones analyzed, clone 14 was one of two cDNAs isolated that appeared to be derived from the same gene based on restriction fragment size analysis (data not shown). Clone 14 appeared to interact specifically with PP-1 because it would not activate transcription of yeast reporter genes either independently or in combination with irrelevant baits. This clone contained an ~3-kilobase cDNA insert and partial nucleotide sequencing followed by data base searches revealed that it represented a novel protein.

Preparation of Bacterial GST Fusion Protein—A 1118-base pair PFM 1 blunted-ended/SalI fragment was purified from clone 14. This was ligated into NotI-blunted/SalI-digested pGEX-4T-2 (Pharmacia) to direct expression of GST fused to residues 309–691 of the PP1-binding protein. The protein was expressed in BL21 DE3 E. coli by induction of log phase cells with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside at 30 °C for 3 h. Cells were resuspended in ice-cold PBS followed by cell lysis using a French press. Triton X-100 was added to 1%, and the lysate was centrifuged for 5 min at 10,000 × g at 4 °C. The supernatant was loaded onto a glutathione-agarose affinity column (Pharmacia) and washed extensively with PBS. The fusion protein was eluted with 5 mM glutathione, 50 mM Tris-HCl, pH 6.0. Protein Phosphatase Activity Assay—[32P]Phosphorylation and purified rabbit muscle PP1 and PP2A were provided by Dr. Hsien-bin Huang. Preparation and activity assays were performed essentially as described (19). The phosphatase assay was carried out with PP1 or PP2A (~150 pm) in 50 mM Tris-HCl, pH 7.0, 0.1% 2-mercaptoethanol, 0.3 mg/ml bovine serum albumin, 0.01% Brj 35, 1 mg/ml [32P]phosphorylase, 5 mM caffeine, 0.1 mM EGTA with or without varied concentrations of GST and GST-14. PP1 and the bacterial GST proteins were pre-incubated at 30 °C for 10 min. The reaction was initiated by addition of substrate, carried out for 10 min at 30 °C, and stopped by trichloroacetic acid precipitation. Radioactivity present in the supernatant was determined by Cherenkov counting.

RESULTS

Identification of a Novel Gene Product Interacting with PP1—To identify proteins capable of interacting with the α isoform of PP1, we performed a yeast two-hybrid screen (20). We used a rat brain cDNA-GAL4 expression plasmid library and a “baits” hybrid protein consisting of PP1α fused to the GAL 4 DNA binding domain. Out of 60 clones analyzed, clone 14 was one of two cDNAs isolated that appeared to be derived from the same gene based on restriction fragment size analysis (data not shown). Clone 14 appeared to interact specifically with PP-1α because it would not activate transcription of yeast reporter genes either independently or in combination with irrelevant baits. This clone contained an ~3-kilobase cDNA insert and partial nucleotide sequencing followed by data base searches revealed that it represented a novel protein.

To obtain an independent evaluation of the protein-protein interaction suggested by the two-hybrid result, we examined the ability of the protein product (residues 309–691) to coimmunoprecipitate with PP1α in 293T cell extracts. Clone 14 was subcloned into a mammalian expression vector designed to include an amino-terminal FLAG tag. The tagged protein was expressed transiently to a high level in 293T cells and immunoprecipitated first using an anti-FLAG antibody. Proteins present in the immunocomplex, as well as SDS extracts

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from the soluble and particulate fractions, were separated by SDS-PAGE and immunoblotted sequentially using anti-FLAG and anti-PP1α antibodies (Fig. 1A). A proportion of the over-expressed clone 14 recombinant protein was present in the particulate fraction, whereas the majority appeared in the soluble fraction at ~65 and ~60 kDa. The lower ~60 kDa species was scarce in the particulate fraction, whereas in the soluble fraction it became relatively more abundant during the immunoprecipitation assay. This suggested that the amino-terminal FLAG tagged ~60 kDa protein is generated by proteolysis and that removal of the carboxyl terminus of the recombinant protein largely prevents partition to the particulate fraction. The strong signal seen for PP1α in immunoprecipitations performed with cells expressing clone 14 indicated that in 293T cell lysates, the two proteins exist in a complex.

The nucleotide sequence near the 5′ end of the partial cDNA gave predicted amino acid sequence that was used to design a peptide for injection into rabbits to raise antisera. Affinity-purified antibody was used for immunoprecipitation and Western blotting. A protein was detected in PC12 cell lysates that migrated in SDS-PAGE with an apparent molecular mass of ~110 kDa (Fig. 1B). Immunoprecipitation using this antibody indicated that in PC12 cell lysates both PP1α and PP1γ isoforms form a complex with the ~110-kDa protein (Fig. 1B). Preabsorption of the anti-clone 14 and anti-PP1α antibodies with their respective peptide immunogens abolished the signals seen in subsequent immunoblots, demonstrating the specificity of the coimmunoprecipitation assay. PP1α is known to undergo a cell cycle-dependent inhibitory phosphorylation of the carboxyl terminus (21). Using synchronized cell cultures and phosphorylation state-specific antibodies, we observed no selective coimmunoprecipitation of the PP1-binding protein with the phospho- or dephospho-form of PP1α (not shown).

**Clone 14 Expression Pattern**—Northern blotting analysis revealed the presence of an ~4.2-kilobase mRNA transcript in all tissues examined, with the highest levels being found in testis (Fig. 2A). The cDNA probe employed did not reveal alternatively spliced transcripts.

The expression pattern of the endogenous protein represented by clone 14 was examined in various rat tissues by Western blotting (Fig. 2B). A protein migrating with an apparent molecular mass of ~110 kDa was detected by anti-peptide antibodies purified from two separate rabbit antisera, as well as by antibody raised against a GST-14 fusion protein (data not shown). Immunoreactivity was present in all tissues examined and was highest in testis, brain, and intestine. Very low levels were seen in heart and skeletal muscle. The human cell lines examined included the embryonic kidney-derived line 293T (Fig. 2B) and the epithelial line HeLa (not shown). In these cells, a protein migrating with a slightly higher molecular mass (~120 kDa) was detected, presumably reflecting a species difference. Cell fractionation studies indicated that in the 293T cell line, both the endogenous protein and the expressed rat protein (see below) were almost entirely associated with the pellet following a 1000 × g spin. This fraction contains large particulate material, including nuclei. Subsequent treatment of the pellet with high salt and lysis of nuclear and vesicular components released much of the ~110/120-kDa proteins into the soluble fraction (Fig. 2C).

The developmental time course of expression in the rat brain was examined by Western blotting of cortical extracts taken at various developmental stages (Fig. 2D). Expression during embryogenesis showed a gradual decrease to a relatively low level in the adult, implying that this protein may play a prominent role in the brain during cortical development.

**Sequence Analysis of the PP1-binding Protein**—Four overlapping cDNA clones were isolated from a rat hippocampal ZAP cDNA library to characterize the full-length cDNA for clone 14. The predicted amino acid sequence is shown in Fig. 3. The putative initiating methionine was identified as the first residue in a long open reading frame of 2616 nucleotides, lying in the context of a sequence possessing 80% compliance with the consensus eukaryotic ribosome binding sequence (22). The predicted molecular mass of this protein is 92.8 kDa, which differs from the apparent molecular mass of ~110 kDa seen in SDS-PAGE for both the expressed cDNA and for the endogenous rat protein. This may be due to an extended conformation and/or low SDS binding capacity. In addition, the glycine residue following the initiating methionine might be subject to ristrylstlation. The protein is predicted to be basic, with a theoretical pl of 9.2.

The human EST data base contains a sequence that presumably represents the human homologue of the rat PP1-binding protein (see “Discussion”). Other than this, data base searches did not reveal any significant similarity to any known proteins or protein domains. However, several noteworthy motifs are present (Fig. 3). Two stretches of basic residues are present toward the amino terminus, either of which may serve as
nuclear localization signals. These motifs are not present in the truncated protein that was expressed from the yeast two-hybrid isolate; their absence may account for the preferentially cytosolic distribution of this recombinant protein in 293T cells (Fig. 1A). Three consensus SH3 domain binding sites are present (23), suggesting a potential for complex formation with proteins other than PP1. In addition, an ATP/GTP phosphate binding (Walker A) motif is found (24). This raises the possibility that nucleotide triphosphate binding may influence the function of the protein.

The carboxyl terminus of the protein contains several closely spaced RGG sequences, motifs that are commonly found in RNA-binding proteins (25). This signature plus the predicted nuclear localization of the protein raise the possibility that it...
plays a role in transcription or RNA processing. Carboxyl-terminal to the RGG boxes is a region rich in histidine, part of which falls into three repeats of 14 residues. Following this, three additional pentamer repeats are found, and a significant number of histidines are again spaced at the interval of 14 residues (i.e. His<sup>598</sup>/His<sup>602</sup>/His<sup>604</sup> and His<sup>614</sup>/His<sup>626</sup>). The structural and functional significance of the organization of this region is unclear at present.

The extreme carboxyl terminus possesses a single putative Zn<sup>2+</sup> finger with the signature C-X<sub>8</sub>-C-X<sub>5</sub>-C-X<sub>3</sub>-H. This motif has been identified in the Nup<sub>475</sub>/Tis11 family of immediate early response genes (26, 27). However, those proteins are distinguished by a tandem repeat of this motif rather than the single example seen here. The functional role of this unusual Zn<sup>2+</sup> finger motif present in the Nup<sub>475</sub>/Tis11 family of proteins has not been reported.

Subcellular Localization of the PP1-binding Protein—We examined the cellular distribution of the PP1-binding protein by indirect immunofluorescence. Freely dividing PC12 cells were fixed, permeabilized, and stained with an antibody (RU154) followed by anti-rabbit IgG conjugated to Texas red as secondary antibody, A (interphase), B (anaphase), and C (telophase) show DAPI-stained cellular DNA. D, E, and F show the RU154 staining of cells in A, B, and C, respectively. The results shown are representative of several individual experiments in which similar results were obtained. G shows an immunoblot of total PC12 cell 1% SDS homogenate probed with antibody RU154 and indicates the absence of significantly cross-reactive species.

The present results provide evidence for a stable complex between PP1 and a novel interacting protein. This protein exhibits a discreet nuclear localization and is a potent modulator of PP1 catalytic activity. We therefore propose to name the protein PNUTS. This acronym reflects its putative role as a phosphatase 1 nuclear targeting subunit. The affinity between PP1 and PNUTS appears to be relatively high. This is suggested both by the strong signals seen in communoprecipitation experiments and by the inhibitory potency in enzyme assays. Preliminary studies suggest that the mode of PP1 binding and inhibition may be complex, possibly involving more than one site, as has been shown for other PP1 regulatory subunits (28–30).

Certain high molecular weight forms of PP1 have previously been characterized biochemically. In particular, Jagiello et al. (5) identified a protein of 111 kDa present in rat liver nuclear extracts that binds to PP1 and inhibits activity toward phospho-Rh. This protein may correspond to PNUTS. In addition, Nelson et al. (35) described unknown proteins of 125 and 110 kDa that are present in mitotic extracts and that bind to PP1. These proteins, one of which may also correspond to PNUTS, are present in cell fractions containing high molecular weight PP1 species that display significant activity toward phosphorylase a and toward phospho-Rh. This raises the possibility that PNUTS may be present in a protein complex containing PP1 activity toward Rh. We performed communoprecipitation experiments to examine the Rh content in PP1 complexes and found a small fraction of hypophosphorylated Rh to be complexed with PP1, as previously reported (31). However, no Rh protein could be detected in an immune complex of PNUTS and vice versa, suggesting that the binding of these two proteins to PP1 is mutually exclusive.2

PNUTS possesses structural signatures that might be expected of an RNA-binding protein. In particular, the RGG boxes present in the carboxyl terminus have been shown to be associated with RNA-binding proteins (25). Previous studies have suggested a role for PP1 in the regulation of RNA splicing (12, 13, 32), and a recent two-hybrid screen identified a splicing

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2 P. B. Allen, Y.-G. Kwon, A. C. Nairn, and P. Greengard, unpublished data.
factor that can form a complex with PP1 (14). PNUTS therefore represents a potential targeting protein for coupling PP1 to the nuclear RNA processing machinery. In addition to these RGG boxes, there is an atypical Zn$^{2+}$ finger and an unusual histidine-rich repeat region. It will be of interest to determine the nucleic acid binding potential of these various regions and their contribution to the targeting of PNUTS to the nucleus. During the incubation of 293T cell lysates for immunoprecipitation experiments (Fig. 1A), the carboxyl terminus of a proportion of the recombinant PNUTS appeared to be lost due to proteolysis. This was accompanied by a shift in the partition of the truncated protein from the particulate to the soluble fraction, implying that the carboxyl terminus is at least partially responsible for the localization of the protein to the particulate fraction. It therefore seems possible that this region could help target the protein to the nucleus, perhaps in addition to the more amino-terminal putative nuclear localization motifs. Curriously, two of the three consensus motifs for SH3 domain binding are found within the region rich in RGG boxes. The presence of both sets of motifs within the same domain of the protein suggests the possibility of a mutually exclusive binding for an SH3 domain-containing protein or nucleic acid. Alternatively, PNUTS may serve to cross-link an SH3 domain-containing protein to nucleic acid.

PP1 has been shown to be important for exit from mitosis both by genetic analyses (7–10) and in microinjection studies (6, 11). However the relevant substrates for PP1 at the exit from mitosis have not been defined. Recent work in both yeast and mammalian cells has indicated that phosphorylation of the carboxyl terminus of PP1 by cdc2 inhibits enzyme activity (33, 34) and that this phosphorylation shows a distinct peak during early to mid-mitosis, with subsequent dephosphorylation presumably reactivating PP1 and allowing exit from mitosis (21, 36). Immunofluorescence microscopy further showed that the phosphorylated form of the enzyme was localized to nonchromosomal regions during anaphase (21). That staining is very similar to that of PNUTS during anaphase, after which phospho-PP1 staining decreases and PNUTS appears to translocate to chromosomal regions. Coimmunoprecipitation studies from synchronized PC12 cell cultures show that although phospho-PP1 was complexed with PNUTS, there was no significant cell cycle-dependent shift in total PP1 complexed. PP1 has been reported to accumulate in the nucleus and to colocalize with chromatin at mitosis (6). Thus, only a proportion of mitotic PP1 is presumably complexed with PNUTS, although the phospho form is preferentially colocalized. It seems likely that a comprehensive description of the role of PP1 during mitosis may require characterization of additional PP1 targeting proteins.

The sequence of what appears to be the human homologue of PNUTS has recently been submitted to the GenBank data base (accession no. Y13247). The human gene for this protein lies in the HLA class 1 region on the short arm of chromosome 6 within a region implicated in hereditary hemochromatosis (37). The only region of extensive diversity between human and rat appears in the carboxyl-terminal histidine-rich region, with the human protein possessing several additional histidine-rich repeat motifs. This divergence presumably explains the slower migration of human PNUTS in SDS-PAGE. In addition, significant sequence divergence occurs within the consensus motif for nucleotide phosphate binding; the serine residue in the consensus GxxGxGxxS is converted to a glycine in the human sequence. Although this casts some doubt on a potential nucleotide binding function for PNUTS, it should be noted that in proteins of the adenylate kinase family the P-loop contains a glycine following the invariant lysine (24), indicating that this residue might be tolerated in a human PNUTS P-loop.

The classical PP1 targeting subunits are thought to function in part by bringing enzyme and substrate into close proximity (3). A major goal underlying the identification of novel targeting subunits for PP1 is to provide insight into the substrates that are responsible for mediating the diverse functions of this enzyme. Future efforts to describe the role of nuclear PP1 will focus on the nucleic acids, potential PP1 substrates, and other proteins that are predicted to interact with PNUTS.

Note Added in Proof—Work describing the interaction of PP1 with what appears to be the human homolog of PNUTS has recently been reported (38).

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Isolation and Characterization of PNUTS, a Putative Protein Phosphatase 1 Nuclear Targeting Subunit
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