Calmodulin Binds to and Inhibits the Activity of the Membrane Distal Catalytic Domain of Receptor Protein-tyrosine Phosphatase α*

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cDNA expression library screening revealed binding between the membrane distal catalytic domain (D2) of protein-tyrosine phosphatase α (PTPα) and calmodulin. Characterization using surface plasmon resonance showed that calmodulin bound to PTPα-D2 in a Ca²⁺-dependent manner but did not bind to the membrane proximal catalytic domain (D1) of PTPα, to the two tandem catalytic domains (D1D2) of PTPα, nor to the closely related D2 domain of PTPμ. Calmodulin bound to PTPα-D2 with high affinity, exhibiting a Kₐ −3 nM. The calmodulin-binding site was localized to amino acids 520–538 in the N-terminal region of D2. Site-directed mutagenesis showed that Lys-521 and Asn-534 were required for optimum calmodulin binding and that restoration of these amino acids to the counterpart PTPε sequence could confer calmodulin binding. The overlap of the binding site with the predicted lip of the catalytic cleft of PTPα-D2, in conjunction with the observation that calmodulin acts as a competitive inhibitor of D2-catalyzed dephosphorylation (Kᵢ −340 nM), suggests that binding of calmodulin physically blocks or distorts the catalytic cleft of PTPα-D2 to prevent interaction with substrate. When expressed in cells, full-length PTPα and PTPα lacking only D1, but not full-length PTPβ, bound to calmodulin beads in the presence of Ca²⁺. Also, PTPα was found in association with calmodulin immunoprecipitated from cell lysates. Thus calmodulin does associate with PTPα in vitro but not with PTPα-D1D2 in vitro, highlighting a potential conformational difference between these forms of the tandem catalytic domains. The above findings suggest that calmodulin is a possible specific modulator of PTPα-D2 and, via D2, of PTPα.

Protein-tyrosine phosphatase α (PTPα)¹ is a ubiquitously expressed, yet brain-enriched, receptor that acts as a positive regulator of the tyrosine kinases Src and Fyn (1–5), as a regulator of the Kv1.2 potassium channel (6), and as a potential modulator of insulin receptor signaling (7). Structurally, PTPα has a short heavily glycosylated extracellular region, a transmembrane region, and an intracellular region containing two conserved catalytic domains. The membrane proximal catalytic domain (D1) is responsible for most if not all of the phosphatase activity measured in vitro and toward an in vivo substrate such as Fyn (8–11). The membrane distal catalytic domain (D2), while possessing a distinct, yet weaker in vitro activity, does not dephosphorylate any cellular substrates identified to date.

A conserved D2 domain is present in many receptor-like PTPs (RPTPs), yet all have undetectable or extremely low activity. This indicates a non-redundant function of the tandem domains, with D2 perhaps playing a regulatory role. The RPTPs CD45, LAR, PTPμ, and PTPα all display altered D1 activity or in vitro substrate specificity in the absence of D2 (9, 12–17). CD45-D2 is specifically required, independent of any catalytic activity, for the in vivo recognition by CD45-D1 of its substrate, the T-cell receptor ζ-chain (18). The in vitro interaction of PTPα-D2 with D1 of PTPα inhibits PTPα-D1 activity, suggesting that D2 may regulate the formation and activity of receptor heterodimers (19). Alternatively, some D2 domains may have a catalytic function but possess a highly restricted or unusual substrate specificity. RPTPs that this could apply to include, for example, PTPα and LAR, the D2 domains of which have minimal substitutions in the 42 residues identified as conserved among all D1 domains and thus likely to be essential for catalysis (20). Both D2 domains assume the folding of their respective D1 domains, as predicted by PTPα-D2 modeling (11) and shown for LAR-D2 within the LAR crystal (21). The D2 domain of PTPα clearly has intrinsic activity, which with some in vitro substrates can be as much as 10% of the D1 activity (8–10). Substitution of two amino acids in PTPα-D2 or LAR-D2 with Tyr and Asp that are conserved in these positions in all D1 domains confers a D1-equivalent catalytic competence, but the mutated D2s retain a distinct non-D1-like substrate specificity (11, 21, 22). Crystal structure determination of PTP1B bound to substrate has shown that the aromatic ring of the conserved Tyr is involved in interactions with substrate phosphotyrosine and that the conserved general acid Asp is in close proximity to the bound phosphotyrosine (23). The equivalent residue in PTPα-D2 and LAR-D2 is hydrophobic but not aromatic, whereas Asp is replaced by the acidic residue Glu which is more distant from the substrate phosphate. Thus, essential catalytic amino acids are intact, and the D2 domain is folded correctly, but positioning and protonation of the conventional phosphotyrosine in the active site pocket is not optimal.

Protein-protein interactions are key determinants of cell regulation and signal transduction events. Several such interactions have been described for PTPα and proposed to modulate its function. The extracellular region of PTPα associates in cis...
with the glycolyosphatidylinositol-linked cell surface molecule cortactin, possibly forming a receptor complex in which PTPα transduces a signal to activate the tyrosine kinase Fyn (24). A proposed dimerization of D1 involves the interaction of the N-terminal “wedge” region of one D1 domain with the active site of the partnering D1 domain, with consequent inhibition of catalytic activity (25). This wedge region can also interact in trans with the D2 domains of PTPα and other RPTPs, perhaps affecting PTPα dimer formation through the wedge-to-D1 interaction described above (26). A phosphotyrosine located in the C-terminal tail region binds to Grb2-SH2 and Src-SH2. The former binding facilitates the subsequent binding of the Grb2-SH3 domain to a region in D1, which is proposed to inhibit D1 catalysis (27–30). Binding of the SH2 domain of Src to the same phosphotyrosine in the PTPα protein was observed.

**Experimental Procedures**

**Expression Plasmids—**Numbering of the PTPα and PTPβ amino acid sequences is according to Krueger et al. (32). The recombinant and mammalian expressed proteins used throughout this study are defined in Table 1. All mammalian expression plasmids pGEX-KG containing PTPα-D1, PTPα-D2, PTPα-D1D2, and PTPα-D2 have been described (9). The mammalian expression plasmids pXJ4-neo containing VSVG-tagged PTPα and PTPα-D2/AD1 have been described (11, 33). PTPα tagged at the C terminus with FLAG was provided by L. Zeng. The plasmid pGEX-4T1-PTPα-D2 was constructed by insertion of a D2 fragment corresponding to aa 453–774 into pGEX-4T1 (Amersham Pharmacia Biotech) to create pGEX-2TK-PTPα. The plasmid pGEX-4T1-PTPα-D2 was constructed by removing the D2 fragment from pGEX-KG-PTPα-D2. Nucleotides encoding PTPα-D2 (aa 486–774) were excised from pGEX-KG-PTPα-D2 and inserted into pGEX-2TK (Amersham Pharmacia Biotech) to create pGEX-2TK-PTPα-D2. The High Fidelity Taq DNA Polymerase kit (Roche Molecular Biochemicals) was used to amplify PTPα-D2 (aa 502–521), PTPα-N2 (aa 520–538), and PTPα-N2 (aa 422–440) as described by the manufacturer. Cells were harvested 24–36 h after transfection. The empty expression plasmid pXJ4-neo was used as a control.

**Immunoprecipitation and Western Blots—**Monoclonal antibodies toward VSVG (Sigma), FLAG (Sigma), and calmodulin (Upstate Biotechnology) were used for immunoprecipitation and Western blotting. Transfected NIH3T3 cells were lysed in buffer A (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Brij-96, 20 μg/ml aprotinin, 2 μg phenylmethylsulfonyl fluoride), and the lysates were clarified by centrifugation. For immunoprecipitation, cell lysates were incubated with antibodies at 4 °C for 12 h. Protein G plus/protein A-agarose suspension (Calbiochem) was then added for another 3 h at 4 °C. The immunoprecipitates were washed five times in buffer B (20 mM Tris, pH 7.5, 150 mM NaCl, 0.2 mM CaCl2, 1% non-fat dry milk) for 15 min and then a total volume of 240 μl. A 20-min dissociation period was provided, and the surface was regenerated by injection of 10 μl of 0.05% SDS. Cycles of injection and regeneration followed by running buffer flow were computer programmed for the binding comparison of different samples. BIA evaluation 3.0 software (BIAcore) was used to analyze data. The Kd value was calculated according to a 1.1 interaction model by direct fitting of ligand binding at multiple concentrations around the Kd.

**Cell Culture and Transient Transfections—**COS-1 cells were obtained from American Type Culture Collection (Manassas, VA). NIH3T3 cells were a gift from P. Lobie. Cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and penicillin/streptomycin in an atmosphere of 5% CO2 at 37 °C. Transient transfections were performed at 50–70% cell confluency (100-mm dishes) using 5 μg of plasmid DNA and liposome-mediated transfection (Life Technologies, Inc.) as described by the manufacturer. Cells were harvested 24–36 h after transfection. Transfection with the empty expression plasmid pXJ4-neo was used as a control.

**Purification and Radiolabeling of GST-PTP Fusion Proteins—**Bacterially expressed GST-PTP fusion proteins were bound to glutathione-Sepharose beads (Amersham Pharmacia Biotech) and purified either by elution with reduced glutathione (Sigma) or by cleavage with thrombin (Sigma). Purified PTPα were quantitated by Bradford or BCA analysis.
formed at 30 °C in 450-μl reactions containing 50 mM sodium acetate, pH 5.5, 0.5 mg/ml bovine serum albumin, 5 mM dithiothreitol, and with 1 mM CaCl2 or EGTA added as indicated in the figure legends. Some reactions contained bovine brain or recombinant calmodulin, and similar results were obtained with either form. The N2 peptide used in the competition experiments was synthesized commercially with an automated peptide synthesizer (BTOC, NUS, Singapore) to >95% purity and added at the indicated concentrations. The apparent Km and Vmax of PTPα-D2 in the absence or presence of calmodulin was manually extrapolated from Lineweaver-Burk inverse plots of PTPα-D2 activity toward pNPP concentrations ranging from 1.25 to 10 mM. For Km determination, the linear regression of the K_{nucp}/V_{max} values (the slopes in the inverse plot) were plotted against inhibitor concentration. The intersection of the linear regression with the x axis gives the negative K_{nucp} value. All reactions were carried out at 30 °C and terminated during the linear portion of the reaction. Released p-nitrophenol was quantitated as described previously (8).

RESULTS

Identification of Calmodulin as a PTPα-D2-binding Protein—Recombinant 35S-labeled PTPα-D2 was used to screen a human cerebellum cDNA expression library. Fifteen positive clones were identified after the secondary screen, and two of these encoded calmodulin.

In Vitro Binding of Calmodulin to PTPα-D2—The recombinant and mammalian expressed proteins used throughout this study are defined in Table I. The interaction of PTPα-D2 and calmodulin was confirmed using surface plasmon resonance. PTPα-D2 bound in a Ca2+-dependent manner to bovine brain calmodulin coated on the sensor chip, with no binding observed in the presence of EGTA (Fig. 1A). In comparison, a greatly reduced but Ca2+-dependent binding occurred between calmodulin and PTPα-D1 (Fig. 1B) or PTPα-D1D2 (Fig. 1C). Increasing concentrations of PTPα-D2 resulted in increased binding (RU) to the calmodulin-coated sensor chip. The kinetics of interaction were fitted to a single interaction site model, and yielded a dissociation constant (K_D) of 2.86 × 10^{-9} M ± 0.31, suggestive of a specific and high affinity interaction.

The Calmodulin-binding Site Is in the N-terminal Region of PTPα-D2—To define the calmodulin-binding site within PTPα-D2, two constructs were generated that expressed an N-terminal portion of D2 (D2-N, aa 486–542) or the remaining C-terminal portion of D2 with the tail region (D2-C, aa 543–774) (Fig. 2A). These regions of PTPα were expressed in bacteria as GST fusion proteins, affinity purified on glutathione-Sepharose beads, and either eluted from the beads (GST-D2-N) or cleaved from the GST with thrombin (D2-C) to give soluble purified polypeptides. Their interaction with a calmodulin-coated sensor chip was analyzed by surface plasmon resonance. The D2-C polypeptide showed little or no interaction with calmodulin, as was found with a control preparation of purified GST protein (Fig. 2, B and C). However, the GST-D2-N polypeptide bound to the immobilized calmodulin with very similar kinetics to the complete D2 polypeptide (Fig. 2B). The irregularities in the D2-N binding curve may be due to the presence of degraded forms of the fusion protein that were detected by SDS-PAGE. The purified GST-D2-N fusion protein was also difficult to produce in any significant quantity. Nevertheless, these results indicate that the calmodulin-binding site resides within the N-terminal amino acids 486–542 of PTPα-D2.

Analysis of the amino acid sequence in D2-N for candidate calmodulin-binding sites identified two overlapping subregions, N1 (aa 502–521) and N2 (aa 520–538) (Fig. 2A), with...
proteins (3 m in PTP and two non-conservative replacements (Lys-521 and Asn-534) in PTP Ce amino acid sequence homology with its closest relative, PTP a. The D2 domains of these PTPs have 72% amino acid sequences of the calmodulin-binding region (αN2) of PTPα-D2 and of the analogous N2 region of PTP-B-D2 (εD2) are shown, together with the point mutations made in these sequences. Numbering of amino acids corresponds to the full-length PTPα or PTPε. B, comparison of the binding of the D2 domains of PTPε (αD2, 3 μM) or PTPα (εD2, 3 μM) to calmodulin. C, chimeric proteins (1 μM) containing the wild-type and mutant PTPε N2 sequences (aa 520–538) (shown in A) fused to the D2-C portion (aa 543–774) of PTPα were evaluated for calmodulin binding. D, chimeric proteins (3 μM) containing the wild-type and mutant PTPε N2 sequences (aa 422–440) (shown in A) fused to the D2-C portion (aa 543–774) of PTPα were evaluated for calmodulin binding. The plots of sensor response (RU) versus the time of protein association and dissociation in B–D were obtained by computerized continuous injection of proteins in running buffer containing 1 mM CaCl2 over a calmodulin-coated sensor chip.

Identification of Amino Acid Residues in PTPα-D2 That Are Essential for Calmodulin Binding—PTPα shares considerable amino acid sequence homology with its closest relative, PTPε (32). The D2 domains of these PTPs have 72% amino acid identity, yet PTPα-D2 exhibited a relatively weak interaction with calmodulin (Fig. 5, A and B). Following the identification of the N2 subregion of PTPα-D2 as the calmodulin-binding site, the corresponding N2 sequence in PTPε (aa 422–440) was examined. The 19-amino acid N2 regions were identical with the exceptions of two conservative substitutions (Asp-530 and Leu-537 in PTPα to Glu-422 and Ile-439, respectively, in PTPε) and two non-conservative replacements (Lys-521 and Asn-534 in PTPα to Asn-423 and Ala-436, respectively, in PTPε). This suggested that these amino acid differences, in particular the non-conserved residues, could be the basis for the differential interactions of PTPα-D2 and PTPε-D2 with calmodulin. Indeed, mutations of the positively charged Lys-521 of PTPα to either a negatively charged glutamic acid (K521E) or to the neutral Asn (K521N) present in this position in PTPε resulted in a greatly reduced binding to calmodulin (Fig. 3C). Mutation of neutral Asn-534 of PTPα to a positively charged lysine residue (N534K) or to the hydrophobic alanine residue (N534A) found in this position in PTPε also significantly reduced the strength of the interaction with calmodulin, although to a lesser extent than did the mutation of Lys-521 (Fig. 3C). This indicates that both Lys-521 and Asn-534 of PTPα play a role in the interaction of PTP-D2 with calmodulin and that substitution of either is sufficient to disrupt binding. Consistent with these results, a single point mutation in position 423 (N423K) of the PTPα N2 sequence (which restored the lysine residue found in this position in PTPα) was unable to restore calmodulin binding to a (PTPα-N2)-(PTPα-D2C) fusion protein. However, two simultaneous mutations of the non-conserved residues 423 and 436 of PTPα (N423K/A436N) that restored the amino acids correspondingly found in PTPα increased calmodulin binding by about 2-fold (Fig. 3D). Since the double mutation in PTPα N2 did not restore binding to a level comparable to that observed with PTPα N2, this indicates that both the conservative and non-conservative amino acid differences between the PTPα and PTPε N2 subregions contribute to the distinct calmodulin binding properties of PTPα-D2 and PTPε-D2.

Calmodulin Inhibits PTPα-D2 Activity—The phosphatase activity of PTPα-D2 was assayed toward two defined D2 substrates, pNPP, and the RR-Src peptide, with and without calmodulin. Although calmodulin alone or in the presence of added EGTA had no effect on PTPα-D2 activity, calmodulin in the presence of added Ca2+ inhibited D2 activity toward pNPP (Fig. 4A) and RR-Src (data not shown). However, neither PTPα-D1 nor PTPα-D1D2 activity was affected by calmodulin in the presence of Ca2+ or EGTA (Fig. 4B and C), consistent with the inability of these recombinant forms of PTPα to bind to calmodulin. In the presence of Ca2+, calmodulin inhibited PTPα-D2 in a concentration-dependent manner (data not shown). The inhibition exerted by calmodulin on PTP-D2 activity could be progressively blocked by the addition to the reaction of increasing concentrations of a synthetic peptide.
corresponding to the N2 sequence (Fig. 4D). Further investigation of the action of calmodulin revealed that it acts as a competitive inhibitor of PTPα-D2, with an apparent Ki of 337 nM (Fig. 5). Thus calmodulin binding reduces the affinity of PTPα-D2 for substrate.

**In Vivo Association of PTPα and Calmodulin**—VSVG-tagged full-length PTPα and VSVG-tagged PTPα lacking only D1 (PTPα-D2ΔD1) were transiently expressed in cells, and the cell lysates were incubated with calmodulin-Sepharose beads in the presence of added EGTA or Ca2+. After washing, proteins bound to the beads were eluted with EGTA. PTPα and PTPα-D2ΔD1 were both eluted from the beads that had been incubated with the lysates in the presence of Ca2+ but were not detected in eluates from beads incubated with lysates and EGTA (Fig. 6A). Thus cellular PTPα possessing the extracellular, transmembrane, and juxtamembrane regions can undergo Ca2+-dependent binding to calmodulin. The two bands that are immunoreactive with the anti-VSVG antibody (Fig. 6, A and B) represent differentially glycosylated forms of PTPα, and it is apparent that there is preferential binding to calmodulin of the lesser glycosylated, faster migrating form. In a similar experiment using lysates of cells expressing full-length PTPε, no binding of PTPε to the calmodulin-Sepharose beads was observed (Fig. 6B), in accord with the lack of binding of PTPε-D2 to calmodulin. To determine whether PTPα could bind to calmodulin in the cell, anti-calmodulin immunoprecipitates of cell lysates were probed for the presence of PTPα. PTPα was found in association with calmodulin (Fig. 6C).

**DISCUSSION**

We have shown that calmodulin binds to and modulates the phosphatase activity of PTPα-D2. Calmodulin binds in a Ca2+-dependent manner and with high affinity (Kp ~ 3 nM) to a 19-amino acid sequence located at the N-terminal region of D2.
affinity calmodulin binding to melittin, a bee venom peptide with an unusual bent coformation and 5% helical content that exhibits an increase in helical content to 72% upon complex formation (38, 39).

Calmodulin can interact with cellular PTPα and with recombinant PTPα-D2 but not with the PTPβ equivalents. Mutagenesis of the calmodulin-binding sequence in PTPα-D2 and of the counterpart non-binding sequence in PTPε-D2 showed that two amino acids near either end of the PTPα-D2 sequence, Lys-520 and Asn-534, are essential for optimal calmodulin binding and are not conserved in PTPβ. All other RPTP D2 domains (except PTP-OST) possess the same Asn residue within a conserved KNR sequence as PTPα-D2, but none have a Lys residue in the equivalent position to that in PTPα-D2. Although PTPβ-D2, PTPα-D2, and LAR-D2 all have a positively charged arginine residue in this latter position, it may not be sufficient to confer calmodulin binding in the absence of other PTPα-D2 features within the N2 sequence, for example a second positively charged residue two positions C-terminal to the Lys/Arg. It will be of interest to determine whether calmodulin can interact with these D2 domains. Nothing resembling the N2 calmodulin-binding sequence is found in a comparable position in any of the RPTP D1 domains, including PTPα. This suggests that the interaction with calmodulin may be a specific property of PTPα-D2, and perhaps a unique means of PTPα-D2, and through this, PTPα regulation.

An intriguing difference is apparent in the abilities of recombinant PTPα-D1ΔD2 and cellular PTPα to bind calmodulin. While recombinant D2 can bind calmodulin, the additional presence of D1 in the recombinant protein appears to preclude binding. Nevertheless, cellular PTPα containing D1 can associate with calmodulin. This suggests that the two catalytic domains may not assume the same relative conformation in the recombinant protein as they do in cellular PTPα, with the calmodulin-binding site being inaccessible in the former conformation. Even so, the D2 catalytic cleft is accessible in both forms, at least to a small molecule substrate such as pNPP, as D2 can contribute to the same extent to the total activity of recombinant PTPα-D1ΔD2 or immunoprecipitated cellular PTPα in in vitro assays (9). Cellular conditions that could modify conformation and thus calmodulin-binding site accessibility include phosphorylation (cellular PTPα is a phosphoprotein, although not all phosphorylation sites are defined), membrane insertion of the mature protein, and intra- or intermolecular interactions, including, for example, those involving the juxtamembrane region of PTPα that is lacking in the recombinant protein.

Calmodulin-binding activates many enzymes. However the phosphatase activity of PTPα-D2 is inhibited upon interaction with calmodulin. Kinetic studies show that calmodulin acts as a competitive inhibitor, likely reflecting the close proximity of the calmodulin-binding site in PTPα-D2 to the substrate-binding site. The C-terminal end of the N2 calmodulin-binding sequence comprises the lip of the D2 catalytic cleft as determined from PTP crystal structures and predicted from PTPα-D2 modeling (11, 21, 23, 25). Thus calmodulin binding could prevent substrate binding, either by direct occlusion of the catalytic cleft, and/or possibly through a distortion of the catalytic cleft resulting from calmodulin pulling the binding site region out from the PTP structure and concomitant rearrangement of the binding site secondary structure. If PTPα-D2 indeed has a catalytic function, this would be an unusual example of calmodulin directly blocking enzymatic activity. If PTPα-D2 acts in a non-enzymatic capacity through binding phosphotyrosyl proteins (20), this function could also be abrogated by association with calmodulin.
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REFERENCES
1. Zheng, X. M., Wang, Y., and Pallen, C. J. (1992) Nature 359, 336–339
2. den Hertog, J. Pals, C. E. G. M., Peppelenbosch, M. P., Tertoolen, L. G. J., de Laat, S. W., and Kruijer, W. (1993) EMBO J. 12, 3789–3798
3. Harder, K. W., Moller, N. P. H., Peacock, J. W., and Jirik, F. R. (1998) J. Biol. Chem. 273, 31890–31900
4. Ponniah, S., Wang, D. Z. M., Lim, K. L., and Pallen, C. J. (1999) Curr. Biol. 9, 535–538
5. Su, J., Muranjan, M., and Sap, J. (1999) Curr. Biol. 9, 505–511
6. Tsai, W., Morielli, A. D., Cachero, T. G., and Peralta, E. G. (1999) EMBO J. 18, 109–118
7. Moller, N. P. H., Moller, K. B., Lammers, R., Kharitonenkov, A., Hoppe, E., Wiberg, F. C., Sures, I., and Ullrich, A. (1995) J. Biol. Chem. 270, 23126–23131
8. Wang, Y. and Pallen, C. J. (1991) EMBO J. 10, 3231–3237
9. Lim, K. L., Lai, D. S. Y., Kalousek, M. B., Wang, Y., and Pallen, C. J. (1997) Eur. J. Biochem. 245, 693–700
10. Wu, L., Buist, A., den Hertog, J., and Zhang, Z.-Y. (1997) J. Biol. Chem. 272, 6994–7002
11. Lim, K. L., Kolatkar, P. R., Ng, K. P., Ng, C. H., and Pallen, C. J. (1998) J. Biol. Chem. 273, 28986–28993
12. Streuli, M., Krueger, N. X., Thai, T., Tang, M., and Saito, H. (1990) EMBO J. 9, 2399–2407
13. Johnson, P., Ostergaard, H. L., Wadsen, C., and Trowbridge, I. S. (1992) J. Biol. Chem. 267, 8035–8041
14. Gehlking, M. F. B. G., Verheijen, M. H. G., Zondag, G. G. C. M., van Etten, I., and Moelenaar, W. H. (1993) Biochemistry 32, 13516–13522
15. Streuli, M., Krueger, N. X., Tsai, A. Y. M., and Saito, H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8698–8702
16. Pot, D. A., Woodford, T. A., Remboutskia, E., Haun, R. S., and Dixon, J. E. (1991) J. Biol. Chem. 266, 19688–19696
17. Cho, H., Ramser, S. E., Inoh, M., Kitas, E., Bannwarth, W., Burn, P., Saito, H., and Walsh, C. T. (1992) Biochemistry 31, 133–138
18. Kashio, N., Matsumoto, W., Parker, S., and Rothstein, D. M. (1998) J. Biol. Chem. 273, 33856–33863
19. Wallace, M., Fladd, C., Batt, J., and Rotin, D. (1996) Mol. Cell. Biol. 18, 2608–2616
20. Lim, K. L., Ng, C. H., and Pallen, C. J. (1999) Biochim. Biophys. Acta 1434, 275–283
21. Nam, H.-J., Poy, F., Krueger, N. X., Saito, H., and Frederick, C. A. (1999) Cell 97, 449–457
22. Buist, A., Zhang, Y.-L., Keng, Y.-F., Wu, L., Zhang, Z.-Y., and den Hertog, J. (1999) Biochemistry 38, 914–922
23. Jia, Z., Barford, D., Flint, A. J., and Tonks, N. K. (1995) Science 268, 1754–1758
24. Zeng, L., D’Alessandri, L., Kalousek, M. B., Vaughan, L., and Pallen, C. J. (1999) J. Cell Biol. 147, 767–773
25. Bilwes, A. M., den Hertog, J., Hunter, T., and Noel, J. P. (1996) Nature 382, 555–559
26. Blanchetot, C., and den Hertog, J. (2000) J. Biol. Chem. 275, 12446–12452
27. den Hertog, J., Tracy, S., and Hunter, T. (1994) EMBO J. 13, 3020–3032
28. Su, J., Batzer, A., and Sap, J. (1994) J. Biol. Chem. 269, 18731–18734
29. Su, J., Yang, L.-T., and Sap, J. (1996) J. Biol. Chem. 271, 28086–28096
30. Zheng, X.-M., Resnick, R. J., and Shalloway, D. (2000) EMBO J. 19, 984–978
31. Krueger, N. X., Streuli, M., and Saito, H. (1990) EMBO J. 9, 3241–3252
32. Bhandari, V., Lim, K. L., and Pallen, C. J. (1998) J. Biol. Chem. 273, 8691–8698
33. Pause, A., Belsham, G. J., Gingras, A. C., Donze, O., Lin, T. A., Lawrence, J. C., Jr., and Sonenberg, N. (1994) Nature 371, 762–767
34. Cox, J. A., Comte, M., Fitton, J. E., and DeGrado, W. F. (1985) J. Biol. Chem. 260, 2527–2534
35. Rhoads, A. R., and Friedberg, F. (1997) FASEB J. 11, 331–340
36. O’Neil, K. T., and DeGrado, W. F. (1990) Trends Biol. Sci. 15, 59–64
37. Terwilliger, T. C., and Eisenberg, D. (1982) J. Biol. Chem. 257, 6016–6022
38. Maulet, Y., and Cox, J. A. (1983) Biochemistry 22, 5680–5686