Receptor Protein Tyrosine Phosphatase rho interacts with components of adherens junctions

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Abstract

Background: Receptor protein tyrosine phosphatase rho (RPTPρ/PTPRT) is a member of the RPTP R2B subfamily, which includes PTPκ, PTPμ and PCP-2. It is a transmembrane protein that is expressed predominantly in the central nervous system, but has also been detected in colon, heart, lung, pancreas and testis. The RPTPρ extracellular segment contains a MAM (meprin, a5, PTPμ) domain, an Ig-like domain and four-fibronectin type III repeats, motifs commonly found in cell adhesion molecules. The intracellular segment consists of a cadherin-like juxtamembrane region and two phosphatase domains, indicating that RPTPρ plays a role in signal transduction via tyrosine dephosphorylation. PTPκ, PTPμ, and PCP-2 modulate cell adhesion by binding to specific proteins in the cadherin/catenin complex.

Results: In the present study, glutathione S-transferase (GST) pulldown assays show that RPTPρ also interacts with several adherens junctional proteins including E-cadherin, N-cadherin, VE-cadherin (cadherin-5), desmoglein, α, β and γ catenin, $p120^{ctn}$ and α-actinin. With the exception of E-cadherin and α-actinin, binding was considerably reduced in high sodium concentrations. Furthermore, immunoprecipitation phosphatase assays indicated that E-cadherin, and to a far lesser extent $p120^{ctn}$, were tyrosine-dephosphorylated by a recombinant RPTPρ intracellular fragment, and thus, were likely to be primary substrates for RPTPρ.

Conclusions: The interaction of RPTPρ with adherens and desmosomal junctional components suggests that this phosphatase may transduce extracellular signals to the actin cytoskeleton and thereby play a role in regulating cadherin-mediated cell adhesion.

Background

Receptor-like protein tyrosine phosphatases (RPTPs) are transmembrane proteins that, in addition to their well-defined role in protein dephosphorylation, have been implicated in cell-cell and cell-matrix interactions, and play a role in neurite extension, axon guidance and axon-target recognition [1]. RPTPρ cDNA (Human Genome Nomenclature Committee designation, PTPRT) encodes an RPTP [2] with high homology to human PTPκ [3], PTPμ [4, 5] and PCP-2 [6, 7], identifying RPTPρ as the most recently characterized member of the RPTP R2B subfamily [8, 9], a small subgroup of phosphatases with adhesive properties mediated through homophilic interactions [10, 11, 12,13].

The RPTPρ gene has been mapped to human chromosome 20q12-13.1 [14] and to a syntenic region on mouse chromosome 2 [2]. Comparison of the RPTPρ cDNA with the genomic sequence predicts a gene with at least 32 exons, several of which are alternatively spliced [8, 9]. The 12.5 kb mRNA nucleotide sequence predicts a 1463 amino acid polypeptide and an 8 kb 3' untranslated region. Motifs in the extracellular segment (a MAM domain, an immunoglobulin-like domain, and four fibronectin type III repeats) are commonly found in cell adhesion molecules. In the intracellular segment, a juxtamembrane region followed by two phosphatase domains suggests that, like other RPTPs, RPTPρ plays a role in signal transduction via protein dephosphorylation. RPTPρ is expressed predominantly in the murine central nervous system [2,15] where the transcript is present at high levels perinatally and becomes rapidly downregulated during postnatal week two. Although the function of RPTPρ in the CNS is unclear, in recent studies, somatic mutations in RPTPρ/PTPRT resulting in impairment of tyrosine dephosphorylation activity were found in colorectal, lung and gastric tumors. Furthermore, the expression of wildtype, but not catalytically impaired PTPRT, inhibited cell growth in human colorectal cancer cell lines, suggesting that RPTPρ/PTPRT may act as a tumor suppressor [16].

We have shown previously that the juxtamembrane segment is the most variable region of the R2B genes [8, 9]. It contains several alternatively spliced exons, is considerably longer than the comparable region in other RPTPs, and shows greater variation in exon size and number. The region displays sequence similarity to the intracellular domain of cadherins, a family of calcium-dependent transmembrane proteins involved in cell adhesion through homophilic binding. Although PTPκ, PTPμ and PCP-2 are known to interact with members of the cadherin/catenin complex [17, 18, 19, 20, 21], similar substrates have not, as yet, been identified for RPTPρ. In the present study, interactions between RPTPρ and junctional proteins were investigated using glutathione S-transferase (GST) pulldown and immunoprecipitation phosphatase (IP) assays. We show that, like other members of the R2B family, RPTPρ interacts with several components of the cadherin/catenin complex.

Results

Expression of GST-RPTPρ fusion proteins

Two RPTPρ-GST fusion proteins were generated: The first contained a wildtype, catalytically active phosphatase domain, and the second, a substrate-trapping mutant (D1026A), contained an enzymatically inactive phosphatase domain. In addition, a PCP-2-GST substrate-trapping mutant (D989A) was also generated [22]. Each construct contained coding sequences encompassing the juxtamembrane domain and the entire first phosphatase domain (Figure 1), both of which are required for full catalytic activity [23]. Fusion proteins were expressed in *E.* coli as 61 kDa soluble proteins and purified to near homogeneity using glutathione sepharose column chromatography (Figure 2A). The GST expression vector encoded a recognition site for the protease, thrombin, which cleaved the recombinant protein into its two components, a GST fragment (26 kDa) and an RPTP phosphatase domain fragment (35 kDa) (Figure 2B).

Phosphatase activity of the GST-RPTPρ fusion proteins

The phosphatase activity of the 61 kDa RPTPρ-GST recombinant fusion proteins was assessed using pNPP, a general synthetic phosphatase substrate. When the compound is cleaved by a phosphatase, a yellow product is formed, the accumulation of which can be measured by monitoring absorbance at 405 nm. Two different concentrations of the elute fraction of the wild-type RPTPρ phosphatase, and a single concentration of the RPTPρ substrate-trapping mutant, were tested. The wild-type protein exhibited phosphatase activity, while the substrate-trapping mutant protein was catalytically inactive (Figure 3). Phosphatase activity was protein concentration-dependent, and was diminished by the addition of the tyrosine phosphatase inhibitor, sodium orthovanadate.

GST pulldown assay to identify RPTPρ interacting proteins

The RPTPρ and PCP-2 GST cDNA fusion constructs were used as "bait" to identify proteins that potentially interact with RPTPρ and PCP-2. In the substrate-trapping mutant, substrate binding is designed to be enhanced due to a single point mutation in the RPTP catalytic domain [22, 24]. This renders the enzyme able to bind native phosphotyrosine substrates, but unable to catalyze the dephosphorylation reaction or release the bound substrate. For this reason, both the substrate-trapping mutant and the wild type recombinant protein were used to identify proteins that bind to RPTPρ. Antibodies directed against proteins previously reported to be present in adherens junctions was used to identify proteins isolated in the GST pulldown assay. RPTPρ interacted with several of these proteins including E- and N-cadherin, cadherin-5 (VE-cadherin), and the desmosomal junction protein, desmoglein (Figure 4); however, RPTPρ did not associate with P-, M- or R-cadherin (data not shown). RPTPρ also interacted with α, β, and γ catenin, $p120^{ctn}$, and with α-actinin. Each of these proteins, except α-actinin and E-cadherin, showed considerably diminished binding under more stringent assay conditions (350 mM versus 150 mM salt concentration). The PCP-2 substrate-trapping mutant interacted with α–, β– and γ-

catenin and with α-actinin, but did not interact with any of the cadherins tested. GST protein alone did not interact with adherens junctional proteins (data not shown).

Identification of a cell line with endogenous RPTPρ expression

Two different primer sets designed to amplify RPTPρ were used to screen various neuronal and non-neuronal cell lines for endogenous RPTPρ expression. Although RPTPρ was not expressed in any of the neuronal cell lines tested (KT98, N2A or PC12), it was detected in MIN6-m9 and βTC-6 neuroendocrine cell lines (Figure 5A), both of which were derived from pancreatic β cells [25]. Furthermore, RT-PCR on mRNA from MIN6-m9 cells indicated that this cell line expresses all four R2B RPTPs (PCP-2, PTPκ, PTPμ, and RPTPρ) (Figure 5B). MIN6-m9 cells, which had higher levels of RPTPρ expression than the βTC-6 cell line, were used for Western blot analysis utilizing a polyclonal antibody raised against the N-terminal region of the RPTPρ protein (Figure 5C). Two bands at approximately 186 kDa and 85 kDa were identified, which correspond to the expected size of the full-length RPTPρ, and the proteolytically cleaved N-terminal fragment, respectively, thus identifying MIN6-m9 cells as a source of endogenous RPTPρ.

Immunoprecipitation of adherens junctional proteins

In order to determine which of the candidate proteins identified in the GST pulldown assays were dephosphorylated by RPTPρ, MIN6-m9 cell lysates, previously treated with sodium pervanadate to increase the fraction of tyrosine-phosphorylated proteins, were immunoprecipitated with antibodies directed against proteins shown previously to interact with GST-RPTPρ. Immunoprecipitates were treated with purified, catalytically active wildtype GST-RPTPρ and probed with an anti-phosphotyrosine antibody. Densitometric analysis revealed that E-cadherin phosphotyrosine levels were diminished by 97% (GE Healthcare, Image Quant TL software) in a dose-dependent manner when treated with active RPTPρ phosphatase (Figure 6), suggesting that E-cadherin is a likely substrate for RPTPρ dephosphorylation. Similarly, $p120^{ctn}$ was also dephosphorylated by RPTPρ, but to a lesser extent (67%). None of the other proteins tested were dephosphorylated, or present in a phosphorylated form.

Discussion

Our experiments suggest that RPTPρ interacts with cell surface and intracellular components of specialized intercellular adherens junctions that mediate both homophilic binding and interactions with the cytoskeleton. These proteins include E-cadherin, N- cadherin, cadherin-5 (VE-cadherin), desmoglein, and α, β and γ catenin/plakoglobin, $p120^{ctn}$ and α-actinin. No difference in binding ability was observed between wildtype and substrate RPTPρ fragments. With the exception of α-actinin and E-cadherin, high salt concentrations generally resulted in considerably reduced binding. The spectrum of interacting molecules differed from that of PCP-2, implying a degree of R2B phosphatase selectivity for junctional proteins. We have also demonstrated that RPTPρ binding leads to dephosphorylation of E-cadherin and,

to a lesser extent, p120^{ctn}, suggesting that the physical interaction between these molecules has functional sequelae.

The interactions of cadherins, catenins and associated cytoskeletal proteins enhance the signaling link between homophilic binding proteins on the extracellular surface, and cytoskeletal proteins in the cytoplasm [26, 27]. Cadherins are transmembrane proteins of which there are multiple tissue-specific isoforms. When dephosphorylated on their intracellular surface, they bind identical molecules located on apposing cells in a calcium dependent manner (reviewed in [28]). Early studies on E-cadherin revealed that in classical cadherins, the cytoplasmic tail interacts with α, β and γ-catenin/plakoglobin, thus coupling cadherins to the actin cytoskeleton [29, 30, 31]. Furthermore, cadherin deletion mutants, lacking the ability to bind catenins, exhibit reduced ability to mediate tight cell-cell adhesion in a variety of cellular contexts [32, 33, 34]. While cadherins are primarily associated with adherens junctions, desmoglein is a cadherin homolog that is found in desmosomal junctions; interactions between the two are regulated by plakoglobin [35].

Catenins are cytosolic proteins that bind to the cytoplasmic surface of cadherins and to cytoskeletal proteins that cross-link actin filaments at specific adhesion sites [36, 37]. The p120^{ctn} protein is a close homolog of β and γ catenin/plakoglobin, but binds at a separate site [38]. Cloning, sequencing, and subsequent structural analysis have revealed that β-and/or γ-catenin/plakoglobin function as a bridge between the intracellular cadherin tail and α–catenin [39], thus enabling α-catenin to couple cadherin to the actin cytoskeleton [40, 41]. The cadherin/catenin complex also may be anchored to actin filaments via the bridging protein, α-actinin [42, 43]. It is through these mechanisms that catenins act as key regulators of cadherin-mediated cell-cell adhesion.

The binding of RPTPρ to adherens junctional proteins is consistent with earlier studies in which the three other members of the RPTP R2B subfamily, PTPμ, PTPκ and PCP-2, were shown to modulate cell adhesion through binding to the cadherin/catenin complex [17, 18, 19, 20, 21]. Brady-Kalnay et al. [44] demonstrated that PTPμ associates directly with N-cadherin, E-cadherin and R-cadherin (cadherin-4) in extracts of rat lung, but not β-catenin [17]. It was noted [44] that although interacting with several cadherins, PTPμ displayed a relatively restricted tissue distribution, suggesting that if regulation of cadherin function by reversible tyrosine phosphorylation were a general phenomenon, there would be additional PTPs in other cell types that might function similarly. Indeed, PTPκ, which is closely related in structure to PTPμ, associates directly with β–catenin and γ–catenin/plakoglobin [18]. PTPκ has a much broader expression pattern [45, 46] than that of PTPμ [46] or RPTPρ [2, 15], and thus may interact with cadherin-catenin complexes in several tissues throughout the body. Most recently, PTPλ (PCP-2) was shown to associate with β-catenin and also colocalized with E-cadherin [21]. Our GST pulldown experiments are in agreement with the latter studies, as GST-PCP-2 interacted with subset of junctional proteins that included β-catenin. However, unlike GST-RPTPρ, GST-PCP-2 did not interact with any of the cadherins tested, indicating a considerable degree of specificity between the two phosphatases. The

pattern emerging is that both RPTPρ and PTPμ bind directly and dephosphorylate E-cadherin, but not β-catenin. In contrast, PTPκ and PCP-2 interact primarily with β or γ catenin, but not the cadherins. Thus, type R2B RPTPs appear to fall into two distinct families defined by their binding specificities.

A recurring feature of our GST-pulldown assays was that both wildtype and substrate-trapping mutant GST-RPTPρ probes exhibited similar binding patterns. The substrate-trapping mutants were designed to form a stable intermediate with tyrosine-phosphorylated substrates, thus allowing for a much higher binding affinity than possible with the wildtype. The lack of differential binding suggests three possibilities: Firstly, that the interactions observed are independent of the phosphorylation state of the substrate; secondly, that binding may be mediated by a site other than the phosphatase catalytic site; or thirdly, the observed binding is indirect and the precipitated component is part of a larger complex.

Conclusions

Our data indicate that RPTPρ interacts with a number of proteins involved in the structure of adherens junctions, and that alterations in RPTPρ activity may contribute to changes in the phosphorylation of junctional components. Except in the case of E-cadherin and α-actinin, binding sensitivity of junctional proteins was considerably reduced under high salt conditions. Because increasing salt concentrations tend to disrupt protein-protein interactions, E-cadherin and α-actinin are likely to be primary binding partners, while the remaining proteins may associate indirectly with RPTPρ. The probability that physical interactions between RPTPρ, E-cadherin and $p120^{ctn}$ have functional consequences was suggested by the immunoprecipitation phosphatase assay in which E-cadherin, and to a lesser extent, $p120^{ctn}$, were dephosphorylated by RPTPρ binding. As $p120^{ctn}$ was not completely dephosphorylated, there may be additional phosphatases involved in the process. This result is analogous to earlier observations that cadherin is dephosphorylated by PTPμ [17], and that $p120^{ctn}$ is dephosphorylated by DEP-1 [47] and PTPμ [48]. Although it is assumed that the RPTPρ intracellular cadherin-like domain is responsible for protein binding, the interacting site remains undefined and deletion experiments would be required to identify the region more precisely. Nonetheless, interactions between RPTPρ and adherens junctional proteins may contribute to changes in the integrity of cell-cell junctions and possibly influence cellular adhesivity.

Methods

Preparation of GST-constructs

Two RPTPρ glutathione S-transferase (GST) fusion proteins (AA#888-1178), a wild type catalytically active phosphatase domain and a substrate-trapping mutant phosphatase domain and a PCP-2 GST substrate trap mutant phosphatase domain protein (AA#855-1141) [22], were expressed in BL21 Codon Plus *E. coli*, by the addition of 0.2 mM IPTG. *E. coli* cells were grown in 50 ml cultures at 16^0C to increase the amount of soluble recombinant fusion protein, and were harvested 18-24 hours after

induction. Cells were pelleted and resuspended in 3.0 ml of lysis buffer containing 50 mM Tris, pH 8.0, 5 mM EDTA, 0.1% Triton X-100 and 150 mM NaCl. Lysozyme was added at a final concentration of 0.2 mg/ml and cells were incubated for 15 minutes at room temperature. Cells were broken apart by sonication (30 x 1-second pulses at 10 W) on ice. The harvested culture was centrifuged at 10,000 rpm for 15 minutes, at 4°C. The supernatant, which contained large amounts of soluble recombinant protein, was distributed into silanized Eppendorf tubes containing 100µl of glutathione sepharose beads (Amersham) and incubated with gentle rocking at 4°C for one hour. The beads were centrifuged briefly at 2,000 rpm, followed by extensive washing in PBS buffer, containing 1% Triton X-100, 2 mM EDTA, 5 mM DTT, 5% glycerol, and 150 mM NaCl, pH 7.4. GST fusion proteins were eluted by the addition of 10 mM glutathione in 50 mM Tris, pH 8.0.

GST pulldown assay

Brain homogenate was prepared as follows: A C57BL/6J mouse brain was homogenized in 10ml lysis buffer (5mM Tris-HCl, 5mM Tris-base, 5mM $MgCl_2$; pH 7.4; 4^0C), containing protease inhibitors (Roche, Cat# 1836153), using a glass homogenizer and Teflon pestle. The homogenate was centrifuged for 15 min at 20,000 rpm, at 4°C, and the supernatant was discarded. The pellet was resuspended in 10 ml lysis buffer containing protease inhibitors. The resuspended pellet was homogenized on ice using a Sonic Dismembrator 60 Tissue homogenizer, and centrifuged for 15 min at 20,000 rpm, at 4°C. The supernatant was discarded and the membrane pellet was resuspended in 2 ml de-ionized water. Protein concentration was determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Sigma, Cat# BCA-1).

GST-RPTP slurry and homogenate (250 µg total protein) were incubated overnight, rocking, at 4°C. Beads were collected by centrifugation at 1000 rpm. The pelleted beads were washed extensively in buffer containing either 150 mM NaCl (low salt condition), or 350 mM NaCl (high salt condition). All liquid was aspirated from the beads, followed by resuspension in 25µl of SDS sample buffer. Beads were boiled for 5 minutes and samples were run on 10% SDS-PAGE for 2.5 hrs, at 65 mA. Proteins from both brain homogenate and cell lysate were transferred to nitrocellulose (1 hour at 100 V) and immunoblotted with antibodies (α, β and γ catenin; $p120^{ctn}$/pp120; E, M, N, P and R-Cadherin, Cadherin-5; Desmoglein) to zonula adherens proteins (BD Transduction Labs, Lexington; cat #611446), and with an α-actinin mouse monoclonal antibody (Sigma, Cat# A-5044).

N-terminal RPTPρ antibody preparation:

A 769 bp nucleotide region (256 amino acids, #25-280 of Genbank NM_021464) coding for the N-terminal MAM and Ig-like protein domains (MIg) (Figure 1) and flanking *Xba* I and *Hind* III restriction sites was amplified by PCR. The region is 60%, 50% and 42% similar to the same region in PTPμ, PTPκ and PCP-2/PTPλ, respectively. The PCR product was subcloned into the pPRO-EX HTb expression vector (Gibco BRL; Bethesda). This vector drives expression in *E. coli* with a *trc* promoter that is induced by isopropyl-β-D-thiogalacto-pyranoside (IPTG), and adds an N-terminal 6x*HIS* tag to the recombinant protein. The plasmid construct (pProB.MIg) was transformed into the DH5α *E. coli* expression strain. Liquid cultures

were grown and expression of a 35 kDa recombinant protein was induced by the addition of IPTG. *E. coli* cells expressing the recombinant MIg protein were grown and harvested according to conventional protocols. Both soluble and insoluble fractions were analyzed by SDS-PAGE, under non-denaturing conditions. Guanidine hydrochloride was required to solublize the RPTPρ N-terminal MIg recombinant protein fragments, which were located entirely in the insoluble (pellet) fraction. The denatured MIg recombinant protein was purified by affinity chromatography using the 6xHIS tag, and was located primarily in fractions eluted with imidazole. The recombinant protein (2 mg) was purified and electrophoresed on 12% SDS-PAGE using a large preparative well. The 35 kDa band (MIg) was excised from the gel, and used as an antigen for injection into rabbits for polyclonal antibody production (Covance, Denver, PA). Two rabbits were injected with 500 μg MIg protein in Freunds Complete adjuvant on day 1 of the protocol, followed by two more injections of 250 μg each on days 21 and 41. Sera were analysed by Western blotting, using the MIg protein as antigen. The MIg N-terminal antiserum was further affinity purified using recombinant MIg protein immobilized on a nitrocellulose membrane.

Immunoprecipitation (IP) phosphatase assay.

MIN6-m9 cells (obtained from Dr. S. Seino, Chiba University, Chiba, Japan) were grown in DMEM (Invitrogen) supplemented with 10% fetal bovine serum, at 37°C in 5% CO_2 in a humid environment in 10cm^2 dishes. In order to generate maximally phosphorylated proteins, cells were treated for 10 minutes with 0.1 mM sodium pervanadate, and lysed in buffer containing 5 mM Tris-Cl, 5 mM $MgCl_2$, and 1% Empigen BB with Complete Protease Inhibitors (Roche). Protein content was adjusted to 500 μg protein per tube. Adherens junctional proteins were immunoprecipitated by adding 5 μg of each antibody to the MIN6-m9 lysate and incubated overnight at 4°C with gentle rocking. Resulting immunoprecipitates were centrifuged, washed in IP buffer containing varying amounts (0, 2.5, 5 μg) of catalytically active RPTPρ-GST fusion protein, and incubated for 30 minutes at 30°C, with occasional mixing. Immunoprecipitates were washed and 25 μl of reducing SDS sample buffer was added; tubes were boiled for 5 minutes and the contents run on 10% SDS-PAGE. The separated proteins were transferred to nitrocellulose membranes and probed with an anti-phosphotyrosine antibody (P-tyr 100; Cell Signaling Technology).

Author's Contributions

JB conducted experiments; RHvH designed and generated the substrate-trapping constructs; AF prepared text and figures and assisted with data analysis; AR supervised experiments and data analysis.

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Figure Legends

Figure 1. RPTPρ protein domain structure showing the location of the GST-RPTPρ fusion proteins and the N-terminal epitope (Mlg). The GST-PCP-2 fusion protein is located at approximately the same relative position.

Figure 2. Expression and solubilization of substrate trapping D1026A mutant RPTPρ in *E. coli*. A. The RPTPρ recombinant protein was expressed as a soluble 61 kDa protein. Lane 1, molecular weight standard (Benchmark ladder, Invitrogen); lane 2, total cell pellet from uninduced culture; lane 3, total cell pellet from culture induced with 0.2 mM IPTG; lane 4, 0.1% Triton X-100 supernatant of induced culture; lane 5, elute fraction from glutathione sepharose column containing a 61 kDa soluble GST-RPTPρ fusion protein. **B.** GST-RPTPρ fusion protein cleaved by thrombin: GST migrated at 26 kDa, and RPTPρ at 35 kDa. Lane 1, molecular weight standard (Benchmark ladder, Invitrogen); lane 2, total cell pellet from uninduced culture; lane 3, total cell pellet from culture induced with 0.2 mM IPTG; lane 4, 16 hour incubation of purified GST-RPTPρ fusion protein with 10U thrombin.

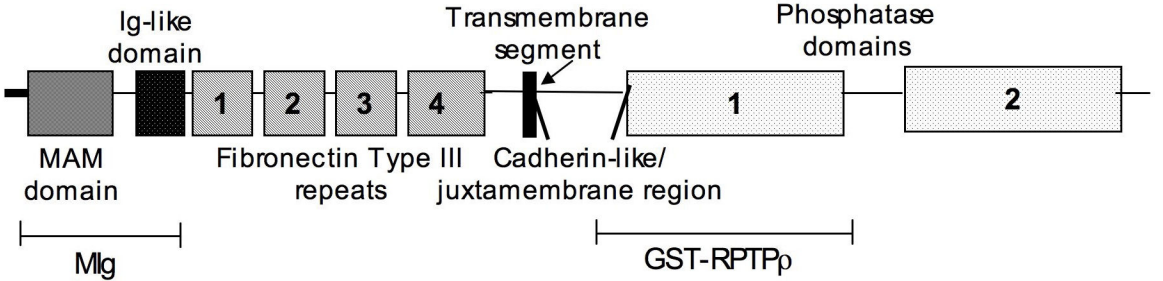
Figure 3. Measurement of phosphatase activity in RPTPρ recombinant proteins. The elute fraction (20 µl) from the RPTPρ recombinant protein purification was assayed with 20 mM pNPP (Sigma) as artificial substrate. Total assay volume was 500µl, at pH 6.8. Both high and low concentrations of wild type (rhoWT) protein showed high catalytic activity, while the substrate trapping protein (rhoMUT) had only background activity.

Figure 4. GST pulldown assays identifying RPTPρ intracellular substrates. Pulldown assays on mouse brain homogenate using wildtype and substrate-trapping mutants were probed with antibodies against proteins found in cell adhesion junctions. The RPTPρ substrate-trapping mutant was also assayed under more stringent (350 mM vs 150mM salt) conditions. GST-RPTPρ associated with 9 of the 12 proteins examined. A PCP-2 substrate-trapping mutant protein interacted with a subset of the catenins and α-actinin. GST protein alone did not interact with any of the adherens junction proteins (data not shown). *Lane 1*, wildtype; *lane 2*, RPTPρ mutant; *lane 3,* RPTPρ mutant + high salt; *lane 4*, PCP-2 mutant.

Figure 5. A. Identification of a cell line (MIN6-m9) expressing RPTPρ mRNA. Several cell lines were examined for the presence of RPTPρ transcripts using RT-PCR. PCR primers amplified a 200 bp juxtamembrane segment of RPTPρ cDNA in lanes 2, 7, and 9, indicating RPTPρ transcripts in brain, βTC-6 and MIN6-m9 samples, respectively. **B. MIN6-m9 cells contain transcripts of all four RPTP type R2B genes**: Lane 1, RPTPρ-negative control; lane 2, RPTPρ PCR alternatively spliced exon 14 doublet at 200 and 257 bp; lane 3, PTPμ negative control; lane 4, PTPμ 171 bp PCR product; lane 5, PTPκ

negative control; lane 6, PTPκ 202 bp PCR product; lane 7, PCP-2 negative control; lane 8, PCP-2 212 bp PCR product. The identity of all PCR products was verified by sequencing. **C. RPTPρ protein is present in MIN6-m9 cells.** When immunoblotted with an N-terminal anti-RPTPρ antibody, MIN6-m9 cell lysates show bands corresponding in size to the predicted RPTPρ protein at 186 and 85 kDa (proteolytically processed segment). Lane 1, positive rabbit IgG; lane 2, negative control lacking primary antibody; lane 3, preimmune N-terminal RPTPρ antiserum; lane 4, N-terminal RPTPρ immune serum.

Figure 6. E-cadherin and p120^{ctn} proteins are dephosphorylated by RPTPρ. Immunoprecipitation phosphatase assays using E-cadherin and p120^{ctn} immunoprecipitates from MIN6-m9 cells showed decreased phosphotyrosine levels, as detected by an anti-phosphotyrosine antibody, when treated with an active RPTPρ phosphatase protein. Lane 1, MIN6-m9 cell lysate; lane 2, IP supernatant; lane 3, 0 μg RPTPρ; lane 4, 2.5 μg RPTPρ; lane 5, 5 μg RPTPρ; lane 6, no primary antibody. Mouse IgG band indicates equal protein concentrations in each lane.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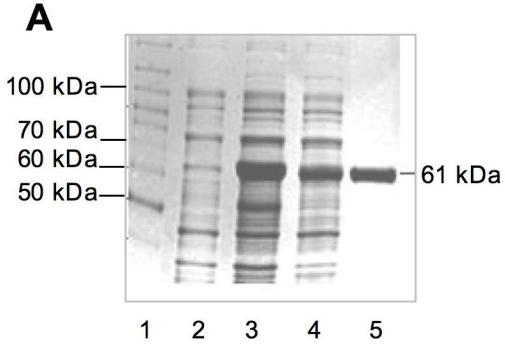
domain
Transmembrane
segment
Phosphatase
domains
1
2
3
4
1
2
MAM
domain
Fibronectin Type III
repeats
Cadherin-like/
juxtamembrane region
Mlg
GST-RPTPρ

Figure 1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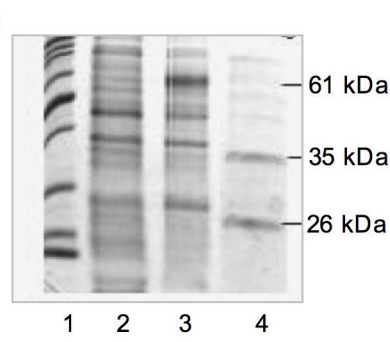
60 kDa
50 kDa
61 kDa
1
2
3
4
5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35 kDa
26 kDa
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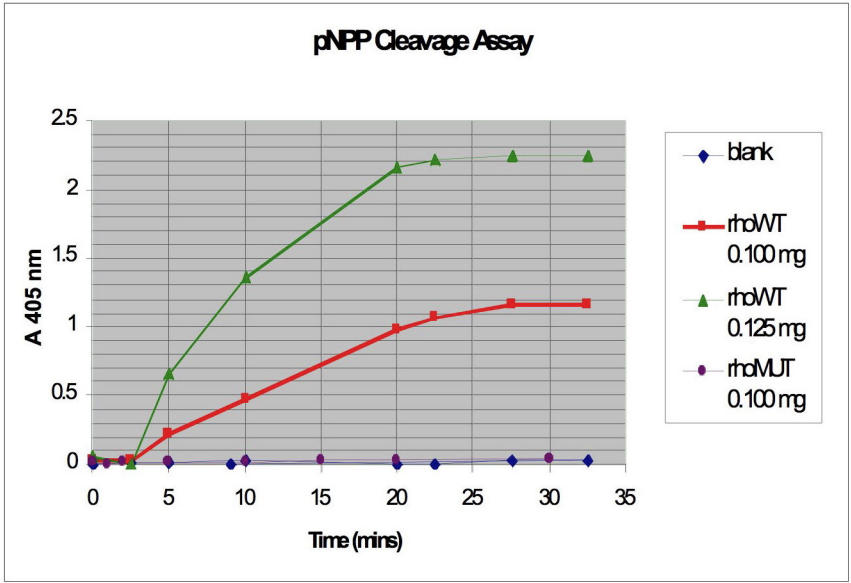
2
3
4

Figure 2


pNPP Cleavage Assay
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2
1.5
1
0.5
0
0
5
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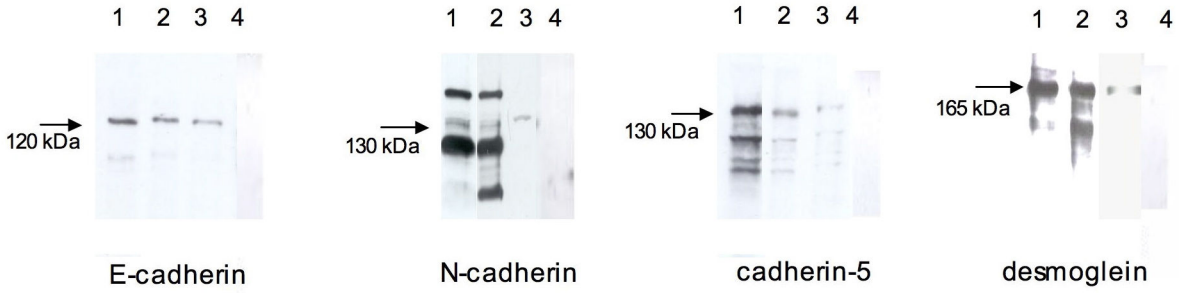
15
20
25
30
35
Time (mins)
blank
rhoWT 0.100 mg
rhoWT 0.125 mg
rhoMUT 0.100 mg

Figure 3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N-cadherin
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130 kDa
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1 2 3 4
165 kDa
desmoglein
1 2 3 4
102 kDa
α-catenin
1 2 3 4
92 kDa
β-catenin
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92 kDa
γ-catenin
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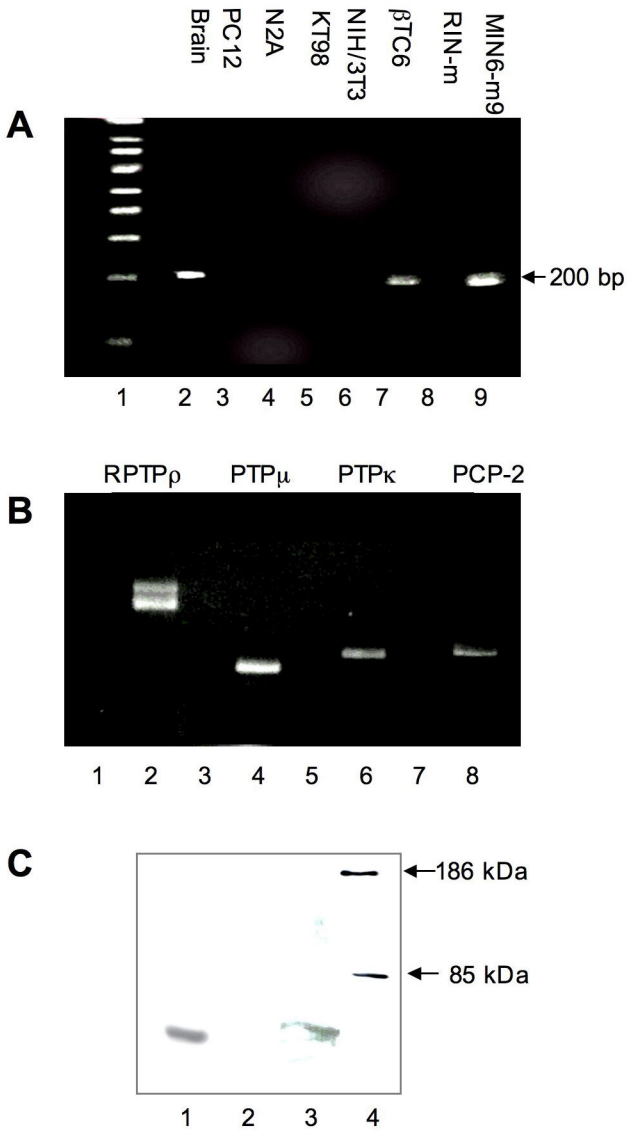
120 kDa
p120ctn
1 2 3 4
103 kDa
α-actinin

Figure 4


Brain
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KT98
NIH/3T3
βTC6
RIN-m
MIN6-m9
A
200 bp
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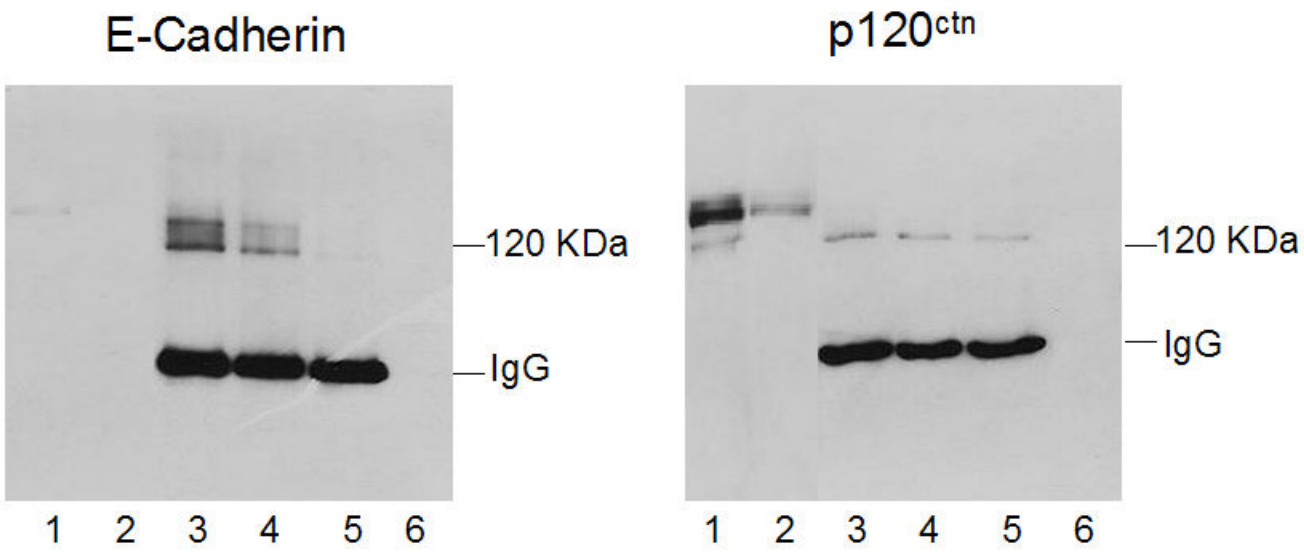
RPTPρ
PTPμ
PTPκ
PCP-2
B
1 2 3 4 5 6 7 8
C
186 kDa
85 kDa
1 2 3 4

Figure 5



Blot: Anti-phosphotyrosine antibody

Figure 6