The receptor protein-tyrosine phosphatase PTPμ is a member of the Ig superfamily of cell adhesion molecules. The extracellular domain of PTPμ contains motifs commonly found in cell adhesion molecules. The intracellular domain of PTPμ contains two conserved catalytic domains, only the membrane-proximal domain has catalytic activity. The unique features of PTPμ make it an attractive molecule to transduce signals upon cell-cell contact. PTPμ has been shown to regulate cadherin-mediated cell adhesion, neurite outgrowth, and axon guidance. Protein kinase C is a component of the PTPμ signaling pathway utilized to regulate these events. To aid in the further characterization of PTPμ signaling pathways, we used a series of GST-PTPμ fusion proteins, including catalytically inactive and substrate trapping mutants, to identify PTPμ-interacting proteins. We identified IQGAP1, a known regulator of the Rho GTPases, Cdc42 and Rac1, as a novel PTPμ-interacting protein. We show that this interaction is due to direct binding. In addition, we demonstrate that amino acid residues 765–958 of PTPμ, which include the juxtamembrane domain and 35 residues of the first phosphatase domain, mediate the binding to IQGAP1. Furthermore, we demonstrate that constitutively active Cdc42, and to a lesser extent Rac1, enhances the interaction of PTPμ and IQGAP1. These data indicate PTPμ may regulate Rho-GTPase-dependent functions of IQGAP1 and suggest that IQGAP1 is a component of the PTPμ signaling pathway. In support of this, we show that a peptide that competes IQGAP1 binding to Rho GTPases blocks PTPμ-mediated neurite outgrowth.

Reversible protein-tyrosine phosphorylation is a primary mode of regulation for several cellular functions including growth, differentiation, adhesion, and protein trafficking. The overall tyrosine phosphorylation state of a protein is regulated by protein-tyrosine kinases (1) and protein-tyrosine phosphatases (2). Similar to the family of protein-tyrosine kinases, the family of protein-tyrosine phosphatases includes both non-receptor and receptor type enzymes. Unlike the tyrosine kinases, however, few substrates and downstream signaling molecules of tyrosine phosphatases have been identified. This manuscript focuses on the receptor protein-tyrosine phosphatase (RPTP). PTPμ, and the identification of novel PTPμ-interacting proteins to aid in the elucidation of the PTPμ signaling pathway.

The extracellular domain of PTPμ contains sequence motifs similar to those in cell adhesion molecules including: a MAM domain, an immunoglobulin (Ig) domain, and four fibronectin type-III repeats (3). The MAM domain (Meprins, A5, PTP Mu) is a sequence motif that is suggested to play a role in protein dimerization (4). Ig domains are disulfide-bonded structures found in many cell surface proteins and have been shown to mediate homophilic and heterophilic binding between cell adhesion molecules. Fibronectin type-III motifs, originally identified in the extracellular matrix protein fibronectin, are present in many cell adhesion molecules. PTPμ has been shown to mediate homophilic binding in non-adhesive SF9 insect cells (5, 6). The juxtamembrane domain of PTPμ contains a region of homology to the conserved intracellular domain of the cadherins. Cadherins are calcium-dependent adhesion molecules that mediate cell-cell adhesion and adherens junction formation (7). Cadherins are anchored to the actin cytoskeleton indirectly through the binding of catenins (7). The catenin family of proteins includes α-catenin, β-catenin, plakoglobin, and p120 catenin. We have demonstrated that PTPμ interacts with classical cadherins such as N-cadherin, E-cadherin, and R-cadherin and associates with these cadherins in a complex containing α-catenin and β-catenin (8, 9). PTPμ was also shown to bind p120 catenin (10). PTPμ has two conserved intracellular catalytic domains, of which only the membrane-proximal domain has been shown to be catalytically active (11). The cell adhesion-like extracellular domain of PTPμ, its intracellular catalytic domain and ability to interact with the cadherin/catenin complex, make PTPμ an attractive candidate for regulating cadherin-mediated adhesion and migration. In support of this idea, we demonstrated that re-expression of PTPμ in LNCAp cells restores E-cadherin-mediated cell-cell adhesion (12). Furthermore, PTPμ expression and catalytic activity are required for N-cadherin mediated neurite outgrowth (13).

In a continuing effort to understand the PTPμ signaling pathway, we set out to identify potential PTPμ substrates and interacting proteins using a previously published strategy (14). PTPs have a conserved aspartate residue, which serves as a general acid during catalysis. The structural data on the PTP catalytic domain suggest that mutation of the aspartate residue to alanine (D→A) creates a "substrate trap" by affecting only the $V_{\text{max}}$ and not the $K_m$ of the phosphatase (15). Holsinger et al. (14) used a fusion protein consisting of an intracellular D1205A mutant of the RPTP, DEP-1, fused to glutathione S-transferase (GST), to isolate substrates from lysates of cells treated with the protein-tyrosine phosphatase inhibitor pervanadate. They found that DEP-1 associates with p120-catenin. Palka et al. (16) performed similar experiments with DEP-1 and identified additional substrates including the hepatocyte kinase C; HA, hemagglutinin; PBS, phosphate-buffered saline; GFP, green fluorescent protein; DSP, dithiobis(succinimidyl)propionate; CA, constitutively active; DN, dominant negative; RGC, retinal ganglion cell.
growth factor receptor (met) and Gab1 as well as p120-catenin. We generated a D1063A mutant using the intracellular domain of PTPμ (iPTPμDA) and expressed it as a bacterial GST fusion protein. The iPTPμDA fusion protein was used in pull-down assays with A549 cell lysates and associated proteins were identified by immunoblotting. Using this strategy, we identified IQGAP1 as a PTPμ-interacting protein.

IQGAP1 was originally identified as a putative RasGAP based on a region of IQGAP1 with significant homology to the catalytic domain of RasGAPs (17). IQGAP1 neither exhibits RasGAP activity nor binds to Ras but directly interacts with the Rho GTPases, Rac1 and Cdc42, in their GTP-bound state (18). IQGAP1 has no GAP activity toward Cdc42 or Rac1. In fact, IQGAP1 stabilizes Cdc42 in its GTP-bound state (19–21). In addition to a RasGAP-related domain, IQGAP1 contains a calponin homology domain, a WW domain, and four IQ motifs, similar to the ones found in unconventional myosins, that mediate interactions of IQGAP1 with calmodulin (17, 19, 22). The presence of several protein-interacting domains suggests IQGAP1 functions as a scaffolding protein. In addition to Cdc42 and Rac1, IQGAP1 interacts with E-cadherin (23, 24), N-cadherin (25), β-catenin (23, 26), CLIP-170 (27), actin (28–30), and ERK2 (p42 MAPK) (31).

It is well established that members of the Rho-family of GTPases regulate actin cytoskeleton remodeling. The most well-known members of this family include Cdc42, Rac1, and RhoA, which generate the formation of filopodia, lamellipodia, and stress fibers, respectively (32). IQGAP1 represents a protein that can link cytoskeletal proteins to Rho-family GTPases, specifically Cdc42 and Rac1 (20, 27–29, 33). In this manuscript, we demonstrate that IQGAP1 is a novel PTPμ-interacting protein. We show that the interaction between PTPμ and IQGAP1 is direct and enhanced by activated Cdc42 and to a lesser extent, Rac1. Furthermore, we demonstrate PTPμ regulates neurite outgrowth via IQGAP1.

EXPERIMENTAL PROCEDURES

Antibodies—Polyclonal antibodies to IQGAP1 (used for immunochemistry) and ERK2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies to IQGAP1 (used for immunoblotting), β-catenin, and E-cadherin were purchased from Transduction Laboratories (San Diego, CA). An antibody to phospho-p44/42 MAPK (p-ERK1/2) was purchased from New England Biolabs (Beverly, MA). An antibody to calmodulin was purchased from Upstate Biotechnology (Lake Placid, NY). An anti-HA-peroxidase antibody used to detect the Rho GTPase fusion proteins was purchased from Roche Applied Science. A polyclonal antibody generated against the extracellular domain of PTPμ (494) and a monoclonal antibody generated against the intracellular domain of PTPμ (SK18) have been described previously (5, 34). A monoclonal anti-phosphoerin antibody was a gift from Nick Tonks (Cold Spring Harbor Laboratory) and has been described previously (35). A monoclonal antibody to N-cadherin was a gift from Margaret Wheelock (University of Nebraska) and has been described previously (36).

Cell Culture—A549 non-small cell lung carcinoma cells were maintained in F-12 media (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM 1-glutamine, and 1 μg/ml gentamicin at 37 °C, 5% CO2. Sf21 insect cells were maintained in SF-900 II SFM media (Invitrogen) at 27 °C.

Expression of Fusion Proteins in Excherichia coli—Plasmids containing intracellular PTPμ GST fusion proteins (iPTPμ-1MD, iPTPμ-Δ765–815, iPTPμWT, iPTPμCS, iPTPμDA, iPTPμWT-ΔD2, iPTPμDA-ΔD2) or GST alone were expressed in E. coli under the control of the lac promoter. The iPTPμ-Δ765–815 (B4), iPTPμWT (B5), and iPTPμCS (B5m) have been described (11). We generated the iPTPμDA construct (D1063A mutation) using site-directed mutagenesis. We generated the iPTPμWT-ΔD2 and iPTPμDA-ΔD2 constructs by restriction digestion of iPTPμWT and iPTPμDA, respectively, with BamHI, which cuts at 3532 bp. iPTPμ-JMD was generated by restriction digest of iPTPμWT-ΔD2 with AccI and Clal. To isolate functional iPTPμ-GST fusion proteins, proteins were isolated from E. coli as follows. Bacteria were resuspended in 10 ml of resuspension buffer (0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) and incubated on ice for 15 min. To lyse bacterial cells 1 ml of 0.5 M EDTA, 1.1 ml of 20% Triton X-100, 55 μl of 1 M dithiothreitol, 10 μl of β-mercaptoethanol, 100 μl of 100 mM phenylmethylsulfon fluoride, and 30 μl of protease inhibitor cocktail (Sigma) was added to 10 ml of resuspended cells. Cells were sonicated and spun at 15,000 rpm for 25 min. GST fusion proteins were isolated from the cleared supernatant using glutathione-Sepharose 4B beads (Amersham Biosciences). Expression and protein concentration of GST fusion proteins was determined by Coomassie stain. Isolated fusion proteins adsorbed onto glutathione-Sepharose were used in the GST pull-down experiments as described below. For pull-down assays performed in the absence of pervanadate treatment, GST fusion proteins were isolated from E. coli using PBST (1% Triton X-100, 1 mM benzamidine, and protease inhibitor cocktail in PBS).

GST Pull-down Experiments—A549 cells were grown to 85–95% confluency. Cells were collected by scraping into lysis buffer containing 20 mM Hepes, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM benzamidine, and protease inhibitor cocktail. Cells were incubated on ice for 30 min and then centrifuged at 3000 rpm for 3 min. The supernatant was saved and its protein concentration determined using the Bradford method (37). Equal amounts of protein (1 mg) were added to equal amounts of the iPTPμ GST constructs or GST alone adsorbed to glutathione-Sepharose. Samples were rocked at 4 °C for 2.5 h, washed four times with lysis buffer, and incubated at 95 °C for 5 min in 2× SDS sample buffer. One-third of the sample was resolved by SDS-PAGE and transferred to nitrocellulose for immunoblot analysis as described previously (8). Pull-down experiments using purified His-IQGAP1 from Sf21 cells were performed in PBS, 1% Triton X-100, 1 mM benzamidine, and protease inhibitor cocktail.

Isolation of TAT Fusion Proteins—TAT fusions of the Rho family GTPases Cdc42, Rac1, and RhoA were obtained from Dr. Steven Dowdy (University of California San Diego, La Jolla, CA). The Rho GTPase fusion proteins contain an N-terminal His6-tag for purification, a TAT-tag for protein transduction, and a HA-tag for protein detection (38). The fusion proteins were expressed in E. coli under the control of the lac promoter and purified on TALON metal affinity resin (Clontech, Palo Alto, CA). Expression and protein concentration of TAT fusion proteins was determined by Bradford assay and verified by immunoblot using anti-HA-tag antibodies.

Purification of His-IQGAP1—IQGAP1 was cloned into pFast Bac HT as follows. The gene for full-length IQGAP1 was cut from pcDNA3-Myc-IQGAP1 (39) with XbaI and partially digested with BamHI, which cuts at 3532 bp. iPTPμ-JMD was transformed into DH10 Bac. The recombinant Bacmid DNA of IQGAP1 was isolated and used to infect Sf21 insect cells. Forty-eight h post-infection, Sf21 cells were lysed and His–IQGAP1 purified using the QIAexpress Ni-NTA Fast Start kit (Qiagen, Inc., Valencia, CA) as indicated in the manufacturer’s protocol with the modification that 1% Triton was included in all buffers with the exception of the elution buffer. Purity of the His-IQGAP1 protein was...
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FIGURE 1. Intracellular PTPμ GST constructs used in pull-down experiments. All constructs contain a GST sequence at the N terminus. iPTPμ-JMD contains intracellular PTPμ amino acid residues 765–958. This construct includes the juxtamembrane domain and 35 residues of the first phosphatase domain. iPTPμ-Δ765–815 contains intracellular PTPμ amino acid residues 816–1452. This construct lacks residues 765–815 of the juxtamembrane domain. iPTPμ WT, iPTPμ CS, and iPTPμ DA contain intracellular residues 765–1452. iPTPμ CS and iPTPμ DA contain C1095S and D1063A mutations, respectively, rendering these constructs catalytically inactive, or substrate trappping, respectively. iPTPμ WT ΔD2 and iPTP μDAD2 contain intracellular residues 765–1178 and lack the second phosphatase domain. Black boxes represent phosphatase domain 1 (PTP D1, residues 923–1153) and phosphatase domain 2 (PTP D2, residues 1213–1447).

determined by silver stain and verified by immunoblot with a monoclonal anti-IQGAP1 antibody.

Immunocytochemistry—A549 cells were plated in Lab Tech II chamber slides (Nalge Nunc International Corp., Rochester, NY). When the cells reached the desired confluence (1–3 days post-plating) they were fixed with 4% paraformaldehyde for 10 min. Cells were then washed with PBS three times and permeabilized using 0.5% saponin in blocking buffer (20% goat serum, 1% bovine serum albumin in PBS) for 30 min. Primary antibodies were diluted in blocking buffer plus 0.5% saponin and incubated overnight at 4°C. After incubation with primary antibody, cells were rinsed five times with TNT buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Tween 20). Secondary antibodies (either goat anti-mouse fluorescein isothiocyanate, goat anti-mouse Texas Red, or goat anti-rabbit fluorescein isothiocyanate from ICN Biochemicals, Irvine, CA) were diluted in blocking buffer plus saponin and incubated for 1 h at room temperature. After incubation with secondary antibody, cells were washed five times with TNT buffer and once with distilled water. Molecular Probes SlowFade® Light Antifade kit was used to minimize quenching. Slides were imaged using a Nikon (Tokyo, Japan) TE200 inverted microscope, and images were collected with a Spot RT digital camera and image acquisition software (Diagnostic Instruments, Inc., Sterling Heights, MI).

Baculovirus Infection of A549 Cells—Baculovirus containing a mammalian expression plasmid for GFP-tagged wild-type PTPμ was generated as previously described using the pBacMam-2 vector from Novagen (40). For co-localization studies, A549 cells were plated in Lab Tech II chamber slides 24 h prior to infection with baculovirus. For infection, 200 μl of viral supernatant was added to chamber slide wells containing cells in 200 μl of medium and incubated 2 h at 37 °C, 5% CO2. After the 2-h incubation, all medium was removed from the cells and replaced with media containing 150 mM trichostatin A (Sigma). Cells were incubated at 37 °C, 5% CO2 overnight. The following day immunocytochemistry for IQGAP1 was performed as described above.

Immunoprecipitations—A549 cells were grown to 90% confluence. Cells were washed twice with PBS and treated with the cross-linking reagent DSP (1 mM, Pierce) for 10 min at room temperature. Tris, pH 7.5, was added to 50 mM for 15 min at room temperature, then the cells were washed once with PBS. Cells were lysed in 50 mM Tris, pH 7.5, 1% Triton X-100, 150 mM NaCl, 1 mM benzamidine, and protease inhibitor cocktail. Samples were sonicated and centrifuged at 10,000 rpm for 5 min. Supernatants were saved and protein concentrations determined using the Bradford method. Immunoprecipitations were performed with equal amounts of supernatant protein (400 μg) using protein A-Sepharose (Amersham Biosciences) preloaded with a rabbit polyclonal antibody directed to an extracellular epitope of PTPμ (49) or non-immune rabbit serum. Samples were rocked at 4°C for 3–4 h. Beads were washed four times with lysis buffer and heated to 37°C for 15 min and 95°C for 5 min in 2X SDS sample buffer. Immunoprecipitated proteins were resolved by SDS-PAGE and immunoblot.

Bonhoeffer Stripe Assay—The stripe assay used was a modified version of the Bonhoeffer method (41) described previously (42). Briefly, tissue cultures dishes were coated with nitrocellulose (43) and allowed to dry before applying a silicon lane matrix to the dish. Alternating stripes of laminin and PTP-Fc chimera containing Texas Red-conjugated bovine serum albumin (for visualization of the lanes) were generated as follows. 80 ng of PTPμ-Fc chimera (described previously; Ref. 44) was injected into the channels of the silicon lane matrix, incubated for 10 min, aspirated, and then replaced with a fresh aliquot of the same substrate two additional times. All remaining binding sites within the lanes were blocked with bovine serum albumin (fraction V; Sigma) and rinsed with calcium-magnesium-free phosphate buffer. The matrix was removed, and 1.75 μg of laminin (Biomedical Technologies Inc., Stoughton, MA) was spread across the lane area and incubated for 20 min. Explants from E8 chick nasal retina were grown on the alternating stripes of laminin and PTPμ for 48 h. Quantitation of the stripe assays was performed using a rating scale previously described (45, 46). Neurites that show no preference for either substrate are assessed at 0. Neurites that grow exclusively on one substrate are assessed at 3. Neurites that grow mainly on the laminin lanes with an occasional neurite crossing over the PTPμ lanes are assessed at 2, while an assessment of 1 is given when there is a significant amount of neurite crossing but a tendency to fasciculate on laminin. To perturb IQGAP1 function, a 6 μM concentration of either a TAT-tagged, scrambled peptide or an IQGAP1 peptide containing an N-terminal TAT protein transduction sequence was added at the time of explant. The IQGAP1 peptide corresponds to amino acids 1054–1077 of IQGAP1 plus the N-terminal TAT sequence (GRKRRQRMMVSVNRGARGQNALQILAPVVK, synthesized by Genemed Synthesis, San Fancisco, CA). A peptide
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comprising amino acids 1054–1077 of IQGAP1 (MK24) is known to compete the interaction of IQGAP1 with Cdc42 and Rac1 (47).

**RESULTS**

**IQGAP1 Associates with PTPμ in Pull-down Assays**—To identify potential PTPμ-interacting proteins we used a previously published strategy employing GST fusion proteins to pull down associated proteins (14, 16). PTPs have a conserved aspartate residue, which serves as a general acid during catalysis. The structural data on the PTP catalytic domain suggest that mutation of the aspartate residue to alanine (Asp → Ala) creates a "substrate trap" by affecting only the $V_{\text{max}}$ and not the $K_m$ of the phosphate (15). The "substrate trapping" mutant (Asp → Ala) retains normal affinity for its substrate but catalysis is reduced; therefore it binds irreversibly to its substrate. We used a series of intracellular PTPμ-GST fusion proteins (Fig. 1), including a D1063A mutant (iPTPμDA), immobilized on glutathione-Sepharose in pull-down assays to identify PTPμ-interacting proteins. Lysates from confluent A549 cells treated with or without the tyrosine phosphatase inhibitor, pervanadate, were incubated with the GST fusion proteins, and associated proteins were resolved by SDS-PAGE and identified by immunoblot. The iPTPμCS construct possesses a mutation in which an essential cysteine residue is mutated to a serine (C1095S), leaving the protein catalytically inactive. The iPTPμ-$\Delta$765–815 construct lacks part of the PTPμ juxtamembrane domain. In our system, both the C1095S and D1063A mutant constructs (iPTPμCS and iPTPμDA) act equally well to "trap" potential substrates based on immunoblot data using a phosphotyrosine antibody to detect associated proteins in the presence of pervanadate (data not shown).

Based on the molecular weight of the proteins identified by the anti-phosphotyrosine immunoblot described above, we made an educated guess as to what proteins may be interacting with PTPμ and probed for a number of these proteins. Among the proteins we identified by immunoblot was IQGAP1 (Fig. 2), a regulator of the Rho GTPases, Cdc42 and Rac1. All the PTPμ constructs pulled down IQGAP1 with approximately equal affinity with the exception of iPTPμ-$\Delta$765–815, which has reduced affinity for several of the proteins we examined (Fig. 2 and data not shown), suggesting the juxtamembrane domain may alter protein folding or the affinity of protein–protein interactions. In addition to IQGAP1, other known IQGAP1-interacting proteins were detected in the pull-down assays including calmodulin and ERK2 (p42 MAPK), in both its unphosphorylated and phosphorylated, active form, suggesting PTPμ interacts with IQGAP1 in a complex. These data do not provide evidence either for or against the possibility that IQGAP1 is a substrate for PTPμ due to the fact that substrates can bind in both the phosphorylated and unphosphorylated form (10, 48, 49). The slight variation in binding of IQGAP1 and ERK2 seen in the absence or the presence of pervanadate is not observed in all experiments. Equal loading of GST fusion proteins was confirmed by Ponceau staining of the nitrocellulose membrane (data not shown). After our initial pull-down experiments, we generated both a wild-type and D1063A mutant construct lacking the second phosphatase domain, iPTPμWT-$\Delta$D2 and iPTPμDA-$\Delta$D2, respectively. These constructs are more efficiently expressed in bacteria due to their smaller size and behave similarly to the full-length intracellular constructs with respect to the binding of IQGAP1 (Fig. 3A). These data indicate that PTPμ interacts with this complex via its juxtamembrane or first phosphatase domain.

**Constitutively Active Cdc42 Enhances the Binding of IQGAP1 to PTPμ**—Members of the Rho family of GTPases are known to induce cytoskeletal changes involved in cell adhesion and motility (50, 51). The most well studied members of this family are Cdc42, Rac1, and RhoA, which induce filopodia, lamellipodia, and stress fibers, respectively (32, 52–54). IQGAP1 is a known regulator of Cdc42, and several IQGAP1 functions show dependence on activated, GTP-bound Cdc42. We were interested in determining the effect of activated Cdc42 on the interaction of IQGAP1 with PTPμ. To address this question, we added purified, constitutively active (CA) or dominant negative (DN) Cdc42, Rac1 and RhoA to pull-down assays using the iPTPμWT-$\Delta$D2 and iPTPμDA-$\Delta$D2 constructs. Constitutively active mutants of the Rho GTPases have defective GTPase activity, keeping them in a GTP-bound...
FIGURE 4. IQGAP1 and PTPμ bind directly via amino acid residues 765–958 of PTPμ.

Equal amounts of protein from A549 cell lysates were incubated with iPTPμ-JMD fusion protein or GST alone immobilized on glutathione-Sepharose. iPTPμ-JMD interacts with IQGAP1, whereas GST alone does not interact with IQGAP1 (4). iPTPμ-JMD, iPTPμ-WT, or GST alone purified from bacteria was incubated with purified His-IQGAP1 isolated from SF21 insect cells. Both iPTPμ-JMD and iPTPμ-WT bind purified His-IQGAP1 as detected by an anti-IQGAP1 monoclonal antibody. GST alone did not bind purified His-IQGAP1. iPTPμ-WT and GST were incubated with purified His-IQGAP1 in the absence or presence of CA-Cdc42. The presence of CA-Cdc42 increased the binding of iPTP μ-WT to purified His-IQGAP1. Values listed below the panel represent densitometry readings relative to the lane where no GTPase was added (8).

state (53). Dominant negative constructs of the Rho GTPases preferentially bind GDP over GTP and inhibit endogenous GTPases by titrating out guanine nucleotide exchange factors required for their activation (52, 55). The data in Fig. 3 show that in the presence of CA-Cdc42 there is a striking increase in the binding of IQGAP1 to both iPTPμ-WT-ΔD2 and iPTPμDA-ΔD2 fusion proteins, 2.4-fold (average of 2.4, n = 3) and 2.6-fold (average of 2.9, n = 3), respectively. CA-Rac1 also caused an increase in the PTPμ-IQGAP1 interaction but to a lesser extent, 1.6-fold (average of 2.0, n = 2) and 1.3-fold (average of 1.5, n = 3), respectively. No significant change in binding is seen with CA-RhoA, consistent with the lack of interaction between IQGAP1 and RhoA (19). Similarly, none of the DN constructs significantly altered the PTPμ-IQGAP1 interaction (Fig. 3). The association of the known PTPμ-interacting protein, RACK1 (56), is not altered by the addition of the Rho GTPases (Fig. 3). These data indicate that CA-Cdc42 and CA-Rac1 association with IQGAP1 enhances its interaction with PTPμ. These data also suggest that PTPμ may regulate Cdc42- and/or Rac1-dependent functions of IQGAP1. Fig. 3D shows that CA-Cdc42 causes an increase in IQGAP1 binding to the full-length intracellular PTPμ constructs similar to that seen with the truncated PTPμ constructs, demonstrating that the increase in binding is not unique to the truncated constructs.

PTPμ and IQGAP1 Bind Directly—To gain additional information regarding the PTPμ-IQGAP1 interaction, we constructed a GST-PTPμ fusion protein (iPTPμ-JMD) containing amino acid residues 765–958, which includes the juxtamembrane domain and the first 35 residues of phosphatase domain one (Fig. 1). This construct was used in pull downs from A549 cells. Data shown in Fig. 4A indicate the iPTPμ-JMD construct binds IQGAP1, indicating that the IQGAP1 binding site on PTPμ lies within amino acid residues 765–958. As expected, GST alone was unable to pull down IQGAP1.

To address whether the PTPμ-IQGAP1 interaction is direct, we purified His-tagged IQGAP1 from SF21 insect cells and performed pull-down experiments using purified, bacterially expressed GST-iPTPμ-JMD or GST alone. Data in Fig. 4B show that GST-iPTPμ-JMD binds directly with His-IQGAP1. GST alone was unable to pull down IQGAP1. These data suggest IQGAP1 directly interacts with PTPμ, and this interaction is mediated by amino acid residues 765–958 of PTPμ. iPTPμ-WT was included as a positive control. To determine whether we could recapitulate our previous data with CA-Cdc42 using purified proteins, we performed pull-down experiments using purified His-IQGAP1 and purified iPTPμ-WT in the presence of CA-Cdc42. Similar to the data presented in Fig. 3, CA-Cdc42 enhances the interaction between purified PTPμ and purified IQGAP1 2-fold. These data suggest the effect of CA-Cdc42 is due to a conformational change in either PTPμ or IQGAP1 upon Cdc42 binding (Fig. 4B).

Endogenous PTPμ and IQGAP1 Interact in Vivo—To further characterize the PTPμ/iQGAP1 interaction, we examined the localization of endogenous PTPμ and IQGAP1 in A549 cells as well as their ability to co-immunoprecipitate. In A549 cells PTPμ accumulates at cell-cell contacts in high cell density cultures (Fig. 5) as described previously in other cell types (8, 57). At low cell density, PTPμ stains diffusely in the cytoplasm of single cells and concentrates where cell-cell contacts have formed in adjacent cells (Fig. 5). IQGAP1 is known to associate with cortical actin filaments (19, 29) and to localize at cell-cell contacts (24, 58). In agreement with these data, IQGAP1 accumulates at cell-cell contacts in high cell density cultures of A549 cells (Fig. 5). At low cell density, IQGAP1 remains mostly cytoplasmic but concentrates in the lamellipodia (Fig. 5) as described in other cell types (19, 58).

To get a better understanding of where PTPμ and IQGAP1 might associate, we did co-localization studies using A549 cells expressing...
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**FIGURE 6. Co-localization of PTPμ-GFP and IQGAP1.** Subconfluent A549 cells were infected with baculovirus expressing WT PTPμ-GFP. One day after infection immunocytochemistry was performed for IQGAP1. Phase images are shown of three different fields (d, h, and l). When cells initially contact one another PTPμ-GFP is concentrated at the tip of filopodial extensions (a), IQGAP1 is concentrated in lamellipodia (b), and there is little or no co-localization of PTPμ-GFP and IQGAP1 (c). As contacts begin to form, PTPμ-GFP is localized in a broad band spanning the contact site (e), and a portion of IQGAP1 redistributes to this site (f) and co-localizes with PTPμ-GFP (g, yellow). Once a cell contact has been established PTPμ-GFP (i) and IQGAP1 (j) are both concentrated at the cell-cell contact (k, yellow).

GFP-tagged full-length PTPμ. When adjacent cells are just touching, PTPμ-GFP is concentrated at the tips of filopodia extensions making initial contacts (Fig. 6a), whereas IQGAP1 is concentrated in the lamellipodia (Fig. 6b). As cell contacts form, PTPμ-GFP accumulates in a broad band spanning the area of contact (Fig. 6d). At this point, a portion of the IQGAP1 redistributes to the nascent contact (Fig. 6e) and co-localizes with PTPμ-GFP (Fig. 6g). As suggested by immunocytochemistry of endogenous proteins (Fig. 5), PTPμ-GFP and IQGAP1 co-localize at cell-cell contacts (Fig. 6f, g, and k).

Based on our immunocytochemistry data, we performed immunoprecipitation studies on A549 cells at high cell density. Confluent A549 cells were grown to 90% confluence (Fig. 6a), whereas IQGAP1 is concentrated in the lamellipodia (Fig. 6b). As cell contacts form, PTPμ-GFP accumulates in a broad band spanning the area of contact (Fig. 6d). At this point, a portion of IQGAP1 redistributes to the nascent contact (Fig. 6e) and co-localizes with PTPμ-GFP (Fig. 6g). As suggested by immunocytochemistry of endogenous proteins (Fig. 5), PTPμ-GFP and IQGAP1 co-localize at cell-cell contacts (Fig. 6f, g, and k).

**FIGURE 7. PTPμ and IQGAP1 interact in vivo.** A549 cells were grown to 90% confluence and treated with 1 mM DSP cross-linking reagent. Immunoprecipitations were performed using a polyclonal antibody to an extracellular epitope of PTPμ or non-immune rabbit serum (NRS). Proteins were resolved by SDS-PAGE and immunoblotted for the presence of the indicated proteins. IQGAP1, N-cadherin (130 kDa), E-cadherin (120 kDa), and β-catenin (92 kDa) specifically co-immunoprecipitate with PTPμ. The PTPμ (200 and 100 kDa) immunoblot is a control for PTPμ immunoprecipitation.

IQGAP1 competitive peptide corresponding to the Cdc42 and Rac1 binding site on IQGAP1 completely abolishes nasal RGC neurite crossing onto the PTPμ substrate without affecting growth on the laminin lanes, suggesting that IQGAP1 is required for PTPμ-mediated crossing of nasal RGC neurites (Fig. 8A, IQGAP1). This peptide, comprising amino acids 1054–1077 of IQGAP1 (MK24), is known to compete the binding site on IQGAP1 completely abolishes nasal RGC neurite crossing onto the PTPμ substrate without affecting growth on the laminin lanes, suggesting that IQGAP1 is required for PTPμ-mediated crossing of nasal RGC neurites (Fig. 8A, IQGAP1). This peptide, comprising amino acids 1054–1077 of IQGAP1 (MK24), is known to compete the binding site on IQGAP1 completely abolishes nasal RGC neurite crossing onto the PTPμ substrate without affecting growth on the laminin lanes, suggesting that IQGAP1 is required for PTPμ-mediated crossing of nasal RGC neurites (Fig. 8A, IQGAP1) and Cdc42 and Rac1 (47). Addition of a membrane-permeable, TAT-tagged scrambled peptide did not inhibit PTPμ-mediated crossing (Fig. 8A, Scrambled). Quantitation of the stripe assays was performed as described previously (45, 46) and is shown in Fig. 8B. These data show that the IQGAP1 competitive peptide induces nasal RGC avoidance of the PTPμ substrate lanes, demonstrating that IQGAP1 is required for PTPμ-mediated neurite outgrowth.

**DISCUSSION**

Protein tyrosine phosphorylation is a key post-translational modification involved in the regulation of several cellular processes. Although several substrates and interacting proteins are known for tyrosine kinases, in comparison, few have been identified for tyrosine phosphatases, especially the RPTP family of enzymes. We have identified IQGAP1 as a novel PTPμ-interacting protein and propose that it is part of a PTPμ signaling pathway leading to changes in the cytoskeleton, cell adhesion, and neurite outgrowth.

Cellular functions known to be regulated by PTPμ include cadherin-mediated cell adhesion (12), N-cadherin-mediated neurite outgrowth (13), and axon guidance (42, 46, 59). Similar to PTPμ, IQGAP1 has been shown to regulate cadherin-mediated cell adhesion (23, 24, 60, 61) and to induce neurite outgrowth (62), suggesting PTPμ and IQGAP1 share a common signaling pathway. In addition, both PTPμ homophilic bind-
to phorbol 12-myristate 13-acetate stimulation, and Ser-1443 was identified as the predominant phosphorylated residue (62). Two groups independently identified the potential kinase as the novel PKC, PKCε (62, 64). Li et al. (62) demonstrated that IQGAP1-induced neurite outgrowth in N1E-115 cells is augmented by phosphorylation at Ser-1443. Phosphorylation of Ser-1443 has also been shown to influence the binding of nucleotide-depleted Cdc42 in vitro by opening up the C terminus of IQGAP1 and revealing novel Cdc42 binding sites (64). The implications of IQGAP1 binding to nucleotide-depleted Cdc42 are currently unclear. The fact that Ser-1443 enhances neurite outgrowth induced by IQGAP1 is of interest because both PTPμ-mediated outgrowth and axon guidance of retinal ganglion cells involve the novel PKC, PKC8 (46, 65). We propose that PKC and IQGAP1 are part of a common signaling pathway initiated by PTPμ homophilic binding. Future studies in our laboratory will explore this possibility.

Regardless of whether IQGAP1 is a tyrosine-phosphorylated substrate for PTPμ, PTPμ could modify IQGAP1 activity by regulating IQGAP1 binding partners. This could be accomplished by conformational changes induced by the binding of IQGAP1 to PTPμ. We have provided evidence that active Cdc42 enhances the binding of IQGAP1 to PTPμ independent of phosphatase activity (Fig. 4). Alternatively, several IQGAP1-associated proteins are potential tyrosine-phosphorylated substrates for PTPμ such as classical cadherins, β-catenin and Cdc42. Changes in the tyrosine phosphorylation status of these proteins could alter their ability to interact with IQGAP1.

Immunocytochemistry data predict that PTPμ and IQGAP1 interact initially at nascent cell-cell contacts. It is intriguing to postulate that IQGAP1, downstream of PTPμ homophilic binding, facilitates changes in the actin cytoskeleton leading to cell adhesion and adherens junction formation. This hypothesis is supported by reports indicating filopodia extensions initiate cell-cell adhesion (66). This scenario is applicable to neurite outgrowth and axon guidance as well as cell adhesion, since all of these processes involve sensing adjacent cells and responding to the environment. This idea is supported by the data presented in Fig. 8, which clearly show IQGAP1 is involved in PTPμ-mediated neurite outgrowth of nasal RGC neurons.

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