PTPμ Regulates N-Cadherin-dependent Neurite Outgrowth

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Abstract. Cell adhesion is critical to the establishment of proper connections in the nervous system. Some receptor-type protein tyrosine phosphatases (RPTPs) have adhesion molecule-like extracellular segments with intracellular tyrosine phosphatase domains that may transduce signals in response to adhesion. PTPμ is a RPTP that mediates cell aggregation and is expressed at high levels in the nervous system. In this study, we demonstrate that PTPμ promotes neurite outgrowth of retinal ganglion cells when used as a culture substrate. In addition, PTPμ was found in a complex with N-cadherin in retinal cells. To determine the physiological significance of the association between PTPμ and N-cadherin, the expression level and enzymatic activity of PTPμ were perturbed in retinal explant cultures. Downregulation of PTPμ expression through antisense techniques resulted in a significant decrease in neurite outgrowth on an N-cadherin substrate, whereas there was no effect on laminin or L1-dependent neurite outgrowth. The overexpression of a catalytically inactive form of PTPμ significantly decreased neurite outgrowth on N-cadherin. These data indicate that PTPμ specifically regulates signals required for neurites to extend on an N-cadherin substrate, implicating reversible tyrosine phosphorylation in the control of N-cadherin function. Together, these results suggest that PTPμ plays a dual role in the regulation of neurite outgrowth.

Key words: neurite outgrowth • protein tyrosine phosphatase • cadherin • cell adhesion • retina

The development of the nervous system is a complex process requiring the formation of a large number of specific connections by a variety of neuronal cell types and target tissues. Some of the factors that regulate axonal pathfinding include contact-mediated growth promoting or repulsive molecules, as well as diffusible attractive and repulsive factors (reviewed by Tessier-Lavigne and Goodman, 1996). Contact-dependent guidance is thought to be mediated by a series of adhesive events between the growth cone, the specialized region at the distal tip of a growing axon, and molecules expressed on other cells or in matrices that form the "substrate" for migration. Presumably, the growth cone responds to these cues by initiation of a local signal that ultimately regulates the direction of axonal extension. The role of adhesion molecules in contact-dependent guidance has been extensively studied, yet the signaling events involved in this process are not well understood. The present study investigates the role of a unique cell adhesion molecule, the receptor-type protein tyrosine phosphatase mu (PTPμ),1 in contact-dependent signaling and neurite outgrowth. PTPμ is expressed in several regions of the central nervous system during development and is capable of mediating adhesion, which suggests it could be one of the adhesion molecules that plays a role in neurite outgrowth.

Due to the complexity of the developing nervous system, many studies, including the one described here, have used in vitro model systems to gain a better understanding of the mechanisms that regulate axonal growth. A number of nervous system–derived cell surface proteins have been isolated, but only a subset of these have been shown to promote neurite outgrowth when used as a substrate in vitro. These proteins are subdivided into three major classes: the integrins and their ligands the extracellular matrix molecules (ECM), cadherins, and the immunoglobulin superfamily of cell adhesion molecules (CAMs). Neuronal cells express several integrin receptors and will extend neurites on a subset of ECM molecules including laminin, vitronectin, thrombospondin, jannusin, and tenascin (R. richard et al., 1990; N. eugebauer et al., 1991; R. richard and T. maselli, 1991; L. ochter and Schachner, 1993; Culp et al., 1997). The cadherins are calcium-dependent adhesion molecules and only N- and R-cadherin have been shown to promote neurite outgrowth (Bixby and Zhang, 1990; Redies and Takeichi, 1993). In addition, some Ig superfamily molecules mediate cell–cell adhesion and have also been shown to promote neurite outgrowth (Brummendorf and Rathjen, 1993; Burden-Gulley and Lem...
mon, 1995). L1 and NCA M are two examples of Ig superfamily CAMs that are abundantly expressed in the developing and adult nervous systems, and are capable of promoting neurite outgrowth (reviewed in Burden-Gulley and Lemmon, 1995; Brummendorf and Rathjen, 1998).

A number of receptor protein tyrosine phosphatases (RPTPs) resemble Ig superfamily CAMs such as NCA M, and appear to directly mediate adhesion or associate with other adhesion molecules (reviewed in Brady-Kalnay and Tonks, 1995; Streuli, 1996; Neel and Tonks, 1997; Brady-Kalnay, 1998). The coupling of CAM-like extracellular domains and phosphatase enzymatic activity within one molecule suggests that changes in the adhesive state of the phosphatase could potentially alter enzymatic activity. One such RPTP, PTPα, is characterized by an extracellular segment that contains one MAC domain (meprins, A5, mu), one Ig domain, and four fibronectin type III repeats (Gebbink et al., 1991). Expression of PTPα induced the aggregation of nonadhesive cells (Brady-Kalnay et al., 1993; Gebbink et al., 1993), and both the MAC domain (Zondag et al., 1995) and the Ig domain (Brady-Kalnay and Tonks, 1994a) have been shown to play a role in PTPα-mediated cell–cell adhesion. Together, these studies demonstrated that the binding is homophilic (i.e., the "ligand" for PTPα is an identical PTPα molecule on an adjacent cell).

The juxtamembrane domain of PTPα has 20% amino acid identity to the conserved intracellular domain of the cadherins (Brady-Kalnay and Tonks, 1994b). The cytoplasmic segment of cadherins binds to catenins that link this complex to the actin cytoskeleton (Gumbiner, 1995; Abel et al., 1996). Previously, we showed that PTPα associates with the cadherin/catenin complex (Brady-Kalnay et al., 1993; Gebbink et al., 1993), and both the MAC domain (Zondag et al., 1995) and the Ig domain (Brady-Kalnay and Tonks, 1994a) have been shown to play a role in PTPα-mediated cell–cell adhesion. Together, these studies demonstrated that the binding is homophilic (i.e., the "ligand" for PTPα is an identical PTPα molecule on an adjacent cell).

However, a role for PTPα in the regulation of cadherin function has not yet been shown. The PTPα protein is abundant in many parts of the central nervous system (Gebbink et al., 1991; Brady-Kalnay et al., 1995; Sommer et al., 1997; Brady-Kalnay, 1998) and is developmentally regulated in the retina. The retina is one of the best characterized and experimentally tractable systems for studying both cell–cell adhesion and development. The retina is comprised of a number of different cell types, and the molecular interactions of multiple CAMs are known to regulate retinal histogenesis and axonal pathfinding (Silver and Rutishauser, 1984; Matsunaga et al., 1988a,b; Brittis et al., 1995; Brittis and Silver, 1995). In the retina, PTPα is primarily expressed on retinal ganglion cells (RGCs) whose sole function is to communicate via their neuronal processes with the brain. RGC axons migrate along the surfaces of neuronal and glial cells in the brain during development, thus using the CAMs on the surfaces of these cells as a substrate for neuronal migration.

Due to the homophilic binding nature of PTPα, its developmental pattern of expression in the retina, and interaction with cadherins in other tissues, we tested the hypothesis that PTPα regulates neurite outgrowth. In this report, we used an in vitro retina explant model system to study neurite outgrowth. We provide evidence that PTPα is capable of promoting neurite outgrowth and cell migration when used as a culture substrate. The neurite outgrowth activity was specific to PTPα, since antibodies against PTPα completely inhibited outgrowth on a PTPα substrate. In addition, PTPα was found in a complex with NCadherin in retinal tissues and RGC neurites. To determine the physiological significance of an association between PTPα and NCadherin, the expression level and enzymatic activity of PTPα were perturbed in retinal explants. Downregulation of PTPα expression through antisense techniques resulted in a significant decrease in neurite outgrowth on an NCadherin substrate, whereas there was no effect on laminin or L1-dependent neurite outgrowth. The overexpression of a catalytically inactive form of PTPα significantly decreased neurite outgrowth on NCadherin, thus illustrating the importance of the enzymatic activity of the PTPα phosphatase in this process. These data indicate that PTPα specifically regulates signals required for neurites to extend on an NCadherin substrate, implicating reversible tyrosine phosphorylation in the control of NCadherin function. Together, these results suggest that PTPα plays a dual role in the regulation of neurite outgrowth during retinal development.

Materials and Methods

Antibodies and Reagents

Monoclonal antibodies against the intracellular (SK15, SK18) and extracellular (BK2, BK9) domains of PTPα have been described (Brady-Kalnay et al., 1993; Brady-Kalnay and Tonks, 1994a). The M 84 monoclonal antibody was generated against the peptide CSH 338 (Brady-Kalnay and Tonks, 1994a) from the immunoglobulin domain of PTPα (amino acids 231–256). The 494 polyclonal antibody generated against a PTPα peptide (amino acids 42–60) has been described (Brady-Kalnay and Tonks, 1994a). A monoclonal pan-cadherin antibody (SMP) generated against the COOH terminus of chick Ncadherin was obtained from Sigma Chemical Co. The NCD-2 monoclonal antibody was generously provided by Dr. Gerald Grunwald (Thomas Jefferson University, Philadelphia, PA.) from hybridoma cells generated by Dr. Masatoshi Takeichi (Hatta and Takeichi, 1986). A polyclonal antibody against Ncadherin (7873) was kindly provided by Dr. J. John Hemperly (Becton Dickinson Labs, Research Triangle Park, NC). A nitrocellulose filter assay against chick NCAM (5e, R0 28 and R0 32) and L1 (R0 21) were generously provided by Dr. U. Rutishauser (Case Western Reserve University). Monoclonal antibodies against chick L1 (BD 9), a bipolar neuron-specific antigen (3G3), and a Müller glia-specific antigen (5A 7) were generously provided by Dr. VANCE LEMMON (Case Western Reserve University). RPMI 1640 medium, laminin, and fetal bovine serum were obtained from Gibco BRL. Aprotinin and leupeptin were obtained from Boehringer Mannheim Biochemicals. TWEEN-20 was obtained from Fisher Scientific Co. All other reagents were obtained from Sigma Chemical Co.

Purification of PTPα from Brain

A dural rat brains were minced and homogenized (Belco Biotechnology) in a solution of 0.32 M sucrose in 50 mM Tris–HCl, pH 8.0, containing protease inhibitors 5 mM EDTA, 10 μg/ml turkey trypsin inhibitor, 2 mM benzamidine hydrochloride, and 200 μM phenylmethylsulfonylfluoride. The homogenate was layered onto a 0.8 M, 1.2 M sucrose gradient and centrifuged at 25,000 rpm for 45 min (SW 28 rotor; Beckman Instruments, Inc.). The membrane layer was diluted with TBS and resuspended at 50,000 rpm for 30 min (Ti 70.1 rotor; Beckman Instruments, Inc.). The pellet was then extracted with 1% sodium deoxycholate in 50 mM Tris–HCl, pH 8.0. The membrane extract was resuspended at 50,000 rpm for 30 min
and the supernatant was incubated overnight at 4°C with CB1r (Biotek Biotech) and CB2r (Biosources) antibodies. The beads were washed extensively with 50 mM Tris-HCl containing 0.5% sodium deoxycholate and 0.5% NP-40, pH 8.0, followed by 10 mM Tris-HCl, pH 8.0. The protein was eluted from the column with 0.1 M diethylamine, pH 11.5, and neutralized with 2 M Tris-HCl, pH 3.6. For SDS-PAGE, sample buffer (4% SDS, 20% glycerol, 0.2 M diethoiothreitol, bromphenol blue, 0.12 M Tris, pH 6.8) was added and boiled at 95°C. Lanes were loaded with 10% of the total Triton extract (30 μl per lane) and then run on a 1% polyacrylamide gel. Protein bands were stained with Coomassie blue R-250.

**Dish Preparation and Culture of Retinal Explants and Dissociated Cells**

35-mm tissue culture dishes (Falcon Labware) were coated with nitrocellulose in methanol (Lagenaur and Lemmon, 1987) and allowed to dry. A small amount of protein (2–4 μg) was spread across the center of the dishes, and they were incubated 30 min at room temperature. Remaining binding sites on the nitrocellulose were blocked with 2% BSA in PBS, and the dishes were rinsed with RPMI-1640 medium.

Retinal explant cultures were made according to a previously described procedure (Hall et al., 1983; Drabak and Lemmon, 1990). In brief, embryonic day 8 (stage 32.5–33 according to Hamburger and Hamilton, 1951) White Leghorn chick eyes were dissected and the retinas were flattened with the photoreceptor side down onto black nitrocellulose filters (0.45–μm pore size; Vanguard International, Inc.) that had previously been incubated in 0.05% concanavalin A to enhance attachment of the retina to the filter. The filter was then cut into 350-μm wide strips perpendicularly to the optic fissure using a Mclain tissue chopper. Strips were inverted onto substrate-coated culture dishes so that the ganglion cell layer was directly adjacent to the substrate. Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum/2% chick serum/penicillin/streptomycin-fungizone and incubated at 37°C in 5% air/3% CO2.

Neurite outgrowth on PTPα, N-cadherin, and L1 substrates was inhibited by the addition of culture supernatant from the BK2 (anti–PTPα), NCD2 (anti–N-cadherin), and 8D9 (anti–L1) hybridoma cells, respectively, into the medium at the time of plating retinal explants as described (Brady-Kalnay et al., 1995).

**Immunoprecipitations**

A retinoblastoma was covalently coupled to CNBr-Sepharose 4B (Pharmacia LKB Biotechnology) using the manufacturer’s protocol, or Protein A beads using a previously described protocol (Brady-Kalnay et al., 1993) and the beads were homogenized with a tissue tearor (200; PR0 Scientific Inc.) in lysis buffer (1% Triton, 20 mM Tris pH 7.6, 2 mM CaCl2, 15 mM NaCl, 1 mM benzamidine hydrochloride, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM sodium orthovanadate, 0.1 mM ammonium molybdate, 0.2 mM phenyl arsine oxide), and incubated on ice for 40 min. Triton-insoluble material was removed by centrifugation at 100,000 × g at 4°C, and the lysate was incubated with antibody-coupled beads overnight at 4°C. The beads were washed extensively with lysis buffer, and then boiled in sample buffer and separated by 6% SDS-PAGE. Proteins were transferred to nitrocellulose membrane and immunoblotted as described (Brady-Kalnay et al., 1995).

**Construction and Expression of the PTPα Retroviruses**

The retroviral system used is a tetracycline-repressible promoter-based (“tet-off”) system (Paulus et al., 1996). HeLa cells, transfected with a constitutively active, tetracycline-dependent promoter, were used. Antibodies recognizing the RGC neurite transfer induced by the retrovirus were used to examine the cells for expression of the PTPα retrovirus. For immunocytochemical labeling of retinal explant cultures, the cultures were fixed, and then rinsed with PBS and incubated with blocking buffer overnight at 4°C. A 1:1000 dilution of rabbit IgG was used as a negative control. The primary antibody was incubated overnight at 4°C.

**Immunolabeling of Retina Sections and Cultured Explants**

For immunohistochemical labeling of retina sections, eyes from E8 chickens were fixed with 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (80 mM Pipes, 5 mM EGTA, 1 mM MgCl2, 3% sucrose), pH 7.4. The tissue was rinsed and cryoprotected with 20% sucrose in PBS and frozen in OCT medium (EM Sciences). Cryostat sections were cut at 7-μm intervals, adhered to gelatin coated slides, and stored at −20°C. Sections were permeabilized and blocked with 1% Triton, 20% goat serum in PBS, and then incubated overnight at 4°C with primary antibodies diluted into blocking buffer (2% for serum, 1% BSA, 0.5% saponin in PBS). A 1:200 dilution of the antibodies in blocking buffer overnight at 4°C. A 1:1000 dilution of rabbit IgG was used as a negative control. The primary antibody was incubated overnight at 4°C.

**Infection of Retinal Explants with Retrovirus**

Retroviral-mediated gene transfer requires that the infected cells are still mitotic in order to incorporate and express the retroviral gene. The density of the bands on the immunoblots was measured using the MicroImage system. For immunocytochemical staining of retinal explant cultures, the cultures were fixed, and then rinsed with PBS and incubated with blocking buffer overnight at 4°C. A 1:1000 dilution of rabbit IgG was used as a negative control. The primary antibody was incubated overnight at 4°C.
rons begin to drop out of cell division at E2–3 at a region just dorso-temporal to the optic fissure, and the wave of maturation continues outward from this region in a spatiotemporal fashion (Halfter et al., 1983; Prada et al., 1991). To infect the greatest number of cells for these experiments, retinas from viral-free E3.5–4 chicks (stage 20–23) (Spafas Inc.) were used. At this age, robust neurite outgrowth occurred on N-cadherin, laminin, and L1 substrates, but not on PTP<sub>m</sub>. Therefore, PTP<sub>m</sub> could not be used as a test substrate for these experiments. The dissection and plating procedure is as described above except that the retinas were cut at 250-μm intervals. Explant strips from each retina were laid in alternating fashion onto two similar substrate-coated dishes, with four explants per dish, then 28 μg of polybrene and 1 ml of virus-containing medium were added for a 6–18-h incubation at 37°C. A 1-μl incubation in virus, the medium was exchanged with normal culture medium. Cultures were examined at ~24 and 48 h after plating, and neurite outgrowth from each explant was photographed.

Quantitation of Neurite Outgrowth
To quantify the neurite outgrowth, the 35-mm negatives were scanned and the digitized images were analyzed using the MetaMorph image analysis program. Lengths of the five longest neurites per explant were measured perpendicular to the explant tissue. To measure the number of neurites per explant, the length of neurite outgrowth was outlined using the threshold function, and the total number of highlighted pixels per region of interest was calculated. This method provided a means to compare density between control and test conditions on each substrate. The neurite length and density measurements were analyzed by Fisher’s PLSD, Scheffe, and Student’s t test (Statview 4.51; Abacus Concepts, Inc.), and similar results were obtained with each of these tests for each experiment. The data from all like experiments were combined and plotted (Cricketgraph III; Computer Associates International, Inc.).

Results
Expression of PTPμ in the Retina
During development, the retina becomes laminated such that cells of a particular type localize to distinct regions within the adult retina. A phase contrast image of an embryonic day 8 (E8) chick retina cross-section illustrates the limits of the neural retina and incomplete lamination at this stage of development (Fig. 1 A). Immunohistochemical labeling of E8 retina sections showed that the greatest level of PTPμ expression was present in the retinal ganglion cell layer and in their processes that make up the optic fiber layer (Fig. 1 B). RGC neurons were verified in this region by labeling with an antibody against chick L1 that specifically recognizes the RGC axons (Fig. 1 C).

Figure 1. Expression of PTPμ in chick retina. E8 chick retina sections were immunohistochemically labeled with antibodies against (B) PTPμ, (C) L1, or (D) a bipolar neuron-specific antigen. (B) PTPμ labeling was present predominantly in regions that labeled positively for retinal ganglion cells (C) and bipolar neurons (D). Phase contrast image is shown in A. OFL, optic fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; PC, precursor cells; PE, pigmented epithelium. Scale bar, 50 μm.

Figure 2. Purification of PTPμ from brain. PTPμ was purified from adult rat brain by immunaffinity methods. (A) The eluted PTPμ protein was separated by 5–15% gradient SDS-PAGE and silver stained. Both the full-length protein (200 kD) and the proteolytically processed extracellular (105 kD) and intracellular (100 kD) fragments were detected (arrows). (B) The purified PTPμ protein was examined by immunoblotting to confirm that the preparation was not contaminated by other cell–cell adhesion molecules that promote neurite outgrowth. N-cadherin, NCAM, L1, and two different preparations of PTPμ were purified and equal amounts of the proteins were separated by 6% SDS-PAGE. Immunoblots using antibodies to PTPμ (top left), NCAM (top right), L1 (bottom left), or N-cadherin (bottom right) are shown.

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PTPμ was also expressed at high levels in a region directly adjacent to the pigmented epithelium, which is the outer limit of the neural retina and is thought to be populated in part by mitotic precursor cells (Fig. 1 B) (Mey and Thanos, 1992). In addition, PTPμ was observed in a region of the retina that labeled positively with the 3G3 antibody against bipolar neurons (Fig. 1 D). This immunolabeling pattern is consistent with that previously described for bipolar neurons (Tsui et al., 1992; Mii et al., 1998). Since the axons of RGCs form the optic nerve and are the sole output from the retina to the brain, the high expression of PTPμ on these cells is consistent with a putative role for PTPμ in neurite outgrowth.

**Purification of PTPμ**

To determine whether PTPμ was capable of promoting neurite outgrowth, it was purified from brain by immunoaffinity methods using a monoclonal antibody against the intracellular domain of the protein (SK15). The PTPμ purification strategy included stringent detergent washes of the column before elution to exclude any associated proteins. The full length PTPμ protein from brain was ~200 kD, and two fragments of ~105 kD (predominantly extracellular form) and 100 kD (the intracellular domain, transmembrane region, and a short stretch of the extracellular domain) were observed (Fig. 2 A, arrows). These fragments have been shown to be due to normal proteolytic processing of the protein into noncovalently associated extracellular and intracellular fragments, respectively (Brady-Kalnay and Tonks, 1994a). The eluted PTPμ protein was examined by immunoblotting to confirm that the preparation was not contaminated by other CAMs that promote neurite outgrowth (Fig. 2 B). The samples included purified proteins from brain: PTPμ (two different preparations), N-cadherin, NCAM, and L1. N-cadherin, NCAM, and L1 were detected in the appropriate purified protein lanes, but were absent from the PTPμ preparations. PTPμ was only detected in the PTPμ preparations. These results clearly show that the PTPμ purified using these stringent conditions was not contaminated with N-cadherin, L1, or NCAM, three CAMs that are highly expressed in brain and have been previously demonstrated to promote neurite outgrowth (Rutishauser, 1983; Lagenaur and Lemmon, 1987; Biixby and Zhang, 1990).

**PTPμ Promotes Neurite Outgrowth**

Retinal ganglion cell axons migrate along the surface of neuronal or glial cells in the brain during development (Mey and Thanos, 1992); therefore, CAMs expressed on the surface of these cells can serve as a substrate for neuronal migration. Due to the complexity of the developing brain, we have used an in vitro model system to gain a better understanding of the mechanisms that regulate axonal growth. The in vitro system uses a purified protein-coated dish as a substrate and measures the ability of neuronal cells to extend neurites on that substrate. The homophilic binding activity of PTPμ and expression by RGC neurons led us to test whether RGC neurons were capable of extending neurites on a PTPμ substrate. The purified PTPμ or N-cadherin preparations from brain (Fig. 2) were individually coated as a substrate on dishes (Lagenaur and Lemmon, 1987) and used to culture E8 chick retinal explants (Fig. 3). Neurite outgrowth on a PTPμ substrate was observed within 2 d of plating, and the length and density of outgrowth increased over the following 2 d. Neurites grew out from explants derived from E6, 8, and 10 chick retinas, but not from E4 (data not shown), suggesting that PTPμ-dependent neurite outgrowth may be developmentally regulated. Alternatively, since the expression of PTPμ is very low at E4 (see Fig. 6), we may be unable to detect neurite outgrowth in vitro at that stage. The most robust outgrowth occurred from E8 retinas and this age was used for the experiments described here. On a PTPμ substrate, the neurites tended to be somewhat fasciulated (Fig. 3, D and E). In addition, the growth cones were small and spiky in nature, with multiple long filopodial processes and a small lamellipodial region (Fig. 3 F).

In contrast, outgrowth on N-cadherin was more robust and was observed within 1 d of culture, suggesting a faster growth rate on N-cadherin than on PTPμ. Additionally, the neurites were less fasciulated on N-cadherin (Fig. 3, A and B), and the growth cones possessed a much larger lamellipodial area and several short filopodial processes (C). The lower density of neurite outgrowth on the PTPμ.
substrate in comparison with the N-cadherin substrate may indicate that only a subset of RGC neurons are able to respond to PTPμ and induce neurite outgrowth. However, PTPμ was expressed at equal levels by all neurites growing on N-cadherin or PTPμ substrates (data not shown). The distinct morphology of neurites on a PTPμ substrate in comparison with N-cadherin suggests that PTPμ may use a unique signaling mechanism to promote neurite outgrowth.

To ensure that the neurite outgrowth activity in the purified PTPμ preparation was mediated by PTPμ, retinal explants were cultured on a PTPμ substrate and N-cadherin or L1 control substrates in the presence or absence of function-blocking antibodies against PTPμ, N-cadherin, or L1 (Fig. 4). Neurite outgrowth on a PTPμ substrate was completely blocked by the addition of antibodies against the extracellular portion of PTPμ (Fig. 4 B), but was unaffected by the addition of antibodies against N-cadherin (C) or L1 (D). These results demonstrate that PTPμ specifically promotes neurite outgrowth. N-cadherin antibodies caused a significant inhibition in neurite outgrowth on N-cadherin (Fig. 4 G). Antibodies against the extracellular domain of PTPμ had no effect on neurite outgrowth on a N-cadherin substrate (Fig. 4 F). Similarly, the L1 antibodies used for these experiments completely blocked neurite outgrowth on an L1 substrate (Fig. 4 K), but antibodies against PTPμ had no effect (J). Therefore, the antibodies used for these experiments were specific. Due to the longer time course to achieve neurite outgrowth on a PTPμ substrate (2-4 d), it was a concern that the retinal explants may secrete factors over time that could deposit on the dish surface and promote neurite outgrowth. For the culture of retinal explants on purified protein substrates, the dishes were first coated with substrate protein, and then the remaining binding sites on the dish were blocked by the addition of BSA before plating. A control experiment was done in which retinal explants were cultured on dishes coated with BSA alone. In this situation, no neurites were observed to extend from any explants over the course of 4 d (Fig. 4 H), suggesting that the neurite outgrowth-promoting activity was due to the purified protein used as the substrate and was not due to a soluble factor originating from the retina tissue.

To determine which cell types extended neurites on PTPμ, the cultures were fixed and processed for immunocytochemistry using antibodies against specific cell types of the retina. All of the long neurites growing on PTPμ were positively labeled with an antibody against L1 (8D9; Fig. 5, E and F), which is only expressed by the RGCs (Lemmon and McLoon, 1986), suggesting that the neurites on PTPμ were derived from RGC neurons. In addition, other cells were observed to migrate from the explants and were identified as bipolar neurons (based on reactivity to the 3G3 antibody; Fig. 5, A and B), or Müller glia (based on reactivity to the 5A7 antibody; Fig. 5, C and D) (Drazba and Lemmon, 1990). Other cells from E8 retinas, which extend neurites on PTPμ substrates, may not be specifically associated with neurite outgrowth.
such as photoreceptor cells, were not observed to migrate on PTP\textsubscript{m}. The cells that grew out onto the PTP\textsubscript{m} substrate all express PTP\textsubscript{m}, suggesting that PTP\textsubscript{m} may be acting homophilically to promote outgrowth.

**PTP\textsubscript{m} Interacts with N-Cadherin in Retina**

PTP\textsubscript{m} is abundant in chick retina, and its expression is developmentally regulated (Fig. 6 A). PTP\textsubscript{m} expression is observed by E4 (the earliest time-point examined), with peak expression by E11. N-cadherin is also detected in retina at E4, and undergoes a similar increase in expression with development (Fig. 6 C) (Inuzuka et al., 1991). The molecular weight of PTP\textsubscript{m} is altered with time, suggesting developmental regulation of glycosylation, proteolytic cleavage, and/or shedding. Similar modifications have been observed for a related RPTP, LAR (Streuli et al., 1992; Serra-Pages et al., 1994; Aicher et al., 1997).

To examine whether PTP\textsubscript{m} associates with N-cadherin, the major cadherin in retina, immunoprecipitation experiments were done using E8 retina lysates. Similar results were obtained using E4 retinas (data not shown). Both the full-length (200 kD) and cleaved (100 kD) forms of PTP\textsubscript{m} were immunoprecipitated by antibodies to PTP\textsubscript{m} (Fig. 6 B, lanes 2 and 3), but not by mouse IgG (Fig. 6 B, lane 1). When immunoprecipitates of PTP\textsubscript{m} were probed on immunoblots with antipancadherin antibodies, an association with N-cadherin was detected (Fig. 6 D, lanes 2 and 3). In the reciprocal experiment, N-cadherin was immunoprecipitated by antibodies to N-cadherin (Fig. 6 D, lane 4), but was not detected when mouse IgG was used for immunoprecipitation (lane 1). N-cadherin immunoprecipitates contained the 200-kD full-length form of PTP\textsubscript{m} (Fig. 6 B, lane 4), and the 100-kD fragment of PTP\textsubscript{m} that was also present in the PTP\textsubscript{m} immunoprecipitates (lanes 2 and 3).

The results from the immunoprecipitation experiments indicated that PTP\textsubscript{m} associates with N-cadherin. Since PTP\textsubscript{m} is expressed at high levels within RGC neurons, it is likely that an association with N-cadherin occurs within these cells. To verify that PTP\textsubscript{m} interacts with cadherins in RGC neurons, an antibody cross-linking experiment was performed using neurites from E8 chick retinal explants growing on the control substrate laminin. In Fig. 7, A–B and E–F, the cells were fixed and processed for immunocytochemistry using the 494 polyclonal antibody against PTP\textsubscript{m} (A and B) and antibodies against N-cadherin (C and D) or NCAM (F). For the cross-linking experiments, the explants were cultured for 24 h to allow significant RGC neurite outgrowth, and the live cultures were incubated with a polyclonal antibody against the extracellular domain of PTP\textsubscript{m} (494) to permit clustering of PTP\textsubscript{m} protein on the cell surface (patching; Fig. 7, C and G). The cells were then fixed and processed for immunocytochemistry with antibodies to N-cadherin (Fig. 7 D) or NCAM (H). The PTP\textsubscript{m} protein was cross-linked into patches along the...
neurites (Fig. 7, C and G). Importantly, N-cadherin protein also became concentrated into the PTP$_{\text{m}}$ patch sites (Fig. 7 D). Extensive colocalization was observed between PTP$_{\text{m}}$ and N-cadherin, suggesting a stoichiometric association between these proteins in neurites. In contrast, another abundantly expressed cell adhesion molecule, NCAM, did not become concentrated into PTP$_{\text{m}}$ patch sites (Fig. 7 H). The results of these experiments show that PTP$_{\text{m}}$, N-cadherin, and NCAM are expressed in a continuous fashion along neurites and into growth cones. Live cells were incubated with a polyclonal antibody against PTP$_{\mu}$ to cluster the PTP$_{\mu}$ protein on the surface of the neurites into “patches” (C and G), and then the cells were fixed and double labeled with a monoclonal antibody against N-cadherin (D) or NCAM (F). These results show that PTP$_{\mu}$, N-cadherin, and NCAM are expressed in a continuous fashion along neurites and into growth cones. Live cells were incubated with a polyclonal antibody against PTP$_{\mu}$ to cluster the PTP$_{\mu}$ protein on the surface of the neurites into “patches” (C and G), and then the cells were fixed and double labeled with a monoclonal antibody against N-cadherin (D) or NCAM (F). These results show that PTP$_{\mu}$, N-cadherin, and NCAM are expressed in a continuous fashion along neurites and into growth cones.

**PTP$_{\mu}$ Is Required for N-Cadherin–dependent Neurite Outgrowth**

Based on previous studies, we hypothesized that PTP$_{\mu}$ maintains a protein in the N-cadherin/catenin complex in a dephosphorylated state that may be important for N-cadherin-mediated adhesion and/or neurite outgrowth. To examine the role of PTP$_{\mu}$ in N-cadherin-mediated adhesion events, a retrovirus encoding the antisense version of the PTP$_{\mu}$ cDNA sequence was used to downregulate PTP$_{\mu}$ expression in dissociated retinal cells. PTP$_{\mu}$ is normally synthesized as a full length (200 kD) precursor that can be expressed at the cell surface or cleaved into a 105 kD form (predominately extracellular) or 100 kD form (the intracellular domain, transmembrane region, and a short stretch of the extracellular domain; Brady-Kalnay and Tonks, 1994a; Gebbink et al., 1995). Immunoblot analysis and densitometric measurement of the gel bands that were immunoreactive with an antibody against PTP$_{\mu}$ demonstrated that the full-length protein (200-kD band) was reduced by 99% in cells infected with PTP$_{\mu}$ antisense virus when compared with cells infected with control virus (Fig. 8 A, lanes 1 and 3). In addition, the 100-kD band was reduced by 77%, whereas a 95-kD immunoreactive band was unchanged (Fig. 8 A, lane 3). These results suggest that PTP$_{\mu}$ antisense expression inhibits the new synthesis of PTP$_{\mu}$. Therefore, the full-length protein made before infection has likely been cleaved over time to form the two smaller fragments that may not turn over rapidly. The retrovirus used for these experiments is negatively regulated by tetracycline. When tetracycline was present in the medium, PTP$_{\mu}$ antisense virus had no effect on PTP$_{\mu}$ expression level (Fig. 8 A, lane 4). In contrast, no change in N-cadherin expression was detected in cells infected with PTP$_{\mu}$ antisense virus when compared with cells infected with control virus (Fig. 8 A, lane 3). These results suggest that PTP$_{\mu}$ antisense expression inhibits the new synthesis of PTP$_{\mu}$. Therefore, the full-length protein made before infection has likely been cleaved over time to form the two smaller fragments that may not turn over rapidly. The retrovirus used for these experiments is negatively regulated by tetracycline. When tetracycline was present in the medium, PTP$_{\mu}$ antisense virus had no effect on PTP$_{\mu}$ expression level (Fig. 8 A, lane 4). In contrast, no change in N-cadherin expression was detected in cells infected with PTP$_{\mu}$ antisense virus when compared with cells infected with control virus (Fig. 8 B). The blot for PTP$_{\mu}$ expression was stripped and reprobed with antibodies to NCAM to verify equal protein loading per lane (Fig. 8 C). These results demonstrate that the PTP$_{\mu}$ antisense retrovirus was able to infect the retina cells, resulting in a significant downregulation of PTP$_{\mu}$ expression.

To determine whether PTP$_{\mu}$ function is required for N-cadherin–mediated neurite outgrowth, E4 retinas were infected with PTP$_{\mu}$ antisense or control retrovirus. Explants from the retinas were then cultured on N-cadherin.
or control substrates and neurite outgrowth was examined at 24 and 48 h after plating. E4 retinas were used for these experiments because a large percentage of the cells are still mitotic at this age, which is a requirement for retroviral-mediated gene transfer. E4 explants infected with PTP\(\mu\) antisense virus displayed a dramatic and significant decrease in neurite outgrowth on N-cadherin in comparison with sister explants infected with control virus (Figs. 9, C–D, and 10, A–B; Table I). A range of effects was detected, including no neurite outgrowth, some short neurites, and in a few cases a small number of long neurites were observed. After infection with antisense virus, there is an \(\sim 50\%\) reduction in PTP\(\mu\) expression in the neurites when they are immunolabeled with antibodies to PTP\(\mu\) (data not shown). Therefore, there is a substantial reduction in the PTP\(\mu\) phosphatase overall, but some residual expression of PTP\(\mu\) in all of the neurites in the explant population. The photograph shown (Fig. 9, C–D) is representative of the median level of neurite outgrowth. Quantitation of all of the results indicated that neurite lengths were reduced by 53% and overall neurite density was reduced by 74% in cultures expressing PTP\(\mu\) antisense in comparison with cells expressing control virus. In contrast, PTP\(\mu\) antisense had no effect on either length or density of neurites growing on the control substrates laminin (Figs. 9, A–B, and 10, A–B), or L1 (Figs. 9, E–F, and 10, A–B; Table I). Since the length and density of outgrowth on laminin and L1 in the presence of PTP\(\mu\) antisense virus was similar to that observed previously (Lemmon et al., 1992), it seems unlikely that the PTP\(\mu\) antisense virus altered RGC differentiation. In addition, no difference in the level of axon fasciculation was observed in cultures infected with control versus PTP\(\mu\) antisense virus on any substrate examined. More importantly, the ability of neurons to extend neurites on other substrates suggests that the PTP\(\mu\) antisense virus was not toxic for the neurons themselves and did not have nonspecific effects on neurite outgrowth in general. These results suggest that PTP\(\mu\) is specifically involved in regulating N-cadherin-mediated neurite outgrowth.

The retroviral expression system used for these experiments is repressed in the presence of tetracycline (Paulus et al., 1996). Retinal explants plated on N-cadherin in the presence of both tetracycline and PTP\(\mu\) antisense virus showed no reduction in either neurite length or density (Fig. 10, A–B; Table I) when compared with explants infected with control virus. These results demonstrate that expression of PTP\(\mu\) antisense is regulated by the presence of tetracycline. In addition, the observed decrease in neurite outgrowth on N-cadherin requires the expression of antisense PTP\(\mu\).

PTP\(\mu\) is capable of acting as both an adhesion molecule and an enzyme; therefore, it was important to determine which of these functions was necessary for the regulation of N-cadherin-mediated neurite outgrowth. To address this issue, we generated a PTP\(\mu\) mutant that contained a single amino acid change (cysteine to serine) in the catalytic domain of the phosphatase. This c \(\rightarrow\) s mutant, which encoded the full-length PTP\(\mu\) protein tagged at the COOH terminus with GFP, preserved the adhesive function of the extracellular segment but rendered the phosphatase catalytically inactive. E4 retinas were infected with a retrovirus encoding the c \(\rightarrow\) s mutant form of PTP\(\mu\), and cultured on an N-cadherin substrate. Explants infected with the c \(\rightarrow\) s mutant virus displayed a dramatic and significant decrease (\(\sim 50\%\)) in neurite outgrowth on N-cadherin in comparison with sister explants infected with control virus (Fig. 10, C–D; Table I). These results were similar to those obtained in cultures infected with PTP\(\mu\) antisense virus (Fig. 10, A–B; Table I). Overexpression of full-length PTP\(\mu\) coupled to GFP did not alter neurite outgrowth on N-cadherin (Fig. 10, C–D; Table I). These results indicate that PTP\(\mu\) tyrosine phosphatase...
activity is a key regulatory component for proper N-cadherin function.

Discussion

To gain an understanding of the function of PTP\(\mu\) in the developing nervous system, we examined the ability of embryonic chick retinal neurons to extend neurites on a PTP\(\mu\) substrate. PTP\(\mu\) promoted neurite outgrowth from RGC neurons, and the migration of bipolar neurons and Müller glia from E8 retinal explants. Neurite outgrowth on a PTP\(\mu\) substrate was blocked by the addition of antibodies against PTP\(\mu\), indicating that the neurite outgrowth activity in the purified PTP\(\mu\) preparation was specific to

Table I. Statistical Analysis of Retroviral-mediated Perturbation of PTP\(\mu\) on Neurite Outgrowth

| Substrate       | Virus type | Length (\(\mu\)m) | \(\pm\) SEM | Density (pixels) | \(\pm\) SEM | \(p\)  |
|-----------------|------------|-------------------|-------------|-----------------|-------------|-------|
| Laminin (n = 20) | Vector     | 2118.7 ± 33.6     | 46027.6 ± 38294.4 |
|                 | Antisense  | 2120.4 ± 43.0     | 47545.6 ± 47267.0 | 0.9764 |
| L1 (n = 21)     | Vector     | 718.3 ± 28.9      | 588344.8 ± 97990.8 | 0.2314 |
|                 | Antisense  | 677.5 ± 33.8      | 635546.3 ± 105779.5 | 0.6659 |
| N-cadherin (n = 37) | Vector | 1244.2 ± 31.1   | 345088.8 ± 47357.5 | <0.0001 |
|                 | Antisense  | 583.2 ± 38.5      | 88339.5 ± 18692.3 | <0.0001 |
| N-cadherin + Tet (n = 11) | Vector | 1270.5 ± 66.6   | 328013.9 ± 75864.6   | 0.4828 |
|                 | Antisense  | 1210.1 ± 85.2     | 330952.2 ± 86113.2  | 0.9785 |
| N-cadherin (n = 20) | Vector | 1058.0 ± 49.4    | 313659.3 ± 61925.4  | 0.7892 |
|                 | PTP\(\mu\)GFP | 1039.4 ± 48.8 | 352796.6 ± 54739.4 | 0.6385 |
| N-cadherin (n = 18) | Vector | 1445.1 ± 41.8   | 432560.8 ± 52316.9  | <0.0001 |
|                 | C -> S mutant | 833.1 ± 62.4 | 179565.2 ± 44197.4 | 0.0008 |

\(n\), number of retinal explants tested per virus type on that substrate; \(p\), values from Fisher’s PLSD test, 99% confidence interval.

Figure 9. PTP\(\mu\) is required for N-cadherin–dependent neurite outgrowth. E4 chick retina explants were infected with control (A, C, and E) or PTP\(\mu\) antisense (B, D, and F) virus and cultured on laminin (A and B), N-cadherin (C and D), or L1 (E and F) substrates for 48 h. The neurites in each dish were examined using dark-field optics. No difference in neurite length or density was observed in cultures infected with vector or PTP\(\mu\) antisense virus when cultured on laminin (A and B) or L1 (E and F) substrates. In contrast, PTP\(\mu\) antisense resulted in a dramatic reduction of both neurite length and density in cultures on a N-cadherin substrate (C and D). Scale bar, 400 \(\mu\)m for images shown in A and B. Scale bar, 200 \(\mu\)m for images shown in C–F.
the PTP<sub>µ</sub> protein. The morphology of neurites growing on PTP<sub>µ</sub> was unique from that observed on other purified CAMs, suggesting PTP<sub>µ</sub> may use a distinct signaling mechanism to promote neurite outgrowth. Within the retina, the expression of PTP<sub>µ</sub> is developmentally regulated and increases over time in a pattern similar to N-cadherin expression (Matsunaga et al., 1988; Inuzuka et al., 1991). Previous studies have demonstrated that cadherins and PTP<sub>µ</sub> associate with one another (Brady-Kalnay et al., 1995, 1998; Hiscox and Jiang, 1998). In this study, we demonstrated that PTP<sub>µ</sub> and N-cadherin form a complex in retinal tissue. An association between PTP<sub>µ</sub> and N-cadherin was also demonstrated in RGC neurites, suggesting that PTP<sub>µ</sub> may be involved in the regulation of N-cadherin-mediated neurite outgrowth. The downregulation of PTP<sub>µ</sub> expression through antisense techniques resulted in a decreased ability of RGC neurites to extend on a N-cadherin substrate, but did not affect neurite outgrowth on laminin or L1 substrates. Overexpression of a catalytically inactive form of PTP<sub>µ</sub> also inhibited N-cadherin-mediated neurite outgrowth, thus providing further evidence that a component of the N-cadherin/catenin complex may be a substrate of PTP<sub>µ</sub>. Together, these results provide evidence that PTP<sub>µ</sub> is capable of promoting neurite outgrowth individually, and specifically regulating neurite outgrowth mediated by N-cadherin.

A xonal pathfinding, fasciculation, target recognition, and synapse formation are all processes that require contact-mediated recognition of cell surface cues. The diversity of the CAMs and other molecules involved in axonal pathfinding reflects the staggering array of decisions an individual axon must make along the way to its target. Many of the guidance molecules are members of the immunoglobulin superfamily. These include CAMs like L1 and NCAM, tyrosine kinases such as Eph family members and FGF receptors and even some RPTPs; for example, DLAR, DPTP69D, and now PTP<sub>µ</sub>. Presumably, these molecules mediate specific recognition events at different

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**Figure 10.** Quantitation of the effect of PTP<sub>µ</sub> perturbation on neurite outgrowth. E4 chick retina explants were infected with control virus (gray bars) or PTP<sub>µ</sub> antisense virus (black bars) and cultured on laminin, N-cadherin, or L1 substrates for 48 h. PTP<sub>µ</sub> antisense virus caused a significant reduction in neurite length (A) and density (B) on N-cadherin, but not on laminin or L1 substrates. The retrovirus used is negatively regulated by tetracycline (tet-off system). Retinal explants plated on N-cadherin in the presence of both tetracycline and PTP<sub>µ</sub> antisense virus showed no reduction in either neurite length (A) or density (B) when compared with explants infected with control virus. For statistical analysis, see Table I. E4 chick retinas were infected with control virus (gray bars) or test virus encoding the c→s (C to S) mutant of PTP<sub>µ</sub> (cross-hatched bars) or PTP<sub>µ</sub>GFP sense control (striped bars), and cultured on N-cadherin for 48 h. The c→s mutant caused a significant reduction in neurite length (C) and density (D), whereas the sense control had no effect on either length or density. For statistical analysis, see Table I.
points during axonal outgrowth and pathfinding. CAMs are not solely involved in adhesion of neurons to one another; they also participate in signal transduction. The interaction of a growth cone with a particular CAM can lead to rapid and specific changes in growth cone morphology (Burden-Gulley et al., 1995). This implies the adhesion molecules are sending signals that result in a transient change in the underlying cytoskeleton (Burden-Gulley and Lemmon, 1996) that guide a neuron toward its target (Lin and Forscher, 1993; Bentley and O’Connor, 1994).

RPTPs Are Involved in Axonal Guidance

Previous inhibitor studies suggested that tyrosine phosphatases are involved in the control of neurite outgrowth in general and on CAM substrates (Bixby and Jhabvala, 1992; Beggs et al., 1994; Igelzi et al., 1994). Recent studies suggest that regulation of tyrosine phosphorylation by RPTPs affects axonal growth possibly by “steering” growth cones along the appropriate pathway (Desai et al., 1997). In Drosophila, two CAM-like RPTPs are expressed in the central nervous system, and knockout experiments have demonstrated that they play critical roles in development. Mutaant embryos for the Drosophila RPTPs, DLAR and DPTP69D, display an inability of specific motorneurons to recognize guidance cues that allow them to innervate appropriate target muscles (Desai et al., 1996; Kueger et al., 1996). In addition, a LA R homologue in leech was shown to accumulate in a subset of axonal growth cones and play a guidance role during outgrowth of these axonal processes (Gershon et al., 1998). Together with the present study, these data provide the first evidence that RPTPs could be directly involved in axonal pathfinding and suggest that tyrosine phosphorylation is a key regulator of axonal guidance and choice point recognition.

In the present study, we demonstrated that PTP[ mu] plays a role in neurite outgrowth at physiological levels of protein expression. This data is important because previous studies on the ability of PTP[ mu] to mediate aggregation were performed when PTP[ mu] was massively overexpressed (Brady-Kalnay et al., 1995). We have now demonstrated that PTP[ mu] promotes neurite outgrowth from RGC neurons, presumably through a homophilic binding mechanism. PTP[ mu] may have several important roles in nervous system development. First, it may act as a cell–cell adhesion molecule necessary for maintenance of nervous system integrity. This could occur through homophilic binding of PTP[ mu] on the surfaces of two opposing axons to promote axon fasciculation, a process required for nerve formation. A similar role has been suggested for other CAMs such as L1 (Lemmon et al., 1989; Tang et al., 1992) and NCA M (Rutishauser et al., 1978). Second, PTP[ mu] may act as a permissive molecule for axonal growth. The expression of PTP[ mu] by axons, astrocytes (our unpublished data) and other nonneuronal cells makes this role a likely possibility. A contact attraction role for PTP[ mu] is a third possibility, such that PTP[ mu] actively guides axons during pathfinding. For example, PTP[ mu] may be expressed at specific choice points where axons must choose the appropriate pathway. RGC growth cones encounter several choice points during outgrowth to their target, the optic tectum (Tessier-Lavigne, 1995). The mechanisms regulating this stereotypical innervation pattern are only partly understood, but involve tyrosine phosphorylation (Cox et al., 1990; Descher et al., 1997). A fourth possible function of PTP[ mu] is as a sensor molecule. For example, changes in the adhesive state of the extracellular environment may be transmitted through PTP[ mu] via regulation of its catalytic domain. PTP[ mu] may then directly regulate the phosphorylation state of a number of cytosolic proteins, including components of the cadherin/catenin complex. This idea is supported by the inhibition of N-cadherin–mediated neurite outgrowth when RGC neurons overexpress a catalytically inactive form of PTP[ mu].

Cadherins Are Regulated by Tyrosine Phosphorylation

A likely target of the PTP[ mu] enzyme is a component of the N-cadherin complex, which was previously postulated to be a substrate of PTP[ mu] (Brady-Kalnay et al., 1995, 1998). The association of cadherins and catenins with receptor and nontransmembrane PTPs has now been observed by many groups (Brady-Kalnay et al., 1995; Balsamo et al., 1996, 1998; Fuchs et al., 1996; Kypa et al., 1996; Aicher et al., 1997; Cheng et al., 1997; Brady-Kalnay et al., 1998; Hiscox and Jiang, 1998). The juxtamembrane domains of PTP[ k] (Fuchs et al., 1996) and PCD2 (Cheng et al., 1997) interact with β catenin. A LA R-like RPTP associates with the cadherin/catenin complex in PC12 cells (Kypa et al., 1996) and the intracellular domain of LA R was shown to bind directly to β catenin and plakoglobin in vitro (Aicher et al., 1997). The PTP1B cytoplasmic phosphatase was shown to interact with the N-cadherin/catenin complex and dephosphorylate β catenin (Balsamo et al., 1996), a process required for N- cadherin–mediated adhesion and actin linkage (Balsamo et al., 1998). Therefore, it is likely that regulation of the cadherin/catenin complex by PTPs will be an important mechanism of control in many cell types, including neurons.

Tyrosine phosphorylation of the cadherin/catenin complex correlates with suppression of cadherin–mediated adhesion (Matuyoshi et al., 1992; Behrens et al., 1993; Hamaguchi et al., 1993), adherens junction disassembly/loss of cytoskeletal association (Warren and Nelson, 1987; Volberg et al., 1991, 1992), invasion and malignant progression (Kemler, 1993; Birchmeier, 1995). Tyrosine phosphorylation of the cadherin/catenin complex can be catalyzed by pp60src, EGF receptor, c-erbB2, or hepatocyte growth factor receptor (Tsukita et al., 1991; Hoschuetzky et al., 1994; Ochiai et al., 1994; Shibamoto et al., 1994). These data indicate that one of the mechanisms the cell uses to regulate the function of the cadherin/catenin complex is tyrosine phosphorylation. In the present study, PTP[ mu] associated with N-cadherin in lysates from retina as demonstrated by immunoprecipitation techniques. A similar association was demonstrated in RGC neurites through antibody cross-linking and immunocytochemistry techniques. In addition, the ability of neurites to migrate on N-cadherin was significantly impaired when PTP[ mu] expression was downregulated. These results provide evidence that N-cadherin–mediated neurite outgrowth requires functional PTP[ mu]. The inhibition of N-cadherin–mediated neurite outgrowth due to overexpression of the catalytically inactive form of PTP[ mu], further supports the idea that cadherins or their associated proteins need to be dephosphorylated to function in adhesion (Brady-Kalnay et al., 1995, 1998).
Therefore, PTP<sub>μ</sub> tyrosine phosphatase activity is a key regulatory component of the N-cadherin/catenin complex. In contrast, PTP<sub>ι</sub> downregulation did not alter neurite outgrowth on L1 or laminin control substrates. These results suggest that the effects of PTP<sub>ι</sub> downregulation were specific to N-cadherin-mediated neurite outgrowth and not due to general alterations in cellular phosphoryrosine that could nonspecifically affect neurite outgrowth.

**Perspectives on the Developmental Role of PTP<sub>μ</sub> in the Retinotectal System**

Our data demonstrate that PTP<sub>μ</sub> can promote neurite outgrowth, which is consistent with a role for PTP<sub>μ</sub> in neuronal pathfinding. This promotion of neurite outgrowth could be mimicking the ability of certain neurons to respond to signaling events initiated by PTP<sub>μ</sub>. The inability of PTP<sub>μ</sub> to promote neurite outgrowth from retinas earlier than E6 suggests that a threshold level of PTP<sub>μ</sub> expression on axons may be required for PTP<sub>μ</sub> to independently promote neurite outgrowth through a homophilic mechanism. At earlier ages, PTP<sub>μ</sub> may play other specific roles; for example, regulation of N-cadherin-dependent adhesion that is required for morphogenetic movements or axonal pathfinding events. N-cadherin is one of the key molecules involved in many aspects of retinal function from histogenesis and lamination to neurite outgrowth and synapse formation (Matsumura et al., 1988; Riedes and Takeichi, 1993; Fannon and Colman, 1996). PTP<sub>μ</sub> may regulate N-cadherin function by modulating signals that allow neurons to respond to N-cadherin-mediated adhesion.

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