A Novel Protein-Tyrosine Phosphatase Related to the Homotypically Adhering κ and μ Receptors*

(Received for publication, July 11, 1996, and in revised form, December 3, 1996)

Jill Cheng‡, Kai Wu‡, Mark Armaninìš, Nancy O’Rourkeš, Donald Dowbenko‡, and Laurence A. Lasky‡‡

From the Departments of ‡Molecular Oncology and §Neuroscience, Genentech, Inc., South San Francisco, California 94080

Here we describe a novel member of the receptor-like protein-tyrosine phosphatases (PTPs) termed PTP κ, which is homologous to the homotypically adherent PTPs κ and μ. Murine PTP κ contains MAM, IgG, fibronectin type III, and dual phosphatase domains. As has been demonstrated for PTPs κ and μ, PTP κ mediates homotypic adhesion in vitro, and PTP κ is associated with β catenin in kidney epithelial cells. The extracellular domain of PTP κ is proteolytically processed in cell culture as well as in vivo. Northern blot analysis reveals that PTP κ is expressed throughout embryonic development and is predominately found in adult brain, lung, and kidney. In situ hybridization to 15.5-day old rat embryos reveals that PTP κ is expressed in a variety of embryonic neuronal sites as well as in the esophagus, lung bronchiolar epithelium, kidney glomerular epithelium, ophthalmic epithelium, and various cartilaginous sites. Analysis of neonatal brain demonstrates expression in cells of the hippocampus, cortex, and the substantia nigra. Finally, immunohistochemical analysis reveals expression of this PTP on specific neurons of the spinal cord as well as on isolated cortical neurons.

Tyrosine phosphorylation is induced by a plethora of receptor-like molecules as well as by a wide range of intracellular enzymes. The effects of tyrosine phosphorylation are numerous, and they modulate a range of developmental as well as other cellular operations. Of course, the importance of tyrosine phosphorylation is underlined by the need for mechanisms that carefully regulate the levels of these events. Thus, protein-tyrosine kinases represent positive mediators of tyrosine phosphorylation, while protein-tyrosine phosphatases (PTPs) induce the removal of phosphate from tyrosine. The balance of the levels of tyrosine phosphate is thus mediated by the relative activities of these two types of enzymes. It is therefore clear that the mechanisms which regulate cellular function via tyrosine phosphorylation require specific proteins that mediate both the up-regulation as well as the down-regulation of the levels of this modified amino acid.

PTPs represent a growing family of enzymes that are found in both receptor as well as non-receptor forms (1–3). The receptor PTPs are a highly diverse group that are unified by the inclusion of a hydrophobic domain that dispose them to the plasma membrane of the cell. Recently, the receptor PTPs have been subdivided into eight types based upon their domain content (4). These subtypes all contain one or two PTP domains on their cytoplasmic sides, with a variety of extracellular motifs including heavily O-glycosylated mucin-like domains (for example, CD45), chondroitin sulfate domains (for example, PTP γ), and short, highly glycosylated segments (for example, PTP α). The largest family of PTPs is the family that contains various motifs related to those found in adhesion molecules. These motifs include immunoglobulin-like (IgG) domains and fibronectin type III (FNIII) regions similar to those found in cell adhesion molecules such as ICAM, N-CAM, and Ng-CAM (5). In addition, a subset of these adhesion-like PTPs, including the PTPs κ and μ, contain a third domain termed the MAM, for meprin/A5/PTP μ, motif (6). The MAM motif has been previously shown to be involved with cell-cell recognition in neurons (7–9). Interestingly, recent data suggest that three of these adhesion-like PTPs appear to be involved with neuronal pathfinding during Drosophila development (10, 11).

PTPs κ and μ are the receptors that are most well characterized as homotypic adhesion molecules (4, 12, 13). Thus, a diversity of assays, including cell- as well as molecule-based, have demonstrated that the extracellular domain of these enzymes can bind with high specificity in a homophilic manner (14–16). Interestingly, mixing experiments have revealed that these related PTPs will not bind to each other in a heterophilic manner, suggesting that the extracellular region is meant to recognize other cells specifically expressing identical receptors, a situation highly reminiscent of the cadherin homotypic adhesion system (17). While the extracellular domains required for this homotypic binding remain controversial, it appears likely that both the MAM motif as well as the IgG region are involved with homophilic interactions (18, 19). While these data suggest that these homophilic adhesion enzymes are involved with the recognition of other cells expressing similar types of receptors, other data have suggested that this recognition event may play a role in the attachment of such cells to each other. Thus, Tonks and coworkers (20) have recently demonstrated that the receptor PTP μ specifically associates with the catenin/cadherin complex of homotypic cell adhesion molecules. They also demonstrated that treatment of cells with the PTP inhibitor pervanadate resulted in the up-regulation of tyrosine phosphorylation of cadherins and catenins, a result which suggested a role for a PTP, potentially PTP μ, in the maintenance of the cadherin/catenin complex in an underphosphorylated state. However, another recent report casts somewhat of a shadow on these findings (21). Interestingly, two other recent reports suggest that PTPs κ and LAR also appear to interact with the catenin/cadherin complex in a specific manner (22, 23). Importantly, previous work suggested that the level of tyrosine phos-
A Novel k-/μ-Like Tyrosine Phosphatase

The finding that PTPs k and μ mediated homotypic adhesion, together with the somewhat restricted tissue distribution of these PTPs (12, 13), has suggested that additional members of this family of adhesive enzymes might exist. Here we report the cloning and characterization of the third member of this receptor PTP family, termed murine PTP λ. The receptor reported here contains structural motifs that are very similar to those found in PTP k and μ and is capable of mediating homotypic adhesion and associating with β catenin. In addition, this receptor PTP reveals a tissue distribution, particularly with respect to its expression on neurons in the central nervous system, which is divergent from that previously described for the other members of this family. These data are consistent with a burgeoning family of MAM-containing receptor PTPs potentially involved with cell adhesion in various tissues.

MATERIALS AND METHODS

RNA Islandation and Polymerase Chain Reaction—Messenger RNA was isolated from the non-adherent Lin-CD34+ fraction of fetal yolk sac hematopoietic cells (25) (Micro-FastTrack, Invitrogen). Poly(A)+ RNA was reverse transcribed with random hexamers (Promega) and Moloney murine leukemia virus reverse transcriptase (SuperScript II, Life Technologies, Inc.). One quarter of this cDNA was amplified by PCR using degenerate mixed oligonucleotides primers. Sense and antisense primers corresponding to the amino acid sequences (H/D)- FWRM(I/V)W (5′-A(C/T)T(C/T)TGG(A/C)GIATG(A/G)TITGG-3′) and WPD/FH/G/VP (5′-GGIAC/GA/TAG/AA/GA/GTCIGGCCA-3′), respectively, in Western blot experiments.

Expression and Purification of the MAM-IgG-FnIII Immunoglobulin—Various truncation mutants of PTP λ, which contained the herpes simplex virus glycoprotein D signal sequence and the monoclonal antibody 5B6 binding epitope, were produced in the PPK 5 vector using the polymerase chain reaction. Constructs were transfected into 293 cells and incubated for up to 4 days. The supernatant was collected and centrifuged to remove cell debris. Then it was incubated with 5B6 at 1 μg/ml for 2 h at 4 °C and protein G-Sepharose (Pharmacia) was added for another hour. The immune complexes were washed 5 times with 0.2% deoxycholic acid, 0.2% Tween 20 in PBS, boiled for 5 min with SDS sample buffer, and analyzed on 4–20% SDS-PAGE. The gel was transferred to a ProBlott membrane (Applied Biosystems) with electrophoretic buffer (10 mM CAPS, pH 11, and 10% Methanol). Immunoblots were probed with the 5B6 antibody and developed using chemiluminescence (ECL, Amersham Corp.). Alternatively, if the cell lysates were used, the transfected cells were washed once with PBS and lysed in E1A lysis buffer (0.25 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 50 mM HEPES, pH 7.6, and 1 μl/melprotinin) at 4 °C. After centrifugation, the incubation of the lysate with antibodies and protein G-Sepharose, SDS-PAGE separation of the immune complex, and immunoblot detection were as described above. Neonatal brains were dounce-homogenized in the same lysis buffer and immunoprecipitated with affinity purified anti-extracellular domain antibody, transferred to membranes, and probed with the same antibody.

Cocapritylation with β Catenin—Lysates of human embryonic kidney (293) cells, which had been previously transfected with either the full-length PTP λ or a truncated version missing the dual phosphatase domains but containing the juxtamembrane region, were immunoprecipitated with an anti-β catenin monoclonal antibody directed against the C terminus of the protein (Transduction Laboratories) using the immunoprecipitation conditions described above. The anti-β catenin immunoprecipitates were run on SDS gels, immunoblotted, and probed with both the receptor and the 5B6 IgG antibody. Immunoprecipitation conditions were as described above. Neonatal brains were dounce-homogenized in the same lysis buffer and immunoprecipitated with affinity purified anti-extracellular domain antibody, transferred to membranes, and probed with the same antibody.

Northern Analysis—A 2.5-kilobase cDNA fragment encoding the cytoplasmic region of PTP λ was used to probe the multi-tissue Northern blot (Clontech).

In Situ Hybridization—Rat E15.5 embryos and postnatal day 1 brains were immersion fixed overnight at 4 °C in 4% paraformaldehyde, then cryoprotected overnight in 15% sucrose. Adult rat brains were fresh frozen with powdered dry ice. All tissues were sectioned at 16 μm and processed for in situ hybridization for PTP λ using [33P]UTP labeled RNA probes. Sense and antisense probes were synthesized from a 2.5-kilobase DNA fragment of PTP λ using SP6 or T7 polymerase, respectively.

Immunohistochemistry—The cerebral cortex from postnatal day 1 (P1) rat pups was dissected out and minced into small pieces in Hanks' balanced salt solution. The tissue was dissociated in papain (Worthington Biochemical Corp.) with a homogenizer in modified medium with B27 supplement (Life Technologies, Inc.) and plated onto chambered microscope slides (Nunc) coated with poly-D-lysine. The cells were maintained in an incubator for 1 day to allow the neurons to extend neurites and then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for at least 15 min. Brains from embryonic day 14 (E14) rat pups were immersion fixed in 4% paraformaldehyde, cryopre-
Results
Isolation and Characterization of the cDNA Encoding PTP λ—In order to isolate novel PTPs expressed in murine primitive hematopoietic cells, we undertook the cloning of PCR fragments produced by priming with sequences directed against conserved protein motifs found in PTPs from a number of different genes and species (28). Analysis of 70 different PCR-derived subclones revealed an array of previously described PTPs as well as two novel PTPs. One of these novel PTPs, termed PTP HSCF, is a member of the PTP PEST family of enzymes, and it has been previously described (29). The second novel PTP fragment was homologous to PTPs κ and μ, both related receptor-type PTPs that mediate homophilic adhesion (4). In order to further characterize the cDNA encoding this novel PTP, a combined cloning approach that utilized RACE as well as cloning from phage cDNA libraries was performed. The composite cDNA and derived protein sequences determined from these various clones is shown in Fig. 1. The ATG start codon utilized for translation of this large open reading frame was embedded within a consensus Kozak sequence, and there are several translational stop codons upstream of this initiator codon. As can be seen from this figure, the protein derived from this cDNA is a large receptor-like molecule of 1,436 amino acids and a molecular mass of approximately 161,176 Da. Closer perusal of the sequence reveals 13 consensus N-linked glycosylation sites in the extracellular region of the receptor, consistent with a potentially significant degree of glycosylation. Fig. 2 illustrates that the novel, hematopoietically derived PTP-related protein reported here shows a high degree of homology to both PTP κ (60%) and PTP μ (53%) throughout their entire lengths (12–13). Thus, the novel PTP contains MAM, IgG, four fibronectin type III, and two cytoplasmically localized phosphatase domains (Fig. 2) (4, 12, 13). These homologies with the novel PTP are somewhat less than the homology between PTP κ and μ (62%), suggesting that the novel PTP reported here is more distantly related to these two PTPs rather than they are to each other. These data suggest that this novel PTP is the third member of the homotypically interacting PTP family containing PTPs κ and μ, and we have therefore named the novel receptor PTP λ.

As can be seen from Fig. 3, the relative sequence homologies in each of the domains of these three receptors suggests that they are indeed closely related. Interestingly, previous data suggested that both the MAM and IgG domains mediated specific homotypic adhesion between PTPs κ and μ (18, 19), and it is clear from the sequence comparisons between these three related proteins that these two domains are homologous. However, the fact that there are a large number of sequence changes between these two motifs is also consistent with the supposition that they can mediate specific homotypic interactions. The overall sequence homologies between the three proteins is also relatively high in the FnIII domains although the homology in the first of these domains is significantly higher than in the others. Previous work has also demonstrated that a juxtamembrane site between the transmembrane domain and the first phosphatase domain is distantly homologous to a similar region in the cadherins (20), and this site shows a high degree of homology between these three receptors, a point that will be discussed further. A significant degree of sequence homology is also found between the first PTPase domains of these three receptors, with a somewhat lower level of homology between the second PTPase domains of these proteins. This latter result may be important since it has been reported that the first phosphatase domain is the critical enzymatic motif of the dual phosphatase regions in the receptor PTPs (30, 31). The homology between these PTPase domains includes many of the residues previously found to be important for substrate recognition and tyrosine dephosphorylation in the PTP 1B (32) although not all of these residues are identically conserved, and immunoprecipitation studies have demonstrated that PTP λ has weak, but detectable, phosphatase activity against two different tyrosine-phosphorylated peptide substrates as well as against the artificial substrate p-nitrophenyl phosphate (data not shown).

Homotypic Adhesion Mediated by PTP λ—Previous data have demonstrated that the PTP λ-related receptor PTPs κ and μ induce homotypic adhesion and that these adhesive events appear to be mediated by the MAM and IgG domains. In order to examine the ability of PTP λ to mediate such homotypic adhesion, a soluble construct containing the MAM, IgG, and first FnIII domain attached to the hinge, CH2, and CH3 regions of human IgG was produced (λ-IgG chimeric protein). This chimeric protein was purified to ~90% by passage over protein A-Sepharose (data not shown) and was used in in vitro binding experiments. As a control, the extracellular domain of the tyrosine kinase receptor FLT 4, in the form of an IgG chimera, was used (FLT 4-IgG) (27). As can be seen in Fig. 4, fluorescent beads containing the FLT 4-IgG were unable to aggregate, while beads containing the λ-IgG protein formed large mixed aggregates. As a further control, the aggregation of a mixture of red λ-IgG and green FLT 4-IgG beads was examined. Fig. 4 shows that only the red beads aggregated in this experiment, with the green beads remaining largely unaggregated. This in vitro binding assay thus demonstrates that a form of PTP λ can mediate homotypic aggregation and is consistent with previous data suggesting that the MAM and IgG domains are critical for this adhesive event (18, 19).

Proteolytic Cleavage of PTP λ in Vitro and in Vivo—A number of previous studies have suggested that various receptor PTPs, including κ and μ, appear to have a regulated, specific proteolytic cleavage that gives rise to a secreted region derived from the extracellular domain (12, 33). We examined this possibility for PTP λ in two different ways. In vivo proteolytic cleavage was examined by probing extracts of neonatal mouse brain with an affinity purified polyclonal antibody directed against a GST fusion of the extracellular domain of PTP λ. This source of tissue was chosen because of the high transcript levels in Northern blots of adult brain as well as the in situ hybridization results (both described below). As can be seen in Fig. 5, immunoprecipitation of brain extracts with this antibody and subsequent Western blotting revealed three specific bands in neonatal brain. The highest molecular mass band (~190 kDa) appears to correspond to a glycosylated full-length form of this PTP. A second band at ~115 kDa and a smaller band at ~70 kDa are observed and are likely to be proteolytic fragments of the full-length receptor since they specifically react with the affinity purified antibody directed against the extracellular domain. These data thus demonstrate that PTP λ appears to be proteolytically processed in brain extracts to two lower molecular weight forms, and additionally demonstrates the specificity of the affinity purified antibody directed against this phosphatase.
To examine this processing event in greater detail, we produced a number of different truncated constructs to examine the cleavage of the protein in vitro. All of the constructs contained a herpes simplex virus glycoprotein D signal sequence and N-terminal monoclonal antibody (5B6) epitope to aid in the analysis. As can be seen in Fig. 6, expression of a full-length form of the receptor in 293 cells results in a 190-kDa protein as well as a 115-kDa proteolytically clipped form. In order to examine this processing event in greater detail, we produced a number of different truncated constructs to examine the cleavage of the protein in vitro. All of the constructs contained a herpes simplex virus glycoprotein D signal sequence and N-terminal monoclonal antibody (5B6) epitope to aid in the analysis. As can be seen in Fig. 6, expression of a full-length form of the receptor in 293 cells results in a 190-kDa protein as well as a 115-kDa proteolytically clipped form.

In order to examine this processing event in greater detail, we produced a number of different truncated constructs to examine the cleavage of the protein in vitro. All of the constructs contained a herpes simplex virus glycoprotein D signal sequence and N-terminal monoclonal antibody (5B6) epitope to aid in the analysis. As can be seen in Fig. 6, expression of a full-length form of the receptor in 293 cells results in a 190-kDa protein as well as a 115-kDa proteolytically clipped form.
A Novel $\kappa/\mu$-Like Tyrosine Phosphatase

2122 GAG GTA ACC ACC ACG TCG GCC ACC AGC TTT GAT TAC TCC GCC GAT CGC TCA CCC
2179 CTC GGC GAG TAC GAC ACC ACT TGT GTC TCC TGG TCG TCG GAG CGG GCC CAG CGG CCA GGA
2236 CCC ACC ATC AGC TGC CAC CAG CAG GAT GAG GAT GAG GAC GGC GCC CAG CCC TGG CCG
2293 CCG GAG CCC GAA GCT CAG GAC TCC TTC TCA GCT CGG ACC ATT TTC GAG ACG GCC CTC
2350 GCT GGC GAG GTG CAA CAC TAC TGG TGG GCT GAA CGG CCG GCT GCC ACC ACG GAG ATG
2407 GCC ATG CCC TTC ACC GTG GGT GAC ACC CAG ACC TAT GGT GTC TGG AAC CAG CGG
2464 CTT GAG CCC AGA AAG GCC TAT CTC ATT TAC TAT CTC GCA GCA ACG CAC CTC GGA AAG
2521 GAA ACC CGA CTT TGC ATC ATG CGG AAA AAG AAT GCT GCC TAC AAG GAG AAC AAG
2578 CUC CCC TTC GAA GTG GCC CAG ATC CAG GAG ATC GGT GGC ATC TTC GAT CAG ATG
2635 GCA GTT GGT TCT GCC TCT CTC ATT CCT CTC CGG GCC ATT CTC GTC ATC ACC ATC GCC
2692 AAC GGG GAG ACG CCA CGA AAC ATG AGC AGG AAC GCC AAC GTC AAC TAC CAC GAG GAG ACT
2749 CAC ATG ATG AGT GCC ATC CAC CAG ACC TTC ACA GAT CAG ACT GTG CAG GAG GAT
2806 GAG CAC TGG TCG TTC CTC GCT ATG CTT GCT GCC TAC TAT CCA GGT GGA CAC CAG
2863 CTA ACC AGT GGT GTC ACC GAG GCC ACC ACC TTC CTC GCG GGG GCC CTA GTG
2920 TGG GCC CGG AAG GTG TCT CGG TAT CAT ACC GCG CAG CTC CAG CTC CCA GCA GCC CTA GTG
2977 TCT GAC CTT CTA CAG CAC ATC ACC GAG ATG AAG AAG AAG GCC GAG GCC TAC GCC TTC AAG
3034 CAG AC GGA TAC ACC GAT ATG ATG ATC CAG AAC TCA GAC GAG CCG GCC ACC AAC AAG AAG GAC CAG ACG
3091 AAC GGC GCC CAA CAG CAG CAG GCA CCA GTC TCT GCC TAT GAG CCA CAG CAT GCT AAG AAA CTA CAC
3148 CCA ATG CTA GCC AAC CTT GAC ACC ATT GCC AAC TAC ATC TTA GCC AAG TAC GCC TAC
3205 CAC AGC TAA ACC CAC TTC ATC ACC ACT CAA GCG CAA CAG CTT TAT GAG TAC ATG CAT
3262 TTC TGG CAC ATG GTG TGG GAG CAA CAG TGT GCG ACC ATC ATG ACC AAG ATC
3319 GTA GAG GTG GCC AGG GTG AAA TGT TCT GCC TAC TGG GCC GAG TAC GCC ATG AGT
3376 GGG GCC ATC AAG ACC AGT ATG GTA AAG AAG AAG GAA GAG ACA ATG GTG GCT CAC
1000 GD IN KIL TUV KTE TEL LA EY VUR
3433 ACC TTT GCC GTG GAG CCG AGA GAT TAC TCA GCC CCC CAT GTC CCG TCG TAC CAT
1019 T P A L R E G Y S A N H E V R Q P E
3480 TTC ACA CGC TGC GAA CAG GAT GGT GTC GCC CCC TTC CAC ACC GCC ACC GCT CGG TGC GCC TCC
1067 T T A W P E H G V P Y H A T G L L A F
3547 ATC CGG CGT GTG AAG TCT TCC ACT CCA CCT GAT GCC GGC GGG GCC ATT ATC ACC TAC
1085 R V K A S T P P A D G F I V H I N C
3604 AGT GCA GAA ACT GCC CAC ACA GCC TTC TAC ATC GTC CAA GAT GTG ATG ATG CAG ATG
1067 S A G T G R T G C Y I V L D V M L D M
3661 TGT GCC TGT GGT GAG GGT GTC GCT GAC ATT TAC AAG TGT GAG ACC TTC TGC CTC CCA
1095 K E C G V D V I N Y E N C V K T L C S R
3718 CGG GTC AAC ATG TAC CAG ACC CGG GAA CAA CAA TAT ATC TTC ATC ATC CAG CAT GCA ATC TGT
1114 R V N M I Q T E B Y I F I N H D A I L
3775 GAG GCC TGC TGT CTC TGT GSG GAG ACC ACC ATC TTC GCC AAG CAG TTC ATG GCC ACC ACC TAC
1133 E A L C G G B T T T P H D N F R A T V
3832 AGG GAG ATC ACG ACT ATC CAC GAT CAG CAC AAT ACC ATC TCC TCG ATC TCT CAG GAA CAG TAC
1152 P H M I S L L K H K M F
3889 CAG ACC ATG ATC CTC ACC CCC GCG CGG ATG GTG GAG GAG TGT GAC ATC ATT GCC CTC
1171 Q T L N S V T P F L D V E R C S I A L
3946 CGG CCC GAA ATG CAA GAC AAG AAG CAA ACC ATG GAT GAT GCG CCA CCA GCC CCC TGC
1190 L P R N R D K N R S M D V L P F D R X
4003 CCG CCC TTC CTC TAC TCC TTC AGT GTG GSG GCG CCC AAT AAC TAC ATT AAT GCA CAC CTA
1209 L P F L S D G D P N Y I N A A L
4060 ACT GAC ACC TAC ACA CGG ACC GCC TTC ATG CTC ACC CAG CTC CAG CAT AGT
1228 T D S Y T R S A A F I V T L H P L Q S

Fig. 1—continued
fragment, very similar to those seen in neonatal brain extracts. Examination of cell supernatants reveals that the smaller −115-kDa fragment appears to be shed from the cells. This cleavage occurs in the absence of the PTP domains since the truncation mutant lacking the dual phosphatase domains also shows a −115-kDa fragment in both the cell lysates as well as in the supernatants. Interestingly, this cleavage appears to occur when the MAM domain is truncated from the receptor (appearing as a smaller band in the cell lysates transfected with construct number 3 in Fig. 6) although this form of the protein does not appear to be secreted, possibly due to incorrect folding of this form of the protein and intracellular degradation. Finally, removal of both the MAM and IgG domains results in 64- and 50-kDa fragments in the cell lysates, with the 50-kDa fragment being very efficiently secreted into the media. The molecular mass of the secreted 50-kDa fragment is consistent with the proteolytic cleavage event occurring within the fourth FnIII repeat (12; also see “Discussion” below).

Furthermore, these experiments demonstrate that the processing site appears to be contained within the fourth FnIII domain, a result similar to that found previously for PTP κ (12).

PTP λ Interacts with β-Catenin in Human Kidney Epithelial Cells—Previous data have suggested that PTPs μ and κ interact specifically with the cadherin/catenin complex (20, 22). In addition, recent data have also suggested that PTP LAR interacts with this homotypic adhesion complex in neuronal PC12 cells (23). However, other investigators have cast some doubts about the interactions between the catenin/cadherin complex and PTP μ, predominantly due to antibody cross-reactivity (21). In order to examine if PTP λ interacts with this adhesion complex, a coprecipitation experiment was performed. Because transcript analysis suggests that PTP λ is expressed in vivo in embryonic kidney epithelial cells (see below), a human 293 embryonic kidney cell line was transfected with either the full-length construct or a construct lacking the phosphatase domains but containing the juxtamembrane domain homologous to cadherin cytoplasmic domains (constructs 1 and 2 in Fig. 6). Lysates from these transfected cells were then immunoprecipitated with a monoclonal antibody directed against β-catenin, and the immunocomplex was electroblotted and probed with antibodies against the glycoprotein D tag contained within the N terminus of PTP λ. As can be seen in Fig. 7, immunoprecipitation with the β-catenin monocalonal antibody resulted in the specific coprecipitation of the full-length...
PTP λ but not of the cleaved extracellular domain. In addition, this figure illustrates that only the intracellular juxtamembrane domain is required for an association with β catenin, consistent with previous data suggesting that this domain of PTP λ is required for catenin association (22). The relatively low percentage (~10%) of PTP λ in the immunoprecipitated β catenin complex can be explained in a number of ways. For example, the system analyzed the coprecipitation of a murine form of the PTP complexed with human β catenin, and the wash conditions utilized may have been overly stringent. While a recent report suggests that some degree of antibody cross-reactivity may have been responsible for the apparent interactions between PTP μ and β catenin or E-cadherin (21), the use of highly specific monoclonal antibodies for both the β catenin immunoprecipitation and PTP λ blotting steps of this experiment eliminates any such potential artifacts. Thus, we have not observed any indication that the anti-β catenin antibody recognizes PTP λ on Western blots, nor have we found that the anti-gD antibody used to detect PTP λ reacts with immunoprecipitated β catenin (data not shown). These data thus support previous studies and suggest that PTP λ, like PTPs μ, κ, and LAR, interacts with the cadherin/catenin adhesion complex.

**Tissue Expression of the PTP λ Transcript**—As can be seen in Fig. 8, Northern blot analysis of fetal as well as adult tissues demonstrates that PTP λ is expressed in a diversity of organs outside of the hematopoietic progenitor cells from which it was originally cloned. Thus, the expression of this PTP is detected throughout embryonic development beginning in the very early embryo at day 7. Interestingly, analysis of adult organs reveals that this receptor is expressed specifically in only a subset of tissues. Thus, there appears to be a very high level of expression of the enzyme in brain, lung, and kidney, a much decreased level in heart, skeletal muscle, and testis, and a lack of obvious expression at this exposure in spleen and liver. The high level expression in lung and brain together with the lack of expression in liver are in contrast to PTP κ, a PTP which is expressed at high levels in liver but is almost undetectable in lung and brain (12). Thus, in spite of the fact that PTP λ was originally isolated from hematopoietic stem cells, there is no obvious expression in two sites which contain hematopoietic

---

**Fig. 2.** Homology between PTP λ, PTP κ, and PTP μ. Illustrated as boxed residues are the homologies between PTP λ, PTP κ, and PTP μ. Also shown above the protein sequences are the domains predicted previously from PTPs κ and μ. These domains include the signal sequence (SS), the MAM (MAM), immunoglobulin-like (IgG), fibronectin-type III-like (FnIII), transmembrane domain (TMD), cadherin-like (cadherin), and dual phosphatase domains (PTPase I and PTPase II).
cells, the spleen, and the liver. The lack of signal in the spleen, an organ which contains mostly mature hematopoietic cells, suggests, therefore, that this receptor may be expressed specifically in earlier hematopoietic progenitor cells and not in the predominately mature cells found in this organ as was previously found for PTP HSCF (34). Interestingly, there appears to also be an alternatively spliced transcript in the lung, which is not detected in the other two organs that express this receptor at high levels or in the embryos although the nature of this alternatively spliced transcript has not been determined. In summary, these data suggest that PTP<sub>λ</sub> is specifically expressed in a subset of adult tissues, some of which are divergent from PTP<sub>κ</sub>.

In Situ Hybridization Analysis—We performed in situ mRNA analysis of the rat E15.5 embryo, and P1 and adult rat brain to determine potential sites of PTP<sub>λ</sub> production. Extensive PTP<sub>λ</sub> expression was observed in developing skeletal, epithelial, and neuronal structures throughout the E15.5 embryo (Fig. 9). Systemic expression was observed in various developing skeletal elements such as vertebral perichondrium, intervertebral discs, teeth, mandible, and maxilla (Fig. 9, A and B). Expression within urogenital structures included the genital tubercle (panels A and B), urethra, and urogenital sinus (not shown). Other positive areas of PTP<sub>λ</sub> expression included the anal canal (not shown), skin, olfactory and oral epithelium, esophagus (panels A and B), pituitary (panels A-C), dura mater (panels A, B, and D), kidney (panels A and B), and lung (panels A and B). Higher magnification reveals expression restricted to developing glomeruli in the cortical region of the kidney (panels F and G), and bronchial epithelium of the lung (panels H and I). Within the E15.5 embryonic nervous system, high levels of expression were observed in the developing cerebral cortex (panels A and B), floor of the midbrain, choroid plexus primordium, gigantocellular reticular nucleus of the brainstem (panels A-C), dura mater, and spinal cord (panels A, B, and D). High magnification of the spinal cord reveals highest expression of PTP<sub>λ</sub> in the ventrolateral motor column (panel D).

In P1 and adult brain, expression of PTP<sub>λ</sub> was localized to regions derived from embryonic anlage that also contained high levels of expression. For instance, expression in the embryonic midbrain preceded the high levels of PTP<sub>λ</sub> expression in the P1 and adult substantia nigra (Fig. 10, C and E, respectively). Expression in the embryonic forebrain (Fig. 9A) preceded expression observed in the inner layers (corticallayers 5 and 6) of the P1 and adult cortex (Fig. 10, A, B, D, and E respectively). Expression in the choroid plexus primordia of the embryo begets high levels of expression in the P1 brain (Fig. 10A) and low levels of expression in the adult brain (Fig. 10D). In general, PTP<sub>λ</sub> expression in the adult brain appears to be down-regulated relative to the P1 brain (Fig. 10). However, other areas of prominent expression in both P1 and adult brain include piriform cortex and endopiriform nucleus (Fig. 10, A
and D, respectively), amygdaloid nuclei, subiculum, and CA1, CA2, and to a lesser extent, CA3 of the hippocampal formation (Fig. 10, B and E, respectively). The P1 brain also exhibits strong expression throughout the septal area, basal ganglia, thalamus, and midbrain (panels A, B, and C). Weak expression is observed in the adult superior colliculus as well as scattered expression throughout the thalamus (panel E).

PTP λ Is Expressed on Neurons of the Central Nervous System and Cortex—The Western blot, Northern blot, and in situ analyses suggested that PTP λ is expressed in the nervous system although the resolution of these techniques was insufficient to determine if the receptor was expressed on neurons. In order to examine this possibility, immunostaining of the spinal cord and isolated cortical neurons of the E14 rat was done. As Fig. 11A shows, a subset of spinal neurons, including ventral and dorsal root neurons extending from the spinal cord as well as those in ventral and ventrolateral funiculi within the spinal cord, were specifically stained with the antibody. Examination of isolated cortical neurons for reactivity with the antibody revealed that both the soma as well as the axons and dendrites extending from the neuron stained with the antibody. In addition, growth cones were also positive for this receptor (Fig. 11, B and C). Together, these data suggest, in agreement with the in situ hybridization data, that spinal cord and cortical neurons express PTP λ, consistent with a function for this receptor in the formation of the nervous system.

DISCUSSION

The relative levels of tyrosine phosphorylation of a diversity of proteins are critical for the regulation of a number of activities during embryonic differentiation and throughout the life of the organism. The absolute levels of this modification are mediated through the balance of the enzymatic activities of tyrosine kinases with those of tyrosine phosphatases. In both cases, these large families of proteins perform their roles through conserved enzymatic domains that are coupled to a plethora of specificity-determining motifs. These various motifs are found in the context of both membrane traversing, receptor-like molecules as well as intracellular forms of the enzymes. These similarities in overall structure of the kinases and phosphatases suggest that they mediate their relative specific activities through the use of these various domains. A subset of receptor phosphatases also contain a diversity of domains, including immunoglobulin- and fibronectin-like, which are associated with cell adhesion and ligand binding activities in other protein families. Among the most interesting of these types of adhesion-associated PTPs are the κ and μ receptors which are involved with homotypic types of interactions. Earlier predictions, based upon the likely function of these receptors in mediating cell adhesion as well as their limited tissue distribution, suggested that there might be other κ and μ-like receptor PTPs with different tissue dispositions (4). We report here the isolation of the third member of this family of homophilically interacting receptor PTPs which may be associated with the construction of epithelial and neural structures during development and in the adult.

The strongest data suggesting that the novel PTP described here, termed PTP λ, is homologous to the κ and μ receptors lies in the high degree of sequence conservation among these three proteins. Analysis of these three receptors clearly revealed that the novel PTP had a significant degree of sequence homology throughout its entire length. This homology included the four major types of domains contained in this family including the MAM, the IgG, the FnIII, and the dual phosphatase (PTP) domains (12, 13). Because previous data have suggested that both the MAM as well as the IgG domain appear to be involved with homotypic adhesion (18, 19), it is likely that these motifs are used for a similar function in PTP λ, a hypothesis that is consistent with a role for this receptor in cell adhesion (see below). However, the degree of sequence homology of these domains between the newly reported receptor and the κ and μ receptors is quite divergent, suggesting that the novel receptor may also specifically mediate a homophilic interaction only to itself and not to these domains in the other family members (19). As will be discussed below, these results, together with the tissue localization of this receptor, suggest that it may be involved with the formation of very specific edifices during development. While it is difficult to currently interpret the significance of the conservation of the FnIII domains, which may act as spacer domains to extend the functionally critical MAM and IgG domains from the cell surface, the conservation of the dual PTP domains lends itself to some comment. Thus, the higher degree of conservation of the first domain as compared with the second substantiates previous work suggesting that the N-terminal PTP motif is the enzymatically active one, while the C-terminal domain may be involved with the regulation of enzyme activity (30). Indeed, recent structural data of the phosphatase domains of PTP α reveal that the second domain may regulate the enzymatic activity of the first domain (31). In summary, the data reported here are consistent with PTP λ being the third member of the homotypically interacting receptor PTP family.

As with other members of this family of PTPs, PTP λ can mediate homotypic adhesion. The in vitro binding studies using the IgG chimeric protein containing the MAM, IgG, and first FnIII domains of PTP λ are consistent with previous data suggesting that the first two of these domains appear to be critical for homotypic adhesion (14, 19). While this paper was under review, Ullrich and colleagues also reported a novel receptor PTP, termed PCP-2, which appears to be the human homologue of PTP λ (35). As predicted by the homotypic adhesion studies reported here, PCP-2 is concentrated at the intracellular contact points of cells expressing this receptor. Together, these data support the hypothesis that PTP λ is the third member of this family of proteins to mediate homotypic adhesion and further suggest that this adhesion is induced by, at most, the first three N-terminal domains of the protein. The proteolytic cleavage studies reported here also are consistent with previous data on this family of homotypically adherent PTPs (12, 33), and have implications as far as the adhesion modulating functions of this receptor. The proteolytic mapping studies suggested that a specific cleavage site within the fourth fibronectin domain was the point where proteolysis occurred, and examination of this domain in PTP λ revealed a furin-like cleavage sequence (His638RLRR639) which is highly conserved in...
all three members of this receptor PTP family (12, 22). Interestingly, this site appears to be missing in the PCP-2 phosphatase in spite of its otherwise high degree of homology with PTP λ, and PCP-2 is apparently not cleaved in transient transfection assays (35), which is in sharp contrast to the results reported here. In addition, analysis of PTP λ expression in neonatal brain extracts revealed that this proteolytic cleavage event also appears to occur at a significant level in vivo at a potentially similar site to that seen in vitro although a further processed form of the extracellular domain was also observed in brain extracts. Because the cleaved form of the extracellular region of PTP λ contains all of the motifs necessary to mediate homotypic adhesion, it is likely that this soluble protein can bind to, and potentially inhibit, the homotypic adhesion mediated by the cell surface form of PTP λ. Thus, it is possible that this specifically cleaved form of the protein acts to regulate the cell-associated form of the enzyme.

Previous data have suggested a role for this category of receptor PTPs in cadherin/catenin regulation, and other investigators have pointed to an intracellular juxtamembrane site with significant homology to a similarly localized region in the cadherins (4, 20). Analysis of β catenin immunocomplexes clearly revealed the association of PTP λ with this adhesion system. We have also found a very high degree of sequence conservation in the juxtamembrane region with the other members of this phosphatase family, consistent with a potential role for this domain in catenin/cadherin interactions. In agreement with this supposition, we have demonstrated that a PTP λ construct containing only this juxtamembrane region intracellularly can associate with β catenin, consistent with previous data demonstrating that a homologous region of PTP κ alone can mediate catenin association in vitro (22). These data thus support the proposal from Tonks and coworkers (4, 20), suggesting a role for this family of PTPs in the regulation of
A Novel κ/μ-Like Tyrosine Phosphatase

Fig. 6. In vitro proteolytic cleavage of PTP λ. A, illustrated are the various constructs produced for analysis of proteolytic cleavage. At the N terminus of each was a herpes simplex virus glycoprotein D tag (gD tag) that was used for immunoprecipitation and detection and allowed for observation specifically of the extracellular domain. Constructs were as follows: 1, full-length PTP λ; 2, PTP λ containing the juxtamembrane motif but lacking both PTPase domains; 3, construct 2 lacking the MAM domain; 4, construct 2 lacking both the MAM and IgG-like domains. B, immunoprecipitation and immunoblotting of the constructs illustrated in A. Shown are immunoprecipitations and immunoblots with anti-gD monoclonal antibody 5B6 of lysates and supernatants of cells transfected with constructs 1–4.

Fig. 7. Coprecipitation of PTP λ with β-catenin. Human embryonic kidney cells (293 cells) were transfected with either full-length or PTP domain-deleted constructs expressing PTP λ as described in Fig. 6 (constructs 1 and 2, respectively). Transfected cells were then immunoprecipitated with a monoclonal antibody directed against the C terminus of β-catenin, and the immunoprecipitates were run on SDS gels and subsequently transferred to membranes. In addition, lysates of the transfected cells were also run on these gels and transferred. Blots were then probed with either the anti-gD monoclonal antibody 5B6, specific for the N terminus of PTP λ (upper panel) or the anti-β-catenin monoclonal antibody (lower panel). The two closed arrows illustrate the full-length and truncated forms of PTP λ, while the open arrow illustrates the proteolytically cleaved form of the receptor (see Fig. 6). Note that the cleaved ~115-kDa form of PTP λ does not appear to be coprecipitated with the anti-β-catenin monoclonal.

Fig. 8. Northern blot analysis of PTP expression during embryonic development and in adult tissues. Commercial Northern blots were probed with a 32P fragment of PTP λ using standard conditions. The blot on the left illustrates the transcript in RNA obtained from murine embryos at the developmental day shown in the figure. The blot on the right illustrates PTP λ expression in RNA isolated from: heart (a), brain (b), spleen (c), lung (d), liver (e), skeletal muscle (f), kidney (g), and testis (h).
FIG. 9. PTP λ mRNA expression in the E15.5 rat embryo. Emulsion autoradiographs of a sagittal embryo section (A) and higher magnifications of embryonic midbrain (C), spinal cord (D), kidney (F), and lung (H) hybridized with a [32P]UTP labeled PTP λ antisense probe are shown. Opposed to the darkfield autoradiographs are the corresponding lightfield images of the sagittal embryo section (B), kidney (G), and lung (I). Hybridization using a PTP λ sense strand control probe is shown in an E15.5 embryonic spinal cord section (E). Bar, 1.0 mm for A-E; bar, 0.2 mm for F-I.
epithelial cells, it is possible that disruptions in the proposed function of this type of adhesion sensing mechanism might be involved with the disorganized morphology and often high rate of metastasis of these tumors (17, 24). Together, these hypotheses suggest a critical role for PTP\textsubscript{\lambda} in the formation of various epithelial-like structures in the embryo.

Recent data from the Drosophila system also suggest interesting possibilities for the function of PTP\textsubscript{\lambda} in the developing nervous system (10, 11). In these reports, three different Drosophila receptor PTPs, termed DPTP69D, DPTP99A, and DLAR, which all contain IgG and fibronectin type III adhesion domains similar to those found in PTP\textsubscript{\lambda}, were shown to be critically involved with neuronal pathfinding in the developing nervous systems. Thus, mutations in either of these receptors resulted in a loss of the ability of certain neural subsets to become reoriented during their formation in the embryo. These data, together with the recent observations on the association of mammalian LAR with the catenin-cadherin complex, are consistent with a role for receptor PTPs in specific neural pathfinding and adhesion. Because PTP\textsubscript{\lambda}, a homotypically adhering PTP associated with the catenin complex, is expressed in a number of developing neural sites, it is possible that it plays a similar role in the pathfinding of nerves in mammals. Thus, the expression of this PTP in the developing midbrain, forebrain, and other neural sites would dispose it to function as a mediator of pathfinding in these maturing sys-
tems. Interestingly, the expression of this receptor in these embryonic anlage was confirmed by expression in the adult sites which arise from these embryonic structures. However, the expression in the adult appeared to be somewhat reduced as compared with that observed in the embryo, and it was far more organized. These data suggest that this enzyme might be utilized during adult neuronal formation although the apparent decrease in adult expression suggests a potentially more critical role during embryogenesis. The observation that PTP λ is expressed on neurites of isolated cortical neurons as well as on the growth-cone like structures at the tips of these processes is also consistent with a potential role for this receptor in neuronal pathfinding in the mammalian nervous system. Interestingly, we have observed that PTP λ is specifically expressed on layer 5 and 6 cortical neurons only during their differentiation and pathfinding phases, again consistent with a role for this phosphatase in aspects of neural architecture. Finally, while the clear observation of the loss of pathfinding in Drosophila will be difficult to recapitulate in the mouse due to the relatively high complexity of the mammalian nervous system, it will nevertheless be potentially of great interest to examine the formation of the nervous system in animals which have been made null for the expression of this receptor.

In summary, the data reported in this paper demonstrate the existence of a third member of the family of receptor PTPs that appear to be involved with homotypic adhesion and, potentially, cadherin/catenin-mediated organ formation. The role that this novel receptor might play in the formation of epithelial sheets and neuronal structures remains to be determined.

However, the existence of three of these types of receptors further suggests that this growing family may be involved with the specific formation of various types of complex structures during development, as well as in the adult.

Acknowledgments—We thank Louis Tamayo and David Wood for production of the figures.

REFERENCES

1. Tonks, N. (1993) Semin. Cell. Biol. 4, 787–793
2. Walton, K. M., and J. E. Dixon. (1993) Annu. Rev. Biochem. 62, 101–120
3. Sun, H., and Tonks, N. (1994) Trends Biochem. Sci. 19, 480–485
4. Brady-Kalnay, S. M., and Tonks, N. K. (1995) Curr. Opin. Cell Biol. 7, 650–657
5. Rao, Y., Wu, X., Rutishauser, U., and Siu, C. (1992) J. Cell Biol. 118, 937–949
6. Beckman, G., and Bork, P. (1993) Trends Biochem. Sci. 18, 40–41
7. Jiang, W., Gebera, C. M., Flannery, A. V., Beynon, R. J., Grant, G. A., and Bond, J. S. (1992) J. Biol. Chem. 267, 9185–9193
8. Takagi, S., Hirata, T., Agata, K., Mochii, M., Eguchi, G., and Fujisawa, H. (1994) Neuron 7, 295–299
9. Hirata, T., Tkagi, S., and Fujisawa, H. (1993) Neurosci. Res. 17, 159–169
10. Desai, C., Gindhart, J., Goldstein, L., and Zinn, K. (1996) Cell 84, 599–609
11. Kreuger, N., Van Vactor, D., Watan, H., Gelbart, W., Goodman, C., and Saito, H. (1996) Cell 84, 611–622
12. Jiang, Y., Wang, H., D’Eustachio, P., Musacchio, J., Schlessinger, J., and Sap, J. (1993) Mol. Cell. Biol. 13, 2942–2951
13. Gebbink, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., Geurts-van-Kessel, G., and Moolenaar, W. H. (1991) FEBS Lett. 290, 123–130
14. Brady-Kalnay, S., Flint, A., and Tonks, N. (1993) J. Cell Biol. 96, 971–976
15. Gebbink, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., van Etten, I., and Moolenaar, W. H. (1993) J. Biol. Chem. 268, 16101–16104
16. Sap, J., Jiang, Y., Friedlander, D., Grumet, M., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 1–9
17. Kemler, R. (1993) Trends Genet. 9, 317–321
18. Brady-Kalnay, S. M., and Tonks, N. K. (1994) J. Biol. Chem. 269, 28472–28477
19. Zondag, G. C. M., Koningstein, G. M., Jiang, Y.-P., Sap, J., Moolenaar, W. H., and Gebbink, M. F. B. G. (1995) J. Biol. Chem. 270, 14247–14250
20. Brady-Kalnay, S. M., Rimm, D. L., and Tonks, N. K. (1995) J. Cell Biol. 130, 977–986
21. Zondag, G., Moolenaar, W., and Gebbink, M. (1996) J. Cell Biol. 134, 1513–1517
22. Fuchs, M., Muller, T., Lerch, M., and Ullrich, A. (1996) J. Biol. Chem. 271, 16712–16719
23. Kypta, R., Su, H., and Reichardt, L. (1996) J. Cell Biol. 134, 1519–1529
24. Behrens, J., Vakaert, L., Friis, R., Winterhager, E., Van Roy, F., Mareel, M., and Birchmeier, W. (1995) EMBO J. 14, 1754–1758
25. Fennie, C., Cheng, J., Dowbenko, D., Young, P., and Laskey, L. A. (1995) Blood 86, 4454–4467
26. Watson, S. R., Inai, Y., Fennie, C., Geffroy, J. S., Rosen, S. D., and Laskey, L. A. (1990) J. Cell Biol. 110, 2221–2229
27. Lee, J., Gray, A., Yuan, J., Lush, S., Avraham, H., and Wood, W. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1898–1992
28. Diam, J. (1995) Ann. N. Y. Acad. Sci. 766, 18–22
29. Cheng, J., Fennie, C., Daimaru, L., and Laskey, L. A. (1996) Blood, 88, 1156–1167
30. Pot, D. A., Woodford, T. A., Rembeautsika, E., Huan, R. S., and Dixon, J. E. (1991) J. Biol. Chem. 266, 19688–19696
31. Bilwes, A. M., Den Hertog, J., Hunter, T., and Noel, J. P. (1996) Nature 382, 555–559
32. Jia, Z., Barford, D., Flint, A. J., and Tonks, N. K. (1995) Science 268, 1754–1758
33. Streuli, M., Krueger, N., Abruinelli, P., Tang, M., Munro, J., Blattler, W., Adler, D., Duseche, C., and Saito, H. (1992) EMBO J. 11, 897–907
34. Cheng, J., Daimaru, L., Fennie, C., and Laskey, L. A. (1996) Blood 88, 1156–1167
35. Wang, H., Lian, Z., Lerch, M., Chen, Z., Xie, W., and Ullrich, A. (1996) Oncogene 12, 2555–2562
36. Klarlund, J. (1985) Cell 41, 707–717

Fig. 11. PTP λ expression in the spinal cord and on isolated cortical neurites. a, immunostaining reveals PTP λ expression along major axon tracts in the embryonic rat central and peripheral nervous system. In transverse sections of E14 rat spinal cord, PTP λ is found in the dorsal root entry zone (arrow, top), and ventral and ventrolateral funiculi (arrow, bottom) are within the spinal cord and in the dorsal (arrowhead, top) and ventral (arrowhead, bottom) roots extending from the cord. b and c, PTP λ staining on cultured cortical neurones from the P1 rat. PTP49 λ immunoreactivity is detected in the soma and along the axons out to the growth cones (arrowheads).