THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 268, No. 14, Issue of May 15, pp. 10573-10581, 1993
Printed in U.S.A.

The Cloning of a Receptor-type Protein Tyrosine Phosphatase Expressed in the Central Nervous System*

(Received for publication, December 23, 1992, and in revised form, January 25, 1993)

J. B. Levy‡, P. D. Canoll‡, O. Silvennoinen‡, G. Barnea‡, B. Morse§, A. M. Honegger‡, J.-T. Huang‡, L. A. Cannizzaro¶, S.-H. Park¶, T. Druck¶, K. Huebner¶, J. Sap‡, M. Ehrlich‖, J. M. Musacchio‡, and J. Schlessinger‡**

From the Departments of ‡Pharmacology and ‖Psychiatry, New York University Medical Center, New York, New York 10016, the §Rhone-Poulenc Rorer, Collegeville, Pennsylvania 19426, and the ¶Jefferson Cancer Institute, Thomas Jefferson Medical College, Philadelphia, Pennsylvania 19107

We have isolated cDNA clones and deduced the complete amino acid sequence of a large receptor-type protein tyrosine phosphatase containing 2307 amino acids. The human gene encoding this phosphatase, denoted RPTPβ (or PTPζ), has been localized to chromosome 7q31-33. RPTPβ is composed of a large extracellular domain, a single transmembrane domain, and a cytoplasmic portion with two tandem catalytic domains. We have also cloned a variant of RPTPβ lacking 859 amino acids from the extracellular domain but with intact transmembrane and cytoplasmic domains. Interestingly, the amino-terminal region of the extracellular domain of RPTPβ contains a stretch of 266 amino acids with striking homology to the enzyme carbonic anhydrase. Immunoprecipitation experiments from a human neuroblastoma cell line indicate that the apparent molecular mass of the core and glycosylated forms of RPTPβ are approximately 250 and 300 kDa, respectively. Northern blot analysis shows that RPTPβ is strictly expressed in the central nervous system. *In situ* hybridization was used to further localize the expression to different regions of the adult brain including the Purkinje cell layer of the cerebellum, the dentate gyrus, and the subependymal layer of the anterior horn of the lateral ventricle. Hence, RPTPβ represents the first mammalian tyrosine phosphatase whose expression is restricted to the nervous system. The high level of expression of RPTPβ transcripts in the ventricular and subventricular zones of the embryonic mouse brain suggests the importance of this tyrosine phosphatase in the development of the central nervous system.

Phosphorylation of proteins on tyrosine residues plays a key role in the signaling of cell growth, differentiation, and transformation (reviewed in Schlessinger and Ullrich (1992)). The net phosphorylation of cellular proteins on tyrosine residues is controlled by the balanced action of protein tyrosine kinases and protein tyrosine phosphatases present in the cell. The family of protein tyrosine kinases has been well characterized and includes ligand-regulated receptor tyrosine kinases such as the EGF[1] receptor and cytoplasmic protein tyrosine kinases such as pp60$^{c\text{-}src}$ (reviewed in Hunter (1987) and in Ullrich and Schlessinger (1990). The tyrosine kinase activity of these proteins has been shown to be essential for their various biological activities (Snyder *et al.*, 1985; Weinmaster *et al.*, 1986; Kmiecik and Shalloway, 1987; Chou *et al.*, 1987; Ullrich and Schlessinger, 1990).

Because tyrosine phosphorylation is a reversible process, it is important to identify and understand the mode of action of protein tyrosine phosphatases. It has been shown that this family of enzymes is composed of two distinct subgroups that include low molecular weight, cytosolic protein tyrosine phosphatases and high molecular weight, receptor-type transmembrane protein tyrosine phosphatases (reviewed by Fischer *et al.* (1991)). The catalytic phosphatase domains are approximately 300 amino acids long and are 30–50% identical in amino acid sequence. The transmembrane protein tyrosine phosphatases resemble growth factor receptors in that they contain a glycosylated extracellular region connected to a cytoplasmic domain via a transmembrane domain. With the exception of one isoform, the intracellular region of the transmembrane protein tyrosine phosphatases contains two tandemly repeated catalytic phosphatase domains. In contrast to the highly conserved protein tyrosine phosphatase domains, the extracellular regions of these proteins are fairly distinct with respect to both size and amino acid sequence. The current hypothesis is that the activity of the phosphatase is regulated by ligands binding to the extracellular domain. Identifying these ligands is a major goal of phosphatase research.

Several lines of evidence indicate that tyrosine phosphorylation is involved in signal transduction pathways in the central nervous system (CNS). High levels of tyrosine kinase activity and several tyrosine kinases have been identified in different regions of the brain (Swarup *et al.*, 1983; Sorge *et al.*, 1984; Sudol and Hanafusa, 1986; Hirano *et al.*, 1988). Moreover, the receptors for nerve growth factor and other neurotrophic factors have been identified as various members of the *trk* family of receptor tyrosine kinases (Cordon-Cardo *et al.*, 1991; Hempstead *et al.*, 1991; Kaplan *et al.*, 1991; Klein *et al.*, 1991; Nebreda *et al.*, 1991). Very little is known about the control of tyrosine phosphorylation in the CNS by protein tyrosine phosphatases. In this report, we describe a novel receptor-type tyrosine phosphatase, designated as RPTPβ (or PTPζ), and a smaller receptor variant. Interestingly, RPTPβ contains a region in the extracellular domain with striking sequence similarity to the enzyme carbonic anhydrase (CAH).

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** Recipient of a grant from Sugen. To whom correspondence should be addressed: Dept. of Pharmacology, New York University Medical Center, 550 First Ave., New York, NY 10016. Tel.: 212-263-7111; Fax: 212-263-7133.

[1] The abbreviations used are: EGF, epidermal growth factor; CNS, central nervous system; CAH, carbonic anhydrase; kb, kilobase(s).

We also provide a preliminary report on the distribution of RPTPβ expression in the adult and developing CNS. The relatively high levels of expression of RPTPβ mRNA in the embryonic CNS suggest that this phosphatase may be involved in the regulation of specific developmental processes in the CNS.

EXPERIMENTAL PROCEDURES

Isolation of cDNA Clones and DNA Sequence Analysis—A cDNA clone containing a portion of the coding sequences for RPTPβ was isolated after screening a λgt11 human infant brainstem cDNA library under conditions of reduced stringency with a nick-translated leukocyte common antigen probe that included both phosphatase domains (Kaplan *et al.*, 1990). Since the 5′ end of this gene was not present in the original clone, the library was rescreened with a DNA fragment that was generated from the 5′ end of the original clone. The probe was labeled with [^{32}P]dCTP utilizing the random priming method (U. S. Biochemical Corp.), and hybridization was performed under moderately stringent conditions at 42 °C in a buffer containing 50% formamide, 5 × SSC, 20 mM Tris-Cl, pH 7.6, 1 × Denhardt's solution, 0.1% SDS, and 100 μg/ml sheared and denatured salmon sperm DNA. After hybridization, phage filters were washed three times for 20 min at 50 °C in a buffer containing 0.1 × SSC, 0.1% SDS and then were processed for autoradiography. The brainstem library was rescreened a total of three times in order to isolate overlapping cDNA clones that contained the entire coding sequence for RPTPβ.

cDNA inserts from positive recombinant plaque-purified phage were subcloned into the plasmid vector, Bluescript (Stratagene, La Jolla, CA), and sequenced by the dideoxy chain termination method using the Sequenase version 2.0 kit (U. S. Biochemical Corp.).

Chromosomal Localization—Isolation, propagation, and characterization of parental and somatic cell hybrids used in this study have been described (Durst *et al.*, 1987; Huebner *et al.*, 1991). The presence of specific human chromosomes or regions of chromosomes has been confirmed by DNA hybridization using probes for genes assigned to specific chromosome regions (see Fig. 3*A*, which diagrammatically depicts the chromosomes or partial chromosomes retained in most of the hybrids used).

Chromosomal *in situ* hybridization was performed as described (Cannizzaro *et al.*, 1991). Slides containing metaphase chromosomes from normal male (46 XY) peripheral blood lymphocytes were aged at 4 °C for 7–10 days and pretreated with ribonuclease A (Sigma) for 1 h at 37 °C. The chromosomal DNA was denatured in a hybridization mixture containing 50% formamide, 2 × SSC, and 10% dextran sulfate (pH 7.0). Hybridization was carried out at 37 °C overnight. After rinsing at 39 °C in three changes of 50% formamide and 2 × SSC and five changes of 2 × SSC, slides were dehydrated, air-dried, subjected to autoradiography, and banded with Wright's-Giemsa stain solution mixed with 1–3 parts of borate buffer, pH 9.2 (Cannizzaro *et al.*, 1991).

Isolation of Mouse Sequences Homologous to Human RPTPβ—Two oligonucleotides in conserved phosphatase domain II were synthesized according to the nucleotide sequence of human RPTPβ. These oligonucleotides in conjunction with phage DNA from a mouse brain cDNA library (Clontech, Palo Alto, CA) were used in the polymerase chain reaction with Taq polymerase (Perkin-Elmer Cetus Instruments) to amplify homologous mouse RPTPβ sequences. The amplified product was purified and cloned into the Bluescript plasmid vector (Stratagene). Homology was confirmed by DNA sequence analysis as described above. This subcloned fragment is called pBSMBDII.

Northern Analysis—Total cellular RNA was prepared with the Stratagene RNA isolation kit. Poly(A$^+$) RNA was further selected utilizing oligo(dT)-cellulose chromatography (Stratagene). For Northern analysis, the RNA was separated on a 1.0% agarose, 2 M formaldehyde gel and transferred to a Nytran membrane (Schleicher and Schuell) by capillary action. The membrane was prehybridized and hybridized in 0.5 M sodium phosphate, pH 7.2, 7% SDS, 1 mM EDTA, 100 μg/ml salmon sperm DNA and then washed in 40 mM sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA at 65 °C. For the blot containing RNA isolated from various mouse tissues, a ^{32}P-labeled probe was made utilizing pBSMBDII as template in the random priming labeling reaction (U. S. Biochemical Corp.). The human glioblastoma and neuroblastoma RNA blots were probed with labeled restriction fragments isolated from different parts of the human RPTPβ cDNA clones.

Antibodies—A peptide derived from the carboxyl-terminal 15 amino acids of human RPTPβ was synthesized and coupled to keyhole limpet hemocyanin according to published procedures (Harlow and Lane, 1988). Two rabbits were inoculated to produce polyclonal antisera against RPTPβ. Anti-EGF receptor immunoprecipitates were performed with RK2 antibody, which recognizes the EGF receptor (Kris *et al.*, 1985).

Cell Labeling and Immunoprecipitation—The human neuroblastoma cell line, Lan 5, was maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cultured cells were incubated with 10 μg/ml tunicamycin (Sigma) for 1 h prior to [^{35}S]methionine labeling. Treated and untreated cells were washed twice with methionine-free Dulbecco's modified Eagle's medium and labeled for 4 h with 0.15 mCi/ml [^{35}S]methionine (Du Pont-New England Nuclear) in Dulbecco's modified Eagle's medium minus methionine containing 1% dialyzed fetal bovine serum. During the labeling period, 10 μg/ml tunicamycin was added to the medium of the treated cells. Cells were then washed with ice-cold phosphate-buffered saline and solubilized in a lysis buffer containing 50 mM Hepes pH 7.5, 150 mM NaCl, 1.0% Triton X-100, 10% glycerol, 1.5 mM $MgCl_2$, 1 mM EGTA, 10 μg of leupeptin/ml, 1 mM phenylmethylsulfonyl fluoride, and 10 μg of aprotinin/ml. Cell lysates were clarified and then immunoprecipitated with normal rabbit antiserum, anti-RPTPβ antiserum, or RK2 antiserum for 2 h at 4 °C. The immune complexes were precipitated with Protein A-Sepharose (Sigma) for 45 min at 4 °C and washed 10 times with RIPA buffer (20 mM Tris-Cl, pH 7.6, 300 mM NaCl, 2 mM EDTA, 1.0% Triton X-100, 1.0% sodium deoxycholate, and 0.1% SDS). The immunoprecipitated material was analyzed on a 7.5% SDS-polyacrylamide gel and by fluorography.

In Situ Hybridization Analysis—Fresh frozen tissue was cut on a cryostat into 20-μm thick sections and thaw-mounted onto gelatin-coated slides. The sections were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4) for 30 min and rinsed three times for 5 min each in 0.1 M sodium phosphate and one time for 10 min in 2 × SSC. Two probes were used in the hybridization analysis, a 49-base oligonucleotide complementary to phosphatase domain II and a 50-base oligonucleotide complementary to the carbonic anhydrase domain. The oligonucleotides were labeled with α-^{35}S-labeled dATP (Du Pont-New England Nuclear) using terminal deoxynucleotidyltransferase (Boehringer Mannheim) and purified using Sephadex G-25 quick spin columns (Boehringer Mannheim). The specific activity of the labeled probes was between 5 × 10^8 and 1 × 10^9 cpm/μg. Prehybridizations and hybridizations were carried out in a buffer containing 50% deionized formamide, 4 × SSC, 1 × Denhardt's solution, 500 μg/ml denatured salmon sperm DNA, 250 μg/ml yeast tRNA, and 10% dextran sulfate. The tissue was incubated for 12 h at 45 °C in hybridization solution containing the labeled probe (1 × 10^6 cpm/section) and 10 mM dithiothreitol. Controls for specificity were performed on adjacent sections by diluting the labeled oligonucleotides with a 30-fold concentration of the appropriate unlabeled oligonucleotide and by hybridization with a sense probe. After hybridization, the sections were washed in two changes of 2 × SSC at room temperature for 1 h, 1 × SSC at 55 °C for 30 min, 0.5 × SSC at 55 °C for 30 min, and 0.5 × SSC at room temperature for 15 min and then dehydrated in 60, 80, and 100% ethanol. After air drying, the sections were exposed to x-ray film for 5–10 days.

RESULTS

The Primary Amino Acid Sequence and Chromosomal Localization of RPTPβ—We have previously isolated a portion of a novel receptor-type phosphatase denoted RPTPβ (Kaplan *et al.*, 1990). This phosphatase was independently cloned by another group and is also called PTPζ (Krueger *et al.*, 1990; Krueger and Saito, 1992). The reason for minor sequence differences between RPTPβ and PTPζ is not yet clear. Four overlapping cDNA clones containing the entire coding sequence for RPTPβ were isolated from a human brainstem library. The deduced amino acid sequence reveals an open reading frame of 2307 amino acids (Fig. 1). RPTPβ belongs to the high molecular weight transmembrane class of tyrosine phosphatases. The sequence contains a signal peptide (*underlined* in Fig. 1) followed by a long extracellular domain of 1611 amino acids containing 21 potential *N*-glycosylation

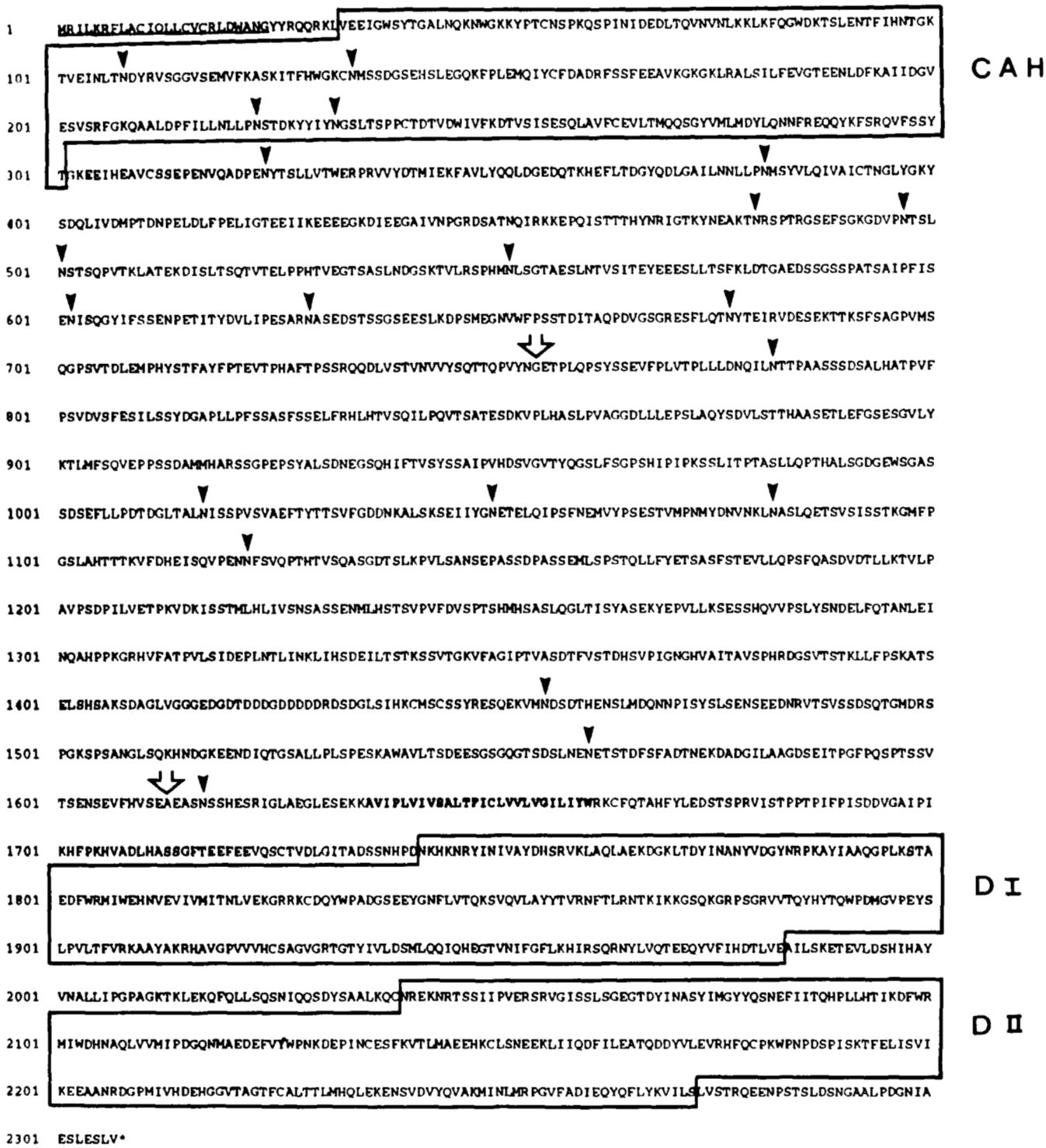
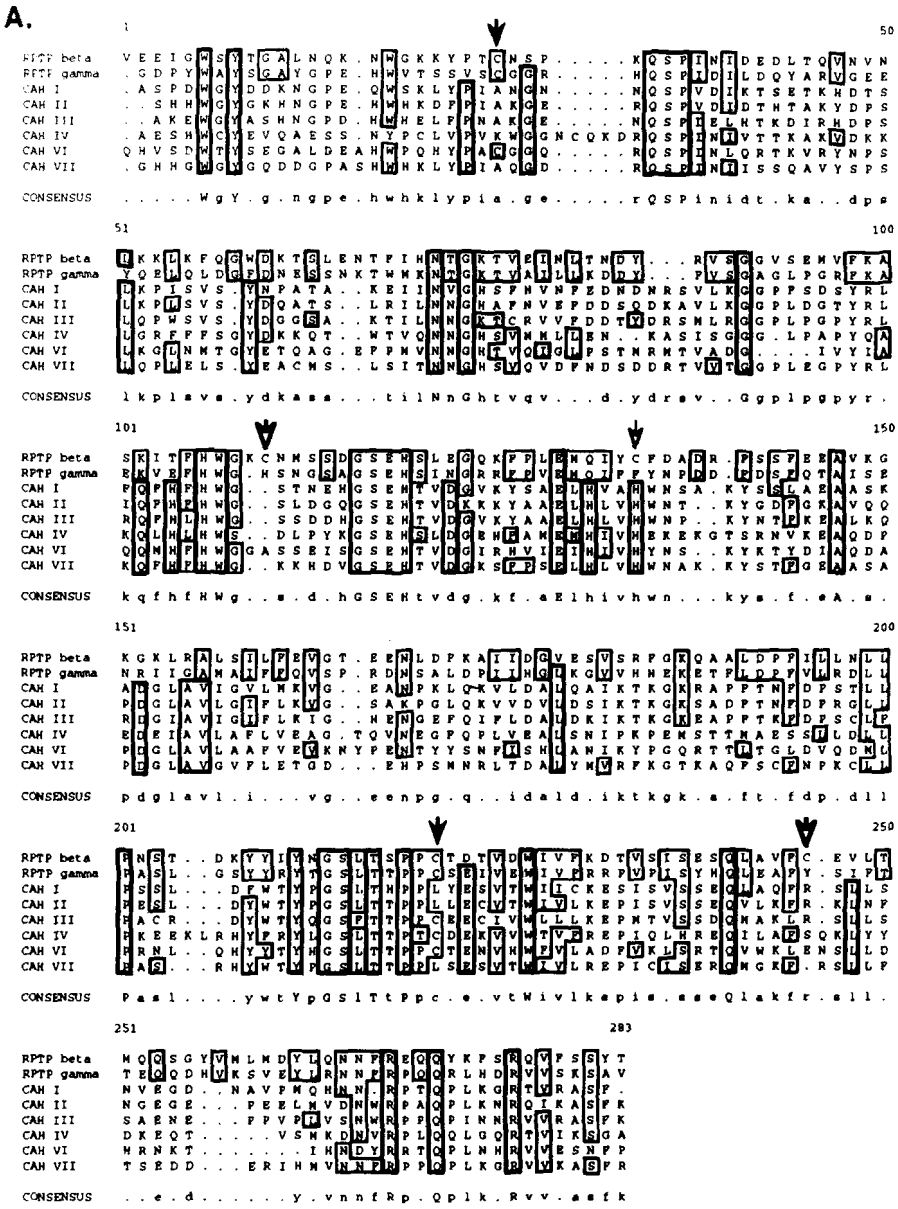


FIG. 1. **The amino acid sequence of RPTPβ.** Protein sequence of RPTPβ is presented. The hydrophobic signal peptide is *underlined*, and the transmembrane peptide is designated in *boldface type*. The 21 potential *N*-glycosylation sites are indicated by the *filled arrows*. The CAH-related domain and the two phosphatase domains, *DI* and *DII*, are indicated by the *boxes*. The *open arrows* represent the boundaries of the deletion in the variant RPTPβ clones.

sites (indicated by *arrows*). A hydrophobic, transmembrane peptide (*boldface sequences*) joins the extracellular portion of the protein to two tandemly repeated and conserved phosphatase domains (designated *DI* and *DII*). One distinguishing feature of this phosphatase is the homology it shares with different isoforms of CAH over a stretch of 283 amino acids located at the extreme amino terminus of the protein (designated in Fig. 1 as *CAH*). In addition to RPTPβ, the extracellular domain of a related transmembrane-type phosphatase, RPTPγ, shares homology with CAH (Barnea *et al.*, 1993). Alignment of the CAH-related domains of RPTPβ and RPTPγ with the six known isoforms of CAH is shown in Fig. 2*A*. Fig. 2*B* shows the percent similarity, taking into account conservative amino acid substitutions between the CAH-related domain of RPTPβ, the corresponding domain of RPTPγ, and the six CAH enzymes. The similarity of the CAH-related domain of RPTPβ to the six different isoforms of CAH ranges from 45 to 50% in amino acid sequence



B.

PERCENT SIMILARITY

	RPTP Beta	RPTP Gamma	CAH I	CAH II	CAH III	CAH IV	CAH VI	CAH VII
RPTP Beta	100.0	58.1	50.4	49.0	48.7	44.8	46.6	47.1
RPTP Gamma		100.0	48.1	51.7	53.7	48.0	45.0	49.0
CAH I			100.0	73.0	69.9	44.6	48.1	65.0
CAH II				100.0	71.0	48.7	51.7	69.9
CAH III					100.0	48.6	53.7	64.5
CAH IV						100.0	47.7	47.1
CAH VI							100.0	51.3
CAH VII								100.0

FIG. 2. **Identification of a CAH-related domain in the extracellular region of RPTPβ.** *A*, the alignment of the amino acid sequence of the CAH-related domain of RPTPβ with the corresponding domain of RPTPγ (Barnea *et al.*, 1993) and the six different isoforms of CAH (*I–VII*) are shown. The amino acid sequences that are *boxed* in *black* are those that are identical in all six isoforms of CAH. The sequences that are *boxed* in *gray hatches* are those that are identical between the CAH-related domains of RPTPβ and RPTPγ. *B*, the percent similarity, taking into account conservative substitutions of amino acids between the CAH-related domains of RPTPβ and RPTPγ and the six isoforms of CAH, is indicated in this grid.

similarity. The highest degree of similarity (58%) exists between the CAH-related sequences of RPTPβ and RPTPγ. Therefore, RPTPβ and RPTPγ represent a new subgroup of tyrosine phosphatases characterized by the presence of CAH-related regions in the amino-terminal regions of their extracellular domains.

The chromosomal localization of the human RPTPβ gene was initially determined utilizing a panel of rodent-human hybrids carrying defined human chromosomes or chromosome regions. The results from screening the rodent-human hybrids, which are summarized in Fig. 3*A*, correlate the presence of the human RPTPβ locus in hybrid cells with human chromosome 7. A more precise localization of the RPTPβ gene was determined by chromosomal *in situ* hybridization to metaphase chromosomes of normal human lymphocytes. This technique places the RPTPβ gene at 7q31-33 with the most likely position at 7q31.3-q32, which is diagramatically shown to the *right* of the chromosome 7 *sketch* in Fig. 3*B*.

Detection of RPTPβ Expression in a Human Neuroblastoma Cell Line, Lan 5—Since all of the clones encoding the entire sequence for RPTPβ were isolated from a human brainstem library, we have examined the expression of RPTPβ mRNA in different human glioblastoma cell lines and a human neuroblastoma cell line, Lan 5 (Sonnenfield and Ishii, 1982). A human RPTPβ probe hybridized to three major transcripts of 8.8, 7.5, and 6.4 kb, respectively (Fig. 4*A*). These transcripts were detected only in RNA isolated from the Lan 5 neuroblastoma cell line and were absent in the RNA isolated from the four glioblastoma cell lines even though similar amounts of total cellular RNA were loaded as revealed by ethidium bromide staining of the 28 S and 18 S ribosomal RNAs (data not shown).

In order to obtain further insights into the nature of the three RPTPβ transcripts, we performed an additional Northern analysis on RNA isolated from Lan 5 cells with probes derived from nonconserved sequences in the extracellular domain of RPTPβ. An identical pattern of transcripts was revealed utilizing these probes (data not shown).

The Lan 5 neuroblastoma cell line was used to study the endogenous expression of RPTPβ protein. Cell lysates prepared from cultures labeled with [^{35}S]methionine for 4 h were immunoprecipitated with normal rabbit serum or anti-RPTPβ antiserum (Fig. 5). A protein with an apparent molecular mass of approximately 300 kDa was recognized by the immune but not by normal rabbit serum (*lanes 1* and *2*). Since there are 21 potential *N*-glycosylation sites, we used

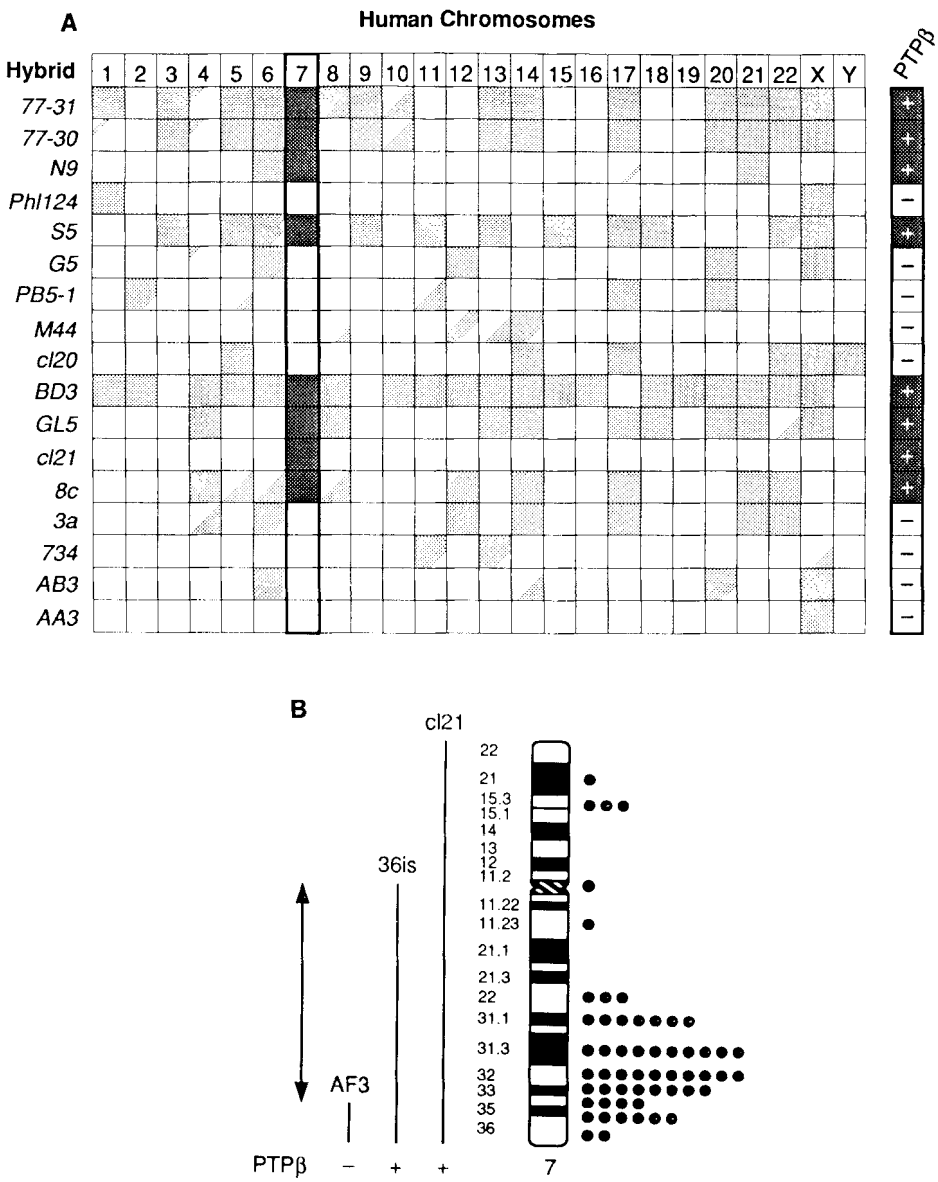


FIG. 3. **Chromosomal localization of human RPTPβ.** *A*, the presence of the RPTPβ (*PTPβ*) gene in a panel of 17 rodent-human hybrids is shown. A *completely stippled box* indicates that the hybrid named in the *left column* contains the chromosome indicated in the *upper row*. *Lower right stippling* indicates the presence of the long arm (or part of the long arm, as indicated by a *smaller fraction* of *stippling*) of the chromosome shown *above* the *column*. *Upper left stippling* indicates the presence of the short arm (or partial short arm) of the chromosome listed *above* the *column*. An *open box* indicates the absence of the chromosome *above* the *column*. The column for chromosome 7 is *boldly outlined* and *stippled* to highlight correlation of the presence of this chromosome with the presence of the RPTPβ gene. The pattern of retention of the RPTPβ sequences in the hybrids is shown to the *right*, where the presence of the gene in the hybrids is indicated by a *stippled box* with a *plus sign*, and the absence of the gene is indicated by an *open box* enclosing a *minus sign*. *B*, RPTPβ maps to 7q31-q33 are shown. Chromosomal *in situ* hybridization of a 1.8-kb RPTPβ cDNA to normal human metaphases confirmed localization of the gene to 7q and revealed a peak of grains centered over region 7q31.3-q32 as illustrated on the right of the chromosome sketch. Each *dot* represents an autoradiographic grain. *AF3* and *36is* represent additional rodent-human hybrids not shown in *A* that were tested for the presence of RPTPβ.

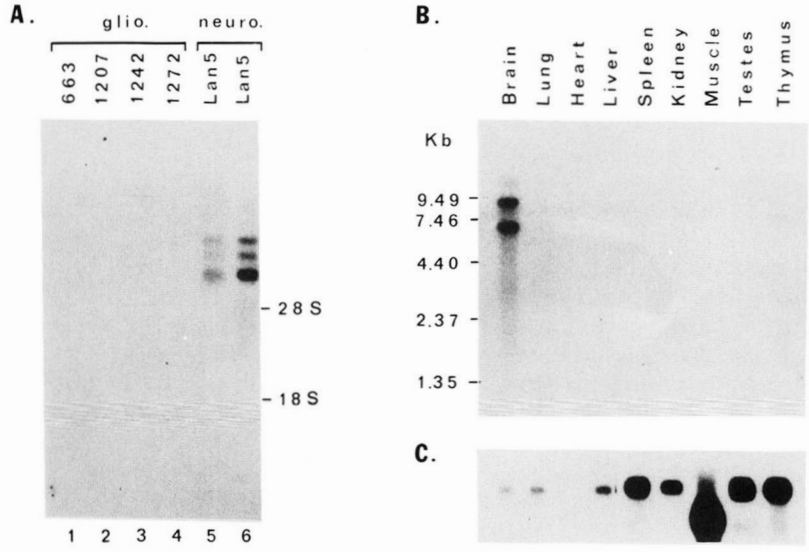


FIG. 4. **Analysis of the mRNA expression of RPTPβ in different human cell lines and various murine tissues.** *A*, 20 μg of total cellular RNA (*lanes 1–5*) and 1 μg of poly(A$^+$) RNA (*lane 6*) isolated from the various glioblastoma (*glio.*) and neuroblastoma (*neuro.*) cell lines indicated were loaded onto a 1.0% agarose, 2.2 M formaldehyde RNA gel and probed with a DNA fragment isolated from the human brainstem cDNA clone that begins with sequences just 5′ of the region encoding the transmembrane region and extends and includes all of the sequences in phosphatase domain I. *B*, poly(A$^+$) RNA (1 μg/sample) from the various murine tissues indicated were loaded onto an RNA gel and probed with the polymerase chain reaction, amplified murine DNA fragment, pBSMBDII (described under "Experimental Procedures"). *C*, the blot in *B* was stripped of probe and rehybridized with a ^{32}P-labeled rat actin probe.

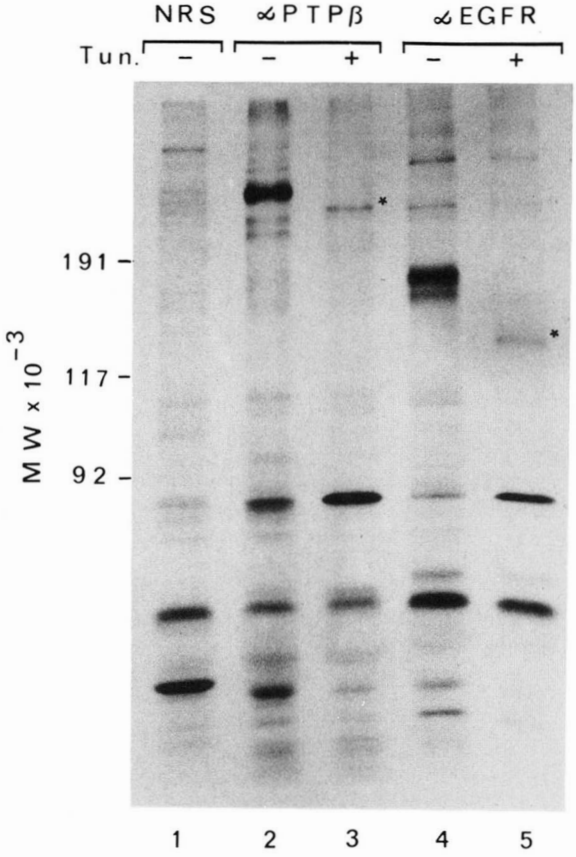


FIG. 5. **Identification of endogenous RPTPβ protein expression in Lan 5 cells.** Immunoprecipitation of RPTPβ with normal rabbit serum (*NRS*, *lane 1*) and immune RPTPβ antiserum (*αPTPβ*, *lanes 2* and *3*) from lysates of [^{35}S]methionine-labeled Lan 5 cells in the absence (*lanes 1* and *2*) or presence (*lane 3*) of tunicamycin, and immunoprecipitation of the EGF receptor with RK2 antibody (*αEGFR*, *lanes 4* and *5*) from lysates of [^{35}S]methionine-labeled Lan 5 cells labeled in the absence (*lane 4*) or presence (*lane 5*) of tunicamycin are shown.

tunicamycin to examine the possibility that the 300-kDa protein immunoprecipitated by the anti-RPTPβ antiserum represents a glycosylated form of RPTPβ. The effects of tunicamycin treatment on the mobility of RPTPβ immunoprecipitated from [^{35}S]methionine-labeled cells were compared with its ability to inhibit the glycosylation of the EGF receptor, which is also expressed in this cell line. Untreated cell lysates and lysates prepared from cells treated with tunicamycin were immunoprecipitated with anti-EGF receptor antibodies (RK2) that recognize the 170-kDa glycosylated and the 135-kDa nonglycosylated forms of the EGF receptor (Kris *et al.*, 1985) (Fig. 5, *lanes 4* and *5*). The protein immunoprecipitated with anti-RPTPβ antiserum from Lan 5 cells that had been metabolically labeled in the presence of tunicamycin (Fig. 5, *lane 3*) migrated with an apparent molecular mass of 250 kDa, a value consistent with the predicted molecular mass of 254 kDa deduced from the amino acid sequence of RPTPβ.

Identification of a Variant Form of RPTPβ—The overlapping human cDNA clones collectively contain approximately 8.1 kb of coding and noncoding sequences and appear to represent the largest transcript that is 8.8 kb in length. In screening the human brainstem library and a human caudate library (Stratagene), we isolated two independent cDNA clones that each contained an identical deletion of 2577 nucleotides from the extracellular domain of RPTPβ. This in-frame deletion joined amino acid 754 to 1614 (shown by the *open arrows* in Fig. 1 and shown in Fig. 6*A*) thereby maintaining the transmembrane domain and the two phosphatase domains. A deletion of this size could account for the difference between the 8.8- and 6.4-kb transcripts expressed in the Lan 5 neuroblastoma cell line (Fig. 4*A*). Hence, duplicate blots were made from RNA isolated from the Lan 5 cell line. One blot was analyzed with a probe that should hybridize to both the full-length and deleted forms of RPTPβ (*P1*). The other blot was analyzed with a probe that should hybridize only to the full-length form (*P2*). The location of probes 1 and 2 in the full-length RPTPβ cDNA is shown in Fig. 6*A*. Northern analysis with the two probes shown in Fig. 6*B* revealed that probe 1 hybridized to the three distinct transcripts (*P1*), whereas probe 2 hybridized only to the 7.5- and 8.8-kb transcripts (*P2*). This experiment suggests that the 6.4-kb transcript represents a deleted variant form of RPTPβ, which could be the result of alternative mRNA splicing. However, only the high molecular weight form of RPTPβ was detected following immunoprecipitation with anti-RPTPβ antibodies and SDS-polyacrylamide gel electrophoresis analysis (Fig. 5). The reason for the inability to detect the low molec-

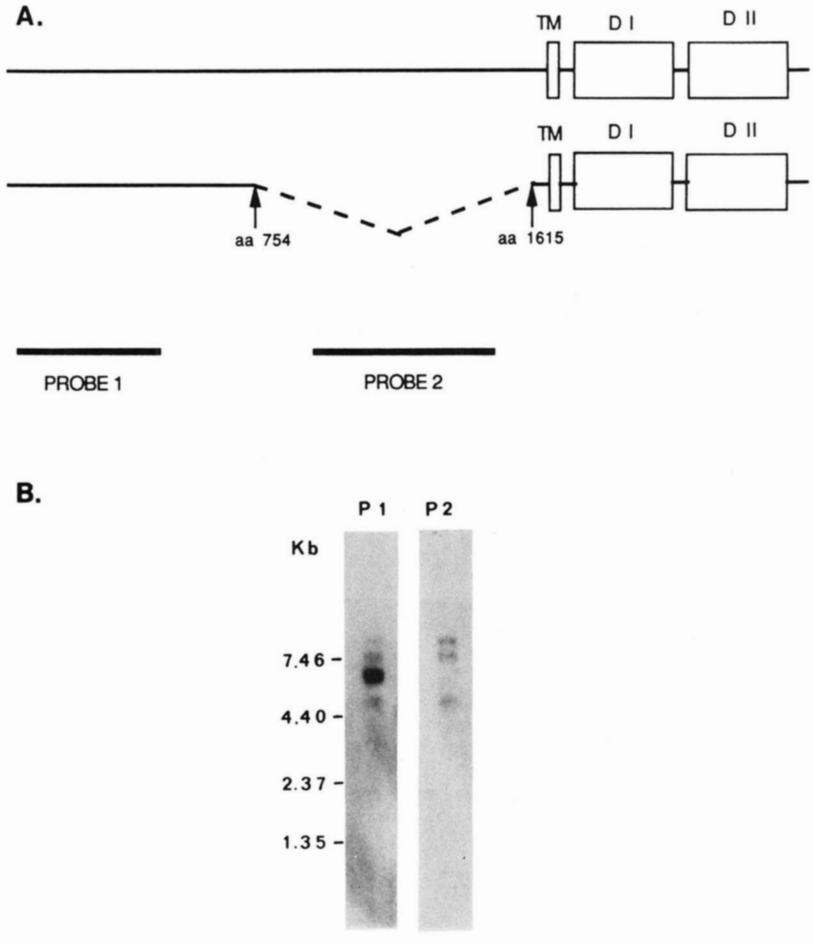


FIG. 6. **Northern blots to identify the variant form of RPTPβ.** *A*, a *schematic diagram* of the protein encoded by the full-length RPTPβ cDNA compared with the putative protein encoded by the two independently isolated cDNA clones that carry an identical deletion of 2577 base pairs in the extracellular region of the protein. The position of the deletion is indicated by the *dotted line* with the number of the amino acid that remains at both the 5′ and 3′ ends of the deletion indicated. The locations of the two probes used in Northern analysis (*probes 1* and *2*) are indicated. *TM*, transmembrane peptide; *DI*, phosphatase domain I; and *DII*, phosphatase domain II. *B*, poly(A$^+$) RNA (1 μg) isolated from the Lan 5 neuroblastoma cell line was separated on an RNA formaldehyde gel and probed with human probe 1 (*P1*) that contains 1.3 kb of sequences derived from the extreme 5′ end of the cDNA clone and human probe 2 (*P2*) that contains 1.6 kb of sequences derived from the portion of the full-length cDNA clone that is deleted in the variant cDNA clones.

ular weight form in lysates from RPTPβ in Lan 5 cells is not clear.

Tissue-specific Expression of RPTPβ—Northern analysis of various tissue RNAs was performed to determine the tissue-specific expression of RPTPβ. The probe used in this analysis was a portion of the murine homolog of RPTPβ that was amplified in the polymerase chain reaction (see "Experimental Procedures") and contains 405 nucleotides encoding 135 amino acids of Domain II. Based on a nucleotide sequence comparison with the equivalent region of the human cDNA clone, the murine and human clones are 88% identical at the nucleotide level in this region of Domain II of RPTPβ. The results of this Northern analysis (Fig. 4*B*) indicate the presence of two major transcripts of 8.8 and 6.4 kb, respectively. These two transcripts are similar in size to the largest and smallest transcripts observed in Lan 5 RNA (see Fig. 4*A*). However, no band corresponding to the 7.5-kb transcript was detected. A minor transcript of approximately 9.4 kb was sometimes observed in RNA prepared from mouse brain tissue and might represent cross-reaction to a highly related phosphatase. RPTPβ transcripts were not detected in the lung, heart, liver, spleen, kidney, muscle, testes, and thymus. The quality of the RNA isolated from the various tissues was compared by parallel hybridization with an actin probe of the same blots (Fig. 4*C*). Hence, Northern hybridization analysis indicates that RPTPβ mRNA is strictly expressed in the brain of the adult mouse.

In Situ Hybridization Analysis—In order to more precisely localize the expression of RPTPβ, we performed *in situ* hybridization in the adult and the developing mouse. The results of this analysis confirm that RPTPβ is expressed in the CNS. In day 20 of the mouse embryo (E 20), a high level of expression was observed in the ventricular and subventricular zones of the brain (Fig. 7*A*) and spinal cord (data not shown). The level of expression is lower in the adult brain and is localized to the Purkinje cell layer of the cerebellum, the dentate gyrus, and the subependymal layer of the anterior horn of lateral ventricle (Fig. 7*B*). Both probes (complementary to the carbonic anhydrase domain and the second phosphatase domain) gave identical results. The addition of a 30-fold excess of unlabeled oligonucleotide completely blocked the labeling in all areas (data not shown). Furthermore, no signal was observed in adjacent sections hybridized with the sense probe (data not shown), indicating that the probes hybridize to mRNA in a sequence-specific manner.

DISCUSSION

We have cloned and characterized a human receptor tyrosine phosphatase that is expressed in the central nervous system and cannot be detected in other murine tissues. Amino acid sequence analysis revealed that RPTPβ shares a striking sequence homology with various isoforms of the enzyme CAH over a stretch of 283 amino acids located at the extreme amino terminus of the phosphatase protein. Recently, we have completed the amino acid sequence of another phosphatase, RPTPγ, that also contains CAH-related sequences near the amino terminus in the extracellular domain (Barnea *et al.*, 1993). Therefore, RPTPβ and RPTPγ are members of a new subgroup of transmembrane phosphatases that can be classified on the basis of a CAH-related domain present in the

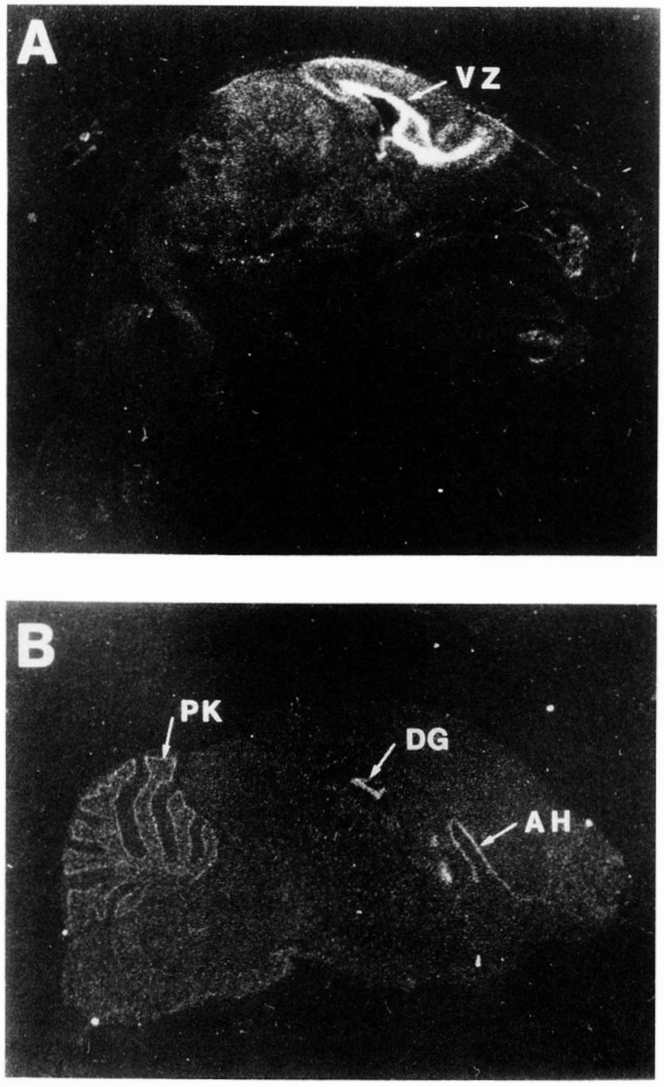


FIG. 7. ***In situ* hybridization analysis of RPTPβ in developing and adult mouse brain.** *A*, a sagittal section through embryonic day 20 (E 20) mouse shows that RPTPβ is expressed in the developing nervous system. The highest level of expression is seen in the ventricular and subventricular zones (*VZ*). *B*, a sagittal section through the adult mouse brain shows discrete bands of expression in the Purkinje cell of the cerebellum (*PK*), the dentate gyrus (*DG*), and the subependymal layer of the anterior horn of the lateral ventricle (*AH*).

amino-terminal regions of their extracellular domain. We have generated a three-dimensional model of the CAH-related domain of RPTPγ based upon the known crystal structure of CAH, and a detailed description of this model is provided in the report by Barnea *et al.* (1993). Since key residues known to participate in the catalytic activity of CAH are missing from RPTPγ and -β, it is likely that the CAH domains in these two phosphatases do not possess classical carbonic anhydrase activity (Barnea *et al.*, 1993).

It has been postulated that underexpression or inactivation of protein tyrosine phosphatases might lead to oncogenesis, suggesting that protein tyrosine phosphatases may function as tumor suppressors (Fischer *et al.*, 1991; La Forgia *et al.*, 1991). In support of this theory, we have shown that the phosphatase RPTPγ maps to chromosome region 3p21. This region is frequently deleted in both renal cell and lung carcinomas (La Forgia *et al.*, 1991). In this report, we have localized the RPTPβ gene to human chromosome 7q31.3-q32. Thus, it would be important to investigate loss or mutations in the RPTPβ gene in certain tumor types, especially those exhibiting deletions of 7q.

Alternative mRNA splicing has been shown to produce six distinct isoforms of CD45 as a result of the differential usage of three exons encoding sequences in the extracellular domain (Streuli *et al.*, 1987; Streuli and Saito, 1989). Alternative splicing within the first catalytic domain of RPTPα has also been described (Matthews *et al.*, 1990). The results of Northern blot analysis reveal the presence of multiple RPTPβ transcripts in both mouse brain and a human neuroblastoma cell line, Lan 5, that appear to result either from alternatively spliced transcripts or from highly related genes. The Northern blot analyses indicate that the 8.8- and 6.4-kb transcripts are strictly expressed in the brain. It remains to be determined whether the two transcripts are expressed in the same region of the CNS. Northern blot analysis described in Fig. 4 in conjunction with the analysis of two independently isolated cDNA clones suggests that the smallest transcript of 6.4 kb may result from a deletion of approximately 2.6 kb encoding a large portion of the extracellular domain of RPTPβ. Definitive proof that this deletion is caused by an alternatively spliced transcript will be provided by analysis of the intron/exon boundaries of the RPTPβ gene upon isolation of genomic clones. Identification of the variant protein encoded by the 6.4-kb transcript is under current investigation.

The importance of tyrosine phosphorylation in the control and regulation of specific neuronal processes is under intense investigation. It was demonstrated that tyrosine phosphorylation is enhanced at the time of synaptogenesis during development (Cudmore and Gurd, 1991; Girault *et al.*, 1992a; Qu *et al.*, 1990) and may play a role in synaptic plasticity in the adult (Girault *et al.*, 1992b). In addition, nerve growth factor and other neurotrophic factors mediate their effects on neuronal survival by binding to and activating receptors with tyrosine kinase activity (Cordon-Cardo *et al.*, 1991; Kaplan *et al.*, 1991; Klein *et al.*, 1991; Schlessinger and Ullrich, 1992). Stimulation of other growth factor receptors with tyrosine kinase activities has profound effects on survival and differentiation of cultured neurons (Aizenman and de Vellis, 1987; Morrison *et al.*, 1987) suggesting a potential role in normal neuronal development. Finally, several cytoplasmic tyrosine kinases, including the products of the protooncogenes c-*src* and c-*yes*, are expressed at high levels in specific regions of the adult brain (Pang *et al.*, 1988a, 1988b; Maness *et al.*, 1988; Sudol *et al.*, 1989).

RPTPβ represents the first cloned mammalian tyrosine phosphatase whose tissue-specific expression is restricted to the nervous system. Several *Drosophila* receptor-linked protein tyrosine phosphatases have been identified that are selectively expressed on central nervous system axons in the embryo (Tian *et al.*, 1991; Yang *et al.*, 1991). The identification of brain-specific protein tyrosine phosphatases, such as RPTPβ, is an important initial step toward understanding the regulation of tyrosine phosphorylation by the protein tyrosine phosphatases in mammalian neural tissues. The relatively high level of RPTPβ expression in the embryonic CNS suggests that it plays a role in the development of the nervous system. It is of note that the ventricular and subventricular zones are the primary sites of cell division in the developing CNS (Altman and Bayer, 1990). Interestingly, RPTPβ is expressed in the few regions of the adult brain that continue to show mitotic activity such as the dentate gyrus (Bayer, 1980) and the subependymal layer of the anterior horn of the lateral ventricle (Altman, 1969). We are currently applying immunocytochemical techniques to determine the precise cel-

lular distribution of RPTPβ protein and to help elucidate its role in the regulation of specific developmental and neuronal processes.

Acknowledgment—We thank Kiki Nelson for the synthesis of oligonucleotides.

REFERENCES

Aizenman, Y., and de Vellis, J. (1987) *Brain Res.* **406,** 32–42
Altman, J. (1969) *J. Comp. Neurol.* **137,** 433–458
Altman, J., and Bayer, S. A. (1990) *Exp. Neurol.* **107,** 23–35
Barnea, G., Silvennoinen, O., Shaanan, B., Honegger, A. M., Canoll, P. D., D'Eustachio, P., Morse, B., Levy, J. B., La Forgia, S., Huebner, K., Musacchio, J. M., Sap, J., and Schlessinger, J. (1993) *Mol. Cell. Biol.* **13,** 1497–1506
Bayer, S. A. (1980) *Science* **216,** 890–892
Cannizzaro, L. A., Skolnik, E., Margolis, B., Croce, C. M., Schlessinger, J., and Huebner, K. (1991) *Cancer Res.* **51,** 3818–3820
Chou, C. K., Dull, T. J., Russell, D. S., Gherzi, R., Lebwohl, D., Ullrich, A., and Rosen, O. M. (1987) *J. Biol. Chem.* **262,** 1842–1847
Cordon-Cardo, C., Tapley, P., Jing, S., Nanduri, V., O'Rourke, E., Lamballe, F., Kovary, K., Klein, R., Jones, K. R., Reichardt, L. F., and Barbacid, M. (1991) *Cell* **66,** 1–20
Cudmore, S. B., and Gurd, J. W. (1991) *J. Neurochem.* **57,** 1240–1248
Durst, M., Croce, C. M., Gissman, L., Schwarz, E., and Huebner, K. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 1070–1074
Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991) *Science* **53,** 401–406
Girault, J.-A., Siciliano, J. C., Robel, L., and Herve, D. (1992a) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 2769–2773
Girault, J.-A., Chamak, B., Bertuzzi, G., Tixier, H., Wang, J. K. T., Pang, D. T., and Greengard, P. (1992b) *J. Neurochem.* **58,** 518–528
Harlow, E., and Lane, D. (1988) in *Antibodies: A Laboratory Manual,* pp. 77–88, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., and Chao, M. V. (1991) *Nature* **350,** 678–683
Hirano, A. A., Greengard, P., and Huganir, R. L. (1988) *J. Neurosci.* 1447–1455
Huebner, K., Druck, T., Croce, C. M., and Thiesen, H-J. (1991) *Am. J. Hum. Genet.* **48,** 726–740
Hunter, T. (1987) *Cell* **50,** 823–829
Hunter, T. (1989) *Cell* **58,** 1013–1016
Kaplan, R., Morse, B., Huebner, K., Croce, C., Howk, R., Ravera, M., Ricca, G., Jaye, M., and Schlessinger, J. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 7000–7004
Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V., and Parada, L. F. (1991) *Science* **252,** 554–557
Klein, R., Jing, S., Nanduri, V., O'Rourke, E., and Barbacid, M. (1991) *Cell* **65,** 189–197
Kmiecik, T. K., and Shalloway, D. (1987) *Cell* **49,** 65–73
Kris, R. M., Lax, I., Gullick, W., Waterfield, M. D., Ullrich, A., Fridkin, M., and Schlessinger, J. (1985) *Cell* **40,** 619–625
Krueger, N. X., and Saito, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 7417–7421
Krueger, N. X., Streuli, M., and Saito, H. (1990) *EMBO J.* **9,** 3241–3252
La Forgia, S., Morse, B., Levy, J., Barnea, G., Cannizzaro, L. A., Li, F., Nowell, P. C., Boghosian-Sell, L., Glick, J., Weston, A., Harris, C. C., Drabkin, H., Patterson, D., Croce, C. M., Schlessinger, J., and Huebner, K. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5036–5040
Maness, P. F., Aubry, M., Shores, C. G., Frame, L., and Pfenninger, K. H. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 5001–5005
Matthews, R. J., Cahir, E. D., and Thomas, M. L. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 4444–4448
Morrison, R. S., Kornblum, H. I., Leslie, F. M., and Bradshaw, R. A. (1987) *Science* **238,** 72–75
Nebreda, A. R., Martin-Zanca, D., Kaplan, D. R., Parada, L. F., and Santos, E. (1991) *Science* **252,** 558–560
Pang, D. T., Wang, J. K. T., Valtorta, F., Benfenati, F., and Greengard, P. (1988a) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 762–766
Pang, D. T., Valtorta, F., DeMarco, M. E., Brugge, J. S., Benfenati, F., and Greengard, P. (1988b) *Soc. Neurosci. Abstr.* **14,** 106
Qu, Z., Moritz, E., and Huganir, R. L. (1990) *Neuron* **2,** 367–378
Schlessinger, J., and Ullrich, A. (1992) *Neuron* **9,** 383–391
Snyder, M. A., Bishop, J. M., McGrath, J. P., and Levinson, A. D. (1985) *Mol. Cell. Biol.* **5,** 1772–1779
Sonnenfield, K. H., and Ishii, D. N. (1982) *J. Neurosci. Res.* **8,** 375–391
Sorge, L. K., Levy, B. T., and Maness, P. F. (1984) *Cell* **36,** 249–257
Streuli, M., and Saito, H. (1989) *EMBO J.* **8,** 787–796
Streuli, M., Hall, L. R., Saga, Y., Schlossman, S. F., and Saito, H. (1987) *J. Exp. Med.* **166,** 1548–1566
Sudol, M., and Hanafusa, H. (1986) *Mol. Cell. Biol.* **6,** 2839–2846
Sudol, M., Kuo, C. F., Shigemitsu, L., and Alvarez-Buylla, A. (1989) *Mol. Cell. Biol.* **9,** 4545–4549
Swarup, G., Dasgupta, J. D., and Garbers, D. L. (1983) *J. Biol. Chem.* **258,** 10341–10347
Tian, S.-S., Tsoulfas, P., and Zinn, K. (1991) *Cell* **67,** 675–685
Ullrich, A., and Schlessinger, J. (1990) *Cell* **61,** 203–212
Weinmaster, G., Zoller, M. J., and Pawson, T. (1986) *EMBO J.* **5,** 69–76
Yang, X., Seow, K. T., Bahri, S. M., Oon, S. H., and Chia, W. (1991) *Cell* **67,** 661–673