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Multiple tyrosine protein kinases in rat hippocampal neurons: Isolation of Ptk-3, a receptor expressed in proliferative zones of the developing brain

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ABSTRACT **Tyrosine protein kinases are likely to play an important role in the maintenance and/or development of the nervous system. In this study we have used the PCR cloning technique to isolate sequences derived from tyrosine kinase genes expressed in cultured hippocampal neurons obtained from 17.5-day-old rat embryos. Nucleotide sequence analysis of 209 independent clones revealed sequences derived from 25 tyrosine kinases, of which two corresponded to previously unreported genes. One of the PCR clones, ptk-2, belongs to the Jak family of cytoplasmic tyrosine kinases. The second clone, ptk-3, was derived from a gene encoding an additional class of tyrosine kinase receptors whose extracellular domains contain regions of homology with coagulation factors V and VIII and complement component C1. Transcripts encoding the Ptk-3 receptor are present in a variety of embryonic and adult tissues with highest levels observed in brain. During development, ptk-3 transcripts are most abundant in the proliferative neuroepithelial cells of the ventricular zone, raising the possibility that this receptor may play an important role in the generation of the mammalian nervous system.**

The complexity of the mammalian nervous system predicts the existence of multiple signaling strategies to generate neuronal cells during embryonic development and to maintain mature neurons during adult homeostasis. However, the signal-transduction mechanisms that govern the growth and survival of neurons remain to be elucidated. The recent discovery that the nerve growth factor family of neurotrophins mediate their trophic activities through the Trk tyrosine protein kinase receptors (1–3) has raised the possibility that tyrosine phosphorylation may play a key role in the development and/or maintenance of neurons. The importance of signaling through the Trk receptors was recently illustrated by generating mice carrying a targeted *trkB* allele (4). Mice homozygous for this mutation have reduced numbers of neurons in various structures of the central and peripheral nervous systems (4). Other growth factors such as acidic fibroblastic growth factor (FGF), basic FGF, insulin-like growth factor, epidermal growth factor, and platelet-derived growth factor, known to signal through tyrosine kinase receptors, also have neurotrophic activity (5, 6). More recently, the glial growth factor family of proteins has been identified as ligands for members of the epidermal growth factor family of tyrosine protein kinase receptors (7, 8). These observations further support the concept that tyrosine kinases play a key role in signaling throughout the mammalian nervous system.

Accumulating evidence indicates that neurons express a large number of tyrosine kinase receptors (9). Lai and Lemke (10) first used PCR-based amplification techniques to isolate a series of tyrosine protein kinases (Tyro-1 to Tyro-13) from enriched cDNA libraries derived from sciatic nerve and Schwann cells. We have used a similar strategy to identify those tyrosine protein kinase receptors expressed in defined neuronal subpopulations of the central nervous system. In this study, we report that at least 25 different tyrosine kinases, including 15 receptors, are present in rat embryonic hippocampal neurons. Two of these tyrosine kinase sequences are derived from previously unreported genes, including a fourth member of the Jak family of cytoplasmic tyrosine kinases and a tyrosine kinase receptor with distinctive structural motifs in its extracellular/ligand-binding domain.

MATERIALS AND METHODS

PCR Amplification. Total RNA (5 μg) was isolated from primary cultures of rat embryonic hippocampal neurons (11) and used to synthesize cDNA with oligo(dT) primers and the Superscript cDNA synthesis system (GIBCO/BRL). The resulting cDNA was submitted to PCR-aided amplification with degenerate primers corresponding to the conserved tyrosine protein kinase sequences HRDLAAR (5′-GGAATTCCAYCGNGAYYTNGCNGCNMCG-3′) and DVWSFG (5′-GATGCGGCCGCNCCRAARSWCCANACRTC-3′). These "amplimers" contain *Eco*RI and *Not* I cleavage sites (underlined) in their 5′ regions for subcloning purposes. Amplified DNA was fractionated on a 2.2% agarose gel; the major 220-bp band was then isolated by electroelution, digested with *Eco*RI and *Not* I, and subcloned into pBluescript. DNAs obtained from randomly selected bacterial transformants were submitted to nucleotide sequence analysis by using the Sequenase system (United States Biochemical).

Isolation of ptk-3 cDNA Clones. A λgt10 neonatal rat brain cDNA library (Clontech) was screened with a ^{32}P-labeled 220-bp *Eco*RI–*Not* I probe derived from a plasmid (pPT9) containing the ptk-3 PCR-amplified clone. Three recombinant phages were isolated, and their respective inserts were subcloned into pBluescript (pMS1, pMS2 and pMS3) and submitted to nucleotide sequence analysis. Because none of these cDNA clones contained the complete ptk-3 cDNA coding sequences, a 510-bp *Eco*RI/*Sac* I probe derived from the 5′ region of pMS3 was used to screen a λgt10 adult rat brain cDNA library (Clontech). One clone (pMS25) contained the missing 5′ cDNA coding sequences. A 729-bp *Eco*RI/*Sac* I fragment corresponding to the 5′ region of pMS25 was ligated to a 3014-bp *Sac* I/*Eco*RI DNA fragment from pMS3 and inserted into pBluescript to generate a full-length ptk-3 cDNA clone pMS35.§

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Abbreviations: FGF, fibroblastic growth factor; E*n*, embryonic day *n*.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L26525).

Immunoprecipitation and Tyrosine Kinase Assays. COS-7 cells were transfected with pBH7 DNA, a pREX2N-derived plasmid (12) that contains the ptk-3 cDNA insert of pMS35, using the DEAE-dextran/chloroquine technique as described (12). To assay for kinase activity, immunoprecipitates were incubated for 10 min at 30°C in the presence of 10 μCi of [γ-^{32}P]ATP (6000 Ci/mmol; NEN/DuPont; 1 Ci = 37 GBq) and 10 mM $MnCl_2$. Samples were analyzed on SDS/8% PAGE gels as described (12).

***In situ* Hybridization.** A 290-bp PCR fragment corresponding to nt 1105–1395 of pMS35 was subcloned into pBluescript and transcribed in the presence of either [α-^{35}S]UTP (Amersham) or [γ-^{33}P]UTP (NEN/DuPont) with T3 and T7 polymerases (Promega) to generate ptk-3 antisense and sense RNA probes, respectively. Frozen coronal sections from adult mouse brain and sagittal sections from embryonic (E) day 12.5 and E17.5 embryos (10-μm thick) were hybridized to the corresponding RNA probes as described (13).

RESULTS AND DISCUSSION

Amplification of Tyrosine Kinase Sequences from Embryonic Hippocampal Neurons. Primary cultures were prepared from E17.5 rat hippocampi as described by Scholz and Palfrey (11). At this time, >90% of the neurons in the developing hippocampus correspond to pyramidal neurons. Cells were plated onto poly(L-lysine)-coated plates and cocultivated with feeder glial cell cultures isolated from the cerebral hemispheres of newborn rats. These glial cells were placed on inverted coverslips, so as not to directly contact the neuronal cultures. After 48 hr, neuritic processes were evident in most (>90%) of the cells in the embryonic hippocampal cultures. Moreover, <5% of these cells were of glial origin, as determined by staining with anti-glial fibrillary acidic protein antibodies.

To amplify sequences related to tyrosine protein kinase genes, we used a PCR cloning strategy based on the use of two degenerate oligonucleotide primers corresponding to the highly conserved sequence motifs (HRDLAAR and DVWSFG) of the catalytic domain of tyrosine protein kinase receptors (10). Of a total of 220 randomly selected plasmid DNAs, 209 contained sequences corresponding to 25 distinct tyrosine protein kinase genes. Twenty-three of these genes were either already known at that time or were subsequently reported by other laboratories. These genes included the ubiquitously expressed Src regulatory kinase, Csk, the Src-related kinases Abl, Arg, Fps, and Fer, the focal adhesion kinase Fak, and the recently identified Jak1 and Jak2 cytoplasmic kinases. The remaining 15 genes were derived from tyrosine kinase receptors. Nine of them were well characterized receptors for FGFs (FGFR-1, FGFR-2, and FGFR-3), plateled-derived growth factors (PDGFRα and PDGFRβ), colony-stimulating factor 1 (CSF-1R), insulin (IR), insulin-like growth factor 1 (IGF-1R), brain-derived neurotrophic factor (TrkB), and neurotrophin 4 (TrkB), all of which, except for the CSF-1R, are expressed in neuronal cells (4, 14, 15). The remaining clones corresponded to six orphan receptors, including three members of the Eph subfamily of tyrosine kinases (Eck, Sek, and Eek) (16–18), Tyro-7 (10), Tyro-10 (10), and Vik (19). To date, there is no additional information regarding the structure of the Tyro-7 and Tyro-10 receptors outside their kinase catalytic domain (10). Vik is a putative tyrosine kinase receptor with a short extracellular domain and unique residues in sequence motifs invariant to all other tyrosine protein kinases (19). Finally, two of the 25 PCR-amplified cDNA clones, ptk-2 and ptk-3, were derived from previously unreported tyrosine protein kinase genes (Fig. 1).

Ptk-2, an Additional Member of the Jak Family of Cytoplasmic Tyrosine Kinases. As illustrated in Fig. 1, the ptk-2 sequence is highly related to the recently identified Jak family of cytoplasmic tyrosine protein kinases. Known members of the Jak kinases include Jak1 (20); Jak2 (21), a kinase recently shown to be associated with the erythropoietin and growth hormone receptors (22); and Tyk2, a kinase involved in the phosphorylation of the interferon-induced transcription factor ISGF3α (23). These cytoplasmic kinases are characterized by the presence of two catalytic domains, of which the most carboxyl-terminal region remains to be functionally defined (20, 21). In addition, they share several regions of high homology, one of which is closely related to the Src homology 2 domains present in the Src kinase family. The deduced amino acid sequence of ptk-2 is most closely related to Jak-2 (82% homology and 67% identity) and contains the WYAPE sequence motif characteristic of this group of kinases (Fig. 1). To establish whether the Ptk-2 kinase has the overall structural features of this subfamily of cytoplasmic tyrosine kinases it will be necessary to isolate full-length ptk-2 cDNA clones.

Ptk-3 Is a Member of a Distinct Class of Tyrosine Protein Kinase Receptors. The ptk-3 PCR-derived clone exhibits the highest level of homology to Tyro-10, another PCR-derived sequence isolated from a sciatic nerve cDNA library (10) (Fig. 1). The ptk-3 kinase region is also highly related to other mammalian tyrosine kinases, such as the three members of the Trk family of neurotrophin receptors (24), the insulin receptor (25), and to a lesser extent, Ror1 and Ror2 (26) (Fig. 1). ptk-3 also displays significant homology to the *Torpedo* RTK (27) and the *Drosophila* Dtrk gene (24).

Molecular cloning of a full-length ptk-3 cDNA clone revealed an open reading frame of 910 residues with a series of structural motifs distinct from those of previously known

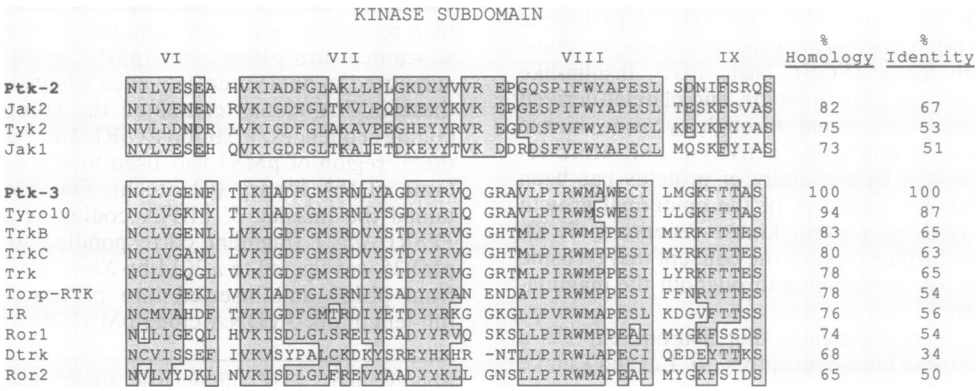

FIG. 1. Sequence homology of the deduced amino acid sequence of the PCR-amplified domain of Ptk-2 and Ptk-3 with other tyrosine protein kinases. Sequences have been aligned using the PILEUP program (Genetics Computer Group). Percentage of homologous and identical amino acid residues is indicated at right. Conserved residues are boxed and shaded. Torp, *Torpedo*; RTK, receptor tyrosine kinase; IR, insulin receptor.

tyrosine protein kinases (Fig. 2). By analogy with other signal peptide sequences, residues 1–19 of the Ptk-3 receptor are likely to correspond to its signal peptide. If so, the mature Ptk-3 protein would be 891 amino acids long. The amino-terminal moiety of the putative extracellular/ligand-binding domain of Ptk-3 (residues 19–409) contains a region (residues 32–185) of significant homology (49–61% homology and 24–35% identity) to structural motifs present in soluble molecules known to associate with cellular membrane proteins, including the light chains of coagulation factors V and VIII and the milk fat globule membrane proteins MFG.E8 and BA46 (28–30) (Fig. 2). These coagulation factor-like motifs are also present in certain membrane proteins, including discoidin I, a *Dictyostelium discoideum* lectin involved in cell aggregation (31), and A5, a *Xenopus* neural recognition molecule (32). The coagulation factor-like motif of Ptk-3 is most related to those of the A5 protein (61% homology) and coagulation factor V (60% homology).

The carboxyl-terminal half of the Ptk-3 extracellular domain also displays an internal repeat (residues 303–347 and 349–388) with limited homology to domains I and III of complement components C1r and C1s (33) (Fig. 2). Interestingly, the related *Xenopus* A5 neural recognition protein also displays these complement-like internal repeats (32). These repeats are contiguous in Ptk-3 and A5 but not in the complement molecules in which they are separated by an epidermal growth factor-like domain (33). However, the Ptk-3 repeats are considerably shorter (40 and 45 residues) than those present in the C1 and A5 (114–135 residues) molecules (Fig. 2). The functional roles of these motifs in the C1 molecules remain to be firmly established. However, it has been proposed that they might be involved in the interaction between the dimeric $C1r_2C1s_2$ complex and the C1q component (34). Whether these complement-like motifs play a role in the dimerization of the Ptk-3 receptors is an interesting possibility that remains to be explored.

The single transmembrane domain of Ptk-3 (residues 410–436) is followed by a juxtamembrane region with unusual structural features. In addition to its length (170 residues), this region contains a high percentage of glycine and proline residues, a feature that predicts a high degree of structural flexibility (Fig. 2). As indicated above, the kinase catalytic domain of Ptk-3 (residues 607–902) is most closely related to Tyro-10 (85% homology and 71% identity), a putative tyrosine kinase receptor primarily expressed in brain tissue (10). Ptk-3 and Tyro-10 also share an unusual 17-residue insert between kinase subdomains I and II. The presence of this unique structural feature along with their high overall sequence homology in their respective kinase domains raises the possibility that Tyro-10 and Ptk-3 may be members of the same receptor subfamily.

Ptk-3 has an unusually short carboxyl-terminal tail of just eight amino acid residues (residues 903–910) (Fig. 2). This feature also resembles the Trk family of neurotrophin receptors (24). However, the tail of the Trk receptors, but not of Ptk-3, contains a tyrosine residue, which is responsible for their interaction with certain substrates such as phospholipase Cγ (35). While we were in the process of writing this manuscript, Johnson *et al.* (36) have reported the isolation of a tyrosine protein kinase receptor from a human breast carcinoma cell line. This tyrosine protein kinase, designated DDR, is 94% identical (96% homologous) to Ptk-3 and exhibits the same structural features (Fig. 2) (36). These observations suggest that DDR may be the human homologue of Ptk-3.

Biochemical Characterization of Ptk-3. COS-7 cells were transfected with pBH7, a mammalian expression plasmid containing the full-length ptk-3 cDNA clone under the control of a Rous sarcoma virus long terminal repeat. As shown in Fig. 3*A*, pBH7, but not mock-transfected cells, expressed a 120-kDa protein specifically immunoprecipitated with two different antisera raised against a synthetic peptide corresponding to the predicted carboxyl terminus of Ptk-3 (residues 897–910) and a bacterial glutathione *S*-transferase–Ptk-3 fusion protein encompassing the entire Ptk-3 tyrosine kinase domain (residues 599–910) (Fig. 2). Immunoprecipitation of the 120-kDa Ptk-3 protein by the antipeptide antibodies was completely abolished by preincubation with the immunizing peptide (Fig. 3*A*). To determine whether this 120-kDa protein had tyrosine kinase activity, immunoprecipitates were incubated with [γ-^{32}P]ATP in the presence of Mn^{2+} ions. As shown in Fig. 3*B*, the 120-kDa Ptk-3 protein became efficiently phosphorylated. Phosphoamino acid analysis of the resulting ^{32}P-labeled Ptk-3 protein indicated that the majority

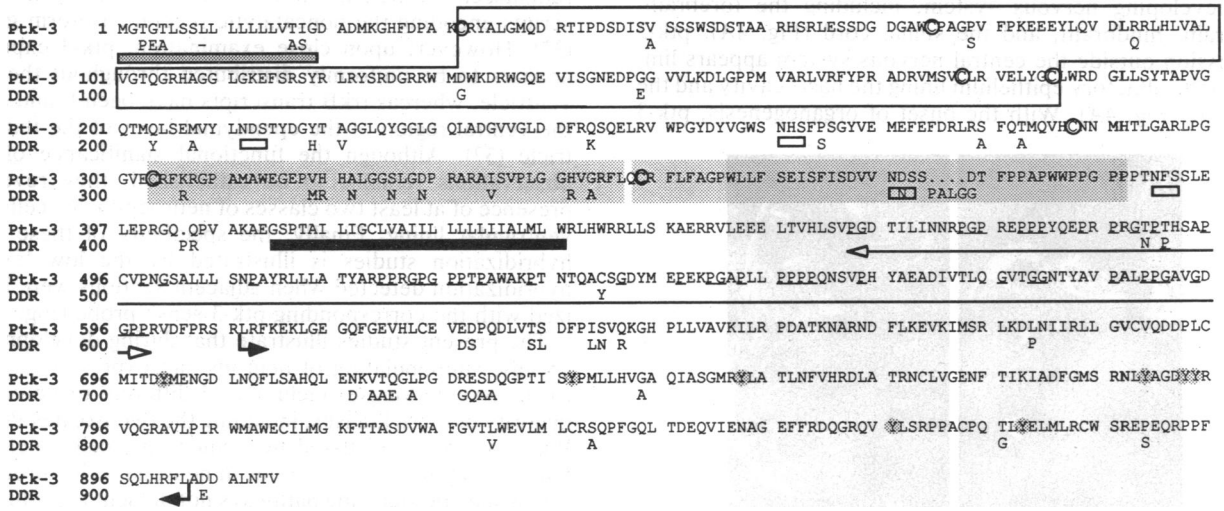

FIG. 2. Deduced amino acid sequence of the rat Ptk-3 tyrosine kinase receptor. The predicted signal peptide is underlined by a stippled bar. A region homologous to coagulation factors is boxed. Two partial internal repeats showing limited homology to complement proteins C1r and C1s are highlighted by stippled boxes. Putative N-glycosylation sites in the extracellular region are underlined by open bars. Extracellular cysteine residues are circled. The single transmembrane domain is underlined by a solid bar. A proline/glycine-rich domain in the juxtamembrane region is indicated by open arrows. Each of the proline and glycine residues in this region are underlined for easier identification. The catalytic tyrosine kinase domain is flanked by solid arrows. Tyrosine residues in the kinase catalytic domain are shaded. Nonidentical residues of the highly related human DDR receptor (36) are shown for comparative purposes. Residues absent in Ptk-3 but present in DDR are indicated by dots. A Ptk-3 residue not present in DDR tyrosine protein kinase is indicated by a dash.

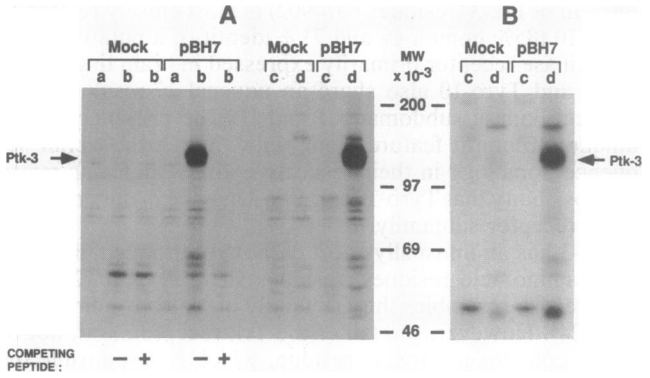

FIG. 3. (*A*) Expression of Ptk-3 in COS-7 cells. COS-7 cells were transfected either with no DNA (Mock) or with pBH7 DNA, a pMEX-neo-derived expression plasmid containing the entire cDNA coding sequences of ptk-3. Transfected cells were labeled with Tran^{35}S-label, lysed, and immunoprecipitated with either preimmune (lanes a and c) or immune (lanes b and d) sera elicited against a peptide corresponding to the carboxyl-terminal sequence of the Ptk-3 protein in the absence (−) or presence (+) of 10 μg of competing peptide (lanes b) and against a bacterial glutathione *S*-transferase Ptk-3 fusion protein (lane d). (*B*) Immunoprecipitates were also assayed for *in vitro* tyrosine kinase activity as described. Samples were loaded onto SDS/8% PAGE gels. Electrophoresed gels were either fluorographed (*A*) or submitted to alkali treatment, dried, and exposed to Kodak X-Omat film (*B*) for either 30 (*A*) or 48 (*B*) hr. Migration of the 120-kDa Ptk-3 protein is indicated by arrows. Molecular-weight markers include myosin (200,000), phosphorylase b (97,000), bovine serum albumin (69,000), and ovalbumin (46,000).

(>90%) of the radioactivity was incorporated as phosphotyrosine (data not shown).

Expression of ptk-3 in Adult and Embryonic Mouse Tissues. The ptk-3-encoding gene directs the synthesis of a single transcript of 4.3 kb in all the tissues analyzed (data not shown). ptk-3 transcripts are also present during mouse development, particularly after E10.5. To better define its pattern of expression, we performed *in situ* hybridization analysis of tissue sections derived from mouse embryos at various stages of development, as well as from adult mouse brain. In E12.5 embryos, ptk-3 is preferentially expressed in the developing nervous system, including the forebrain, midbrain, hindbrain, and the spinal cord (Fig. 4*A*). ptk-3 expression outside the central nervous system appears limited to the olfactory epithelium lining the nasal cavity and the yolk sac (Fig. 4*A*). With the onset of organogenesis, ptk-3 expression expands to most developing organs of the embryo. However, at E15.5, the strongest ptk-3 hybridization signal was still detected in the ventricular proliferative zones of the brain (data not shown).

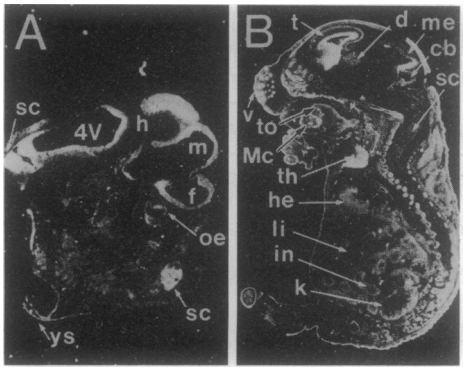

FIG. 4. *In situ* hybridization analysis of ptk-3 expression during mouse embryonic development. Sagittal sections of 12.5-day-old (*A*) and 17.5-day-old (*B*) mouse embryos. cb, Cerebellum; d, diencephalon; f, forebrain; h, hindbrain; he, heart; in, intestine; k, kidney; li, liver; Mc, Meckel's cartilage; m, midbrain; me, mesencephalon; oe, olfactory epithelium; sc, spinal cord; t, telencephalon; th, thymus; to, tongue; v, vibrissae; ys, yolk sac; 4V, fourth ventricle. (*A*, ×4.5; *B*, ×2.2).

In E17.5 embryos, ptk-3 is widely expressed in nonneural tissues. High levels of ptk-3 expression can be observed in olfactory and nasal structures, vibrissae, ossifying Meckel's cartilage, tooth primordia, tongue, thyroid cartilage, thymus, bone, heart, intestine, pancreas, and kidney (Fig. 4*B*). Skin, adipose, and liver tissue also display significant levels of ptk-3 transcripts (Fig. 4*B*). In the nervous system, ptk-3 expression is highly localized to well-defined structures. In the telencephalon, there are abundant ptk-3 transcripts in the caudate putamen, as well as in the ventricular zone of the cerebral cortex, where those mitoses giving rise to the younger cortical neurons are still in progress. These observations indicate that ptk-3 is preferentially expressed in proliferative zones of the developing cortex, whereas there is little or no ptk-3 expression in regions of neuronal migration and differentiation. In the diencephalon, low levels of ptk-3 transcripts can be detected in the dorsal thalamus. However, none of the other diencephalic structures displays detectable levels of ptk-3 expression. In the midbrain, only the inferior colliculus shows a high density of ptk-3 hybridization. In the hindbrain, ptk-3 transcripts can be found in the cerebellum as well as in some dorsal structures of the medulla. The choroid plexus of the lateral and fourth ventricles, a region of continuous cellular turnover, also shows a high density of ptk-3 transcripts. Finally, moderate levels of ptk-3 expression could be observed in the pituitary gland and in the spinal cord (Fig. 4*B*), as well as in the peripheral nervous system, where we observed homogeneous distribution of ptk-3 hybridization signals in all the ganglia analyzed (data not shown).

In the adult brain, the highest density of ptk-3 transcripts are present in the white matter (Fig. 5 *A* and *C*), suggesting that the Ptk-3 receptor is expressed in glial cells. Significant levels of ptk-3 mRNA can also be observed in the pyramidal layer of the hippocampus (Fig. 5 *A* and *C*), as well as in the choroid plexus and ependymal cell layer surrounding the cerebral ventricles (Fig. 5*D*). Interestingly, the expression of ptk-3 in the choroid plexus and the ependymal layers is reminiscent of the pattern of expression of those trkB transcripts encoding the noncatalytic receptor isoform gp95^{trkB} (37). However, upon close examination, ptk-3 expression appears to be uniformly distributed throughout the entire ventricle, whereas trkB transcripts have been found only in the ventral region of the ependymal layer of the third ventricle (37). Although the functional significance of these observations remains to be established, they reveal the presence of at least two classes of neuroepithelial cells in the ventricular lining. Finally, the specificity of these *in situ* hybridization studies is illustrated by the low levels of hybridization detected when adjacent sections were hybridized with the corresponding ptk-3 sense probe (Fig. 5*B*).

The present studies illustrate that during development, a specific subpopulation of neurons can express multiple tyrosine protein kinases including 15 different receptors. The majority of these kinases, including the two isolates Ptk2 and Ptk-3, are also expressed in nonneuronal cells. Yet, these kinases may play distinct roles in neuronal cells through the use of specific signaling pathways not present in proliferating cells. This scenario was recently illustrated with the Trk family of neurotrophin receptors, which can induce the neuronal differentiation of PC-12 and neuroblastoma cells, as well as the malignant transformation of mouse fibroblasts. A similar situation may occur with the Ptk-3 receptor, which has been recently found to be overexpressed in human breast carcinoma cells (36). The high levels of expression of ptk-3 transcripts in the proliferating structures of the developing

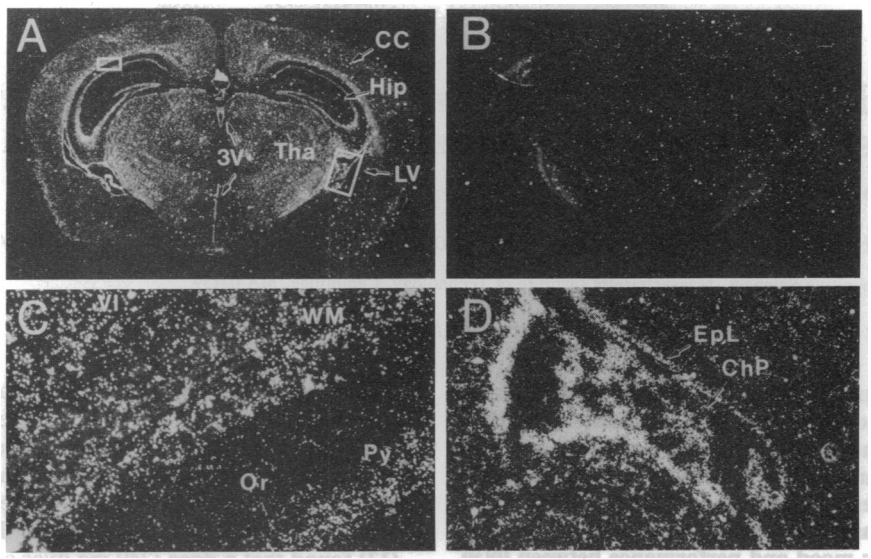

FIG. 5. *In situ* hybridization analysis of ptk-3 expression in adult mouse brain. Frozen coronal sections (10 μm) were hybridized with either antisense (*A*, *C*, and *D*) or sense (*B*) ptk-3 probes. (*C*) Expanded areas indicated by an open square in upper left corner of *A*. (*D*) Expanded area indicated by an open square in lower right part of *A*. CC, cerebral cortex; ChP, choroid plexus; EpL, ependymal cell layer; Hip, hippocampus; LV, lateral ventricle; Or, stratum oriens of the hippocampus; Py, pyramidal cell layer of the hippocampus; Tha, thalamus; WM, white matter; 3V, third ventricle; VI, cortical layer VI. (*A*, ×5.5; *B*, ×5.5; *C*, ×85; *D*, ×45).

nervous system compared with those in the adult brain raises the possibility that Ptk-3 may play a role during the proliferative stages of the developing brain. Identification of its cognate ligand should provide important information regarding the role of the Ptk-3 receptor in the ontogeny of the mammalian nervous system.

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1. Kaplan, D. R., Martin-Zanca, D. & Parada, L. F. (1991) *Nature (London)* **350**, 158–160.
2. Klein, R., Jing, S., Nanduri, V., O'Rourke, E. & Barbacid, M. (1991) *Cell* **65**, 189–197.
3. Barbacid, M. (1993) *Oncogene* **8**, 2033–2042.
4. Klein, F. R., Smeyne, R. J., Wurst, W., Long, L. K., Auerbach, B. A., Joyner, A. L. & Barbacid, M. (1993) *Cell* **75**, 113–122.
5. Barde, Y.-A. (1989) *Neuron* **2**, 1525–1534.
6. Walicke, P. A. (1989) *Annu. Rev. Neurosci.* **12**, 103–126.
7. Falls, D. L., Rosen, K. M., Corfas, G., Lane, W. S. & Fischbach, G. D. (1993) *Cell* **72**, 801–815.
8. Marchionni, M. A., Goodearl, A. D. J., Su Chen, M., Bermingham-McDonogh, O., Kirk, C., Hendricks, M., Danehy, F., Misumi, D., Sudhalter, J., Kobayashi, K., Wroblewski, D., Lynch, C., Baldassare, M., Hiles, I., Davis, J. B., Hsuan, J. J., Totty, N. F., Otsu, M., McBurney, R. N., Waterfield, M. D., Stroobant, P. & Gwynne, D. (1993) *Nature (London)* **362**, 312–318.
9. Maness, P. F. & Cox, M. E. (1992) *Semin. Cell Biol.* **3**, 117–126.
10. Lai, C. & Lemke, G. (1991) *Neuron* **6**, 691–704.
11. Scholz, W. K. & Palfrey, H. C. (1991) *J. Neurosci.* **11**, 2422–2432.
12. Jing, S., Tapley, P. & Barbacid, M. (1992) *Neuron* **9**, 1067–1069.
13. Bustelo, X. R., Rubin, S. D., Suen, K.-L., Carrasco, D. & Barbacid, M. (1993) *Cell Growth Differ.* **4**, 297–308.
14. Fantl, W. J., Johnson, D. E. & Williams, L. T. (1993) *Annu. Rev. Biochem.* **62**, 453–481.
15. Schlessinger, J. & Ullrich, A. (1992) *Neuron* **9**, 383–391.
16. Lindberg, R. A. & Hunter, T. (1990) *Mol. Cell. Biol.* **10**, 6316–6324.
17. Chan, J. & Watt, V. M. (1991) *Oncogene* **6**, 1057–1061.
18. Gilardi-Hebenstreit, P., Nieto, M. A., Frain, M., Mattéi, M.-G., Chestier, A., Wilkinson, D. G. & Charnay, P. (1992) *Oncogene* **7**, 2499–2506.
19. Kelman, Z., Simon-Chazottes, D., Guenet, J. L. & Yarden, Y. (1993) *Oncogene* **8**, 37–44.
20. Wilks, A. F., Harpur, A. G., Kurban, R. R., Ralph, S. J., Zurcher, G. & Ziemiecki, A. (1991) *Mol. Cell. Biol.* **11**, 2057–2065.
21. Harpur, A. G., Andres, A.-C., Ziemiecki, A., Aston, R. R. & Wilks, A. F. (1992) *Oncogene* **7**, 1347–1353.
22. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O. & Ihle, J. N. (1993) *Cell* **74**, 227–236.
23. Velazquez, L., Fellous, M., Stark, G. R. & Pellegrini, P. (1992) *Cell* **70**, 313–322.
24. Barbacid, M., Lamballe, F., Pulido, D. & Klein, R. (1991) *Biochim. Biophys. Acta Rev. Cancer* **1072**, 115–127.
25. Ullrich, A., Bell, J. R., Chen, E. Y., Herrera, R., Petruzzelli, L. M., Dull, T. J., Gray, A., Coussens, L., Liao, Y.-C., Tsubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M. & Ramachandran, J. (1985) *Nature (London)* **313**, 756–761.
26. Masiakowski, P. & Carroll, R. D. (1992) *J. Biol. Chem.* **267**, 26181–26190.
27. Jennings, C. G. B., Dyer, S. M. & Burden, S. J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2895–2899.
28. Kane, W. H. & Davie, E. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6800–6804.
29. Stubbs, J. D., Lekutis, C., Singer, K. L., Bui, A., Yuzuki, D., Srinivasan, U. & Parry, G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8417–8421.
30. Larocca, D., Peterson, J. A., Urrea, R., Kuniyoshi, J., Bistrain, A. M. & Ceriani, R. L. (1991) *Cancer Res.* **51**, 4994–4998.
31. Poole, S., Firtel, R. A. & Lamer, E. (1981) *J. Mol. Biol.* **153**, 273–289.
32. Takagi, S., Hirata, T., Agata, K., Mochii, M., Eguchi, G. & Fujisawa, H. (1991) *Neuron* **7**, 295–307.
33. Tosi, M., Duponchel, C., Meo, T. & Julier, C. (1987) *Biochemistry* **26**, 8516–8524.
34. Arlaud, G. J., Colomb, M. G. & Cagnon, G. (1987) *Immunol. Today* **8**, 106–111.
35. Obermeier, A., Halfter, H., Weismuller, K. H., Jung, G., Schlessinger, J. & Ullrich, A. (1993) *EMBO J.* **12**, 933–941.
36. Johnson, J. D., Edman, J. C. & Rutter, W. J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 5677–5681.
37. Klein, R., Conway, D., Parada, L. F. & Barbacid, M. (1990) *Cell* **61**, 647–656.