*trk*B, a Neural Receptor Protein-Tyrosine Kinase: Evidence for a Full-Length and Two Truncated Receptors

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We have screened an adult rat cerebellar cDNA library in search of novel protein tyrosine-kinase (PTK) cDNAs. A cDNA for a putative PTK, *trkB*, was cloned, and its sequence indicates that it is likely to be derived from a gene for a ligand-regulated receptor closely related to the human *trk* oncogene. Northern (RNA) analysis showed that the *trkB* gene is expressed predominantly in the brain and that *trkB* expresses multiple mRNAs, ranging from 0.7 to 9 kb. Hybridization of cerebral mRNAs with a variety of probes indicates that there are mRNAs encoding truncated *trkB* receptors. Two additional types of cDNA were isolated, and their sequences are predicted to encode two distinct C-terminally truncated receptors which have the complete extracellular region and transmembrane domain, but which differ in their short cytoplasmic tails.

There is increasing evidence that many receptor proteintyrosine kinases (PTKs) may be important in both development and maintenance of neural tissue. This has attracted our attention to the role of protein-tyrosine phosphorylation in neural and cellular signal transduction in the nervous system. The nervous system contains high levels of PTK activity, although there are variations in the relative levels of PTK activity in different regions of the brain (12). The finding that basic fibroblast growth factor (bFGF) has neurotrophic properties that are presumably mediated by neuronal receptors for bFGF (37) was extended recently by the discovery that the bFGF receptor is a PTK (18). The fact that bFGF mRNA is also expressed in brain tissue reinforces the premise that the FGF receptor plays a critical role in the central nervous system (7). In Drosophila cells, the product of the Dabl gene, which is localized in axons (9) interacts with fasciclin I, a homophilic cell adhesion molecule, in the formation of central nervous system axon pathways. Drosophila embryos with mutations in both Dabl and fasI display a defect in growth cone guidance, although mutants with single mutations in either Dabl or fasI display no gross morphological changes in the nervous system (6). In vertebrates, PTKs may be involved in differentiation of neurons since expression of the v-src product in PC12 cells causes cessation of cell division and neurite extension (1). c-src protein is found in neuronal growth cones (19), which suggests c-src involvement in neurite outgrowth.

Ion channels, which play a paramount role in neuronal signal transduction, may be substrates for receptor PTKs in neurons. The rate of desensitization of the nicotinic acetylcholine receptor, isolated from *Torpedo californica* electric organ, is increased by phosphorylation on tyrosine in vitro (13). Denervation of the rat diaphragm produces a timedependent decrease in phosphorylation of the nicotinic acetylcholine receptor, which implies that innervation may regulate its state of tyrosine phosphorylation (27).

To investigate the role of PTKs in neuronal signal transduction, we searched for new members of the receptor PTK family that are expressed in the nervous system. Since all known protein kinases have extensive sequence homology in

MATERIALS AND METHODS

Probe design. A probe corresponding to the HRDLAAR region conserved in receptor PTKs was synthesized by using phosphoramidite chemistry; it had the following composition: 5'-C(TG)-(ATG)GC-(ATGC)GC-CA(AG)-GTC-(ATGC) CG-GTG-3'. The probe was end labeled by using T4 polynucleotide kinase and [γ -³²P]ATP (ICN Radiochemicals) and used directly (31).

Cloning PTKs. An adult rat cerebellar λ ZAP cDNA library (obtained from Jim Boulter, Salk Institute) was plated on a lawn of *Escherichia coli* BB4 (5 × 10⁴ plaques per 15-cm dish). Replicas of the plaques were made on Hybond N (Amersham) by using standard methods (31). Prehybridization, hybridization, and washes were performed as described previously with tetramethylammonium chloride (10). Positive bacteriophages were plaque purified. Bluescript SK(-) phagemid, containing the target cDNA insert, were excised and circularized as specified by the manufacturer (Stratagene).

Identification of PTK clones. Rapid strategies for DNA sequence determination in the probe target region were developed. cDNA inserts were excised by digestion with *Eco*RI and purified by agarose gel electrophoresis. DNA was collected on NA45 paper (Schleicher & Schuell) and eluted as specified by the manufacturer. Southern transfer of the DNA onto GeneScreen Plus (Du Pont) followed by hybrid-

their catalytic domains (11), degenerate oligonucleotide probes were designed that would recognize the known receptor PTKs. The probe described in this paper is a 20-mer, which corresponds to the region made up of the sequence of amino acids HRDLAAR that is notably conserved amongst the receptor PTKs. This region differs from the corresponding regions in both the c-*src* PTK family and all known protein-serine/threonine kinases. Using this probe to screen a cDNA library made from adult rat cerebellar RNA, we have cloned *trk*B, which is closely related to the human oncogene *trk*. In fact, murine *trk*B has also been recently identified by using a *trk* probe (16). Northern (RNA) analysis indicates that *trk*B is expressed predominantly in the nervous system as multiple mRNAs, some of which encode truncated receptors.

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ization with the degenerate probe reaffirmed a positive cDNA. The insert was purified and digested with Sau3A and then ligated into BamHI-digested M13mp19. Recombinant phages, containing small fragments of the original cDNA insert, were plated on a lawn of *E. coli* JM101. Replica lifts of the phages were made on Hybond N and screened by using the degenerate probe described above. Sequence analyses of positive phages were used to determine whether a cDNA could encode a PTK. Alternatively, sequencing reactions were performed by using the degenerate oligonucleotide as a primer to identify regions near the HRDLAAR sequence.

DNA sequencing. cDNA sequences were determined by using dideoxyribonucleotide chain termination sequencing reactions (32) with $[\alpha^{-35}S]dATP$ (Dupont, NEN) and Sequenase (United States Biochemical Corp.) as specified by the manufacturers. The reaction products were resolved on denaturing polyacrylamide gels developed with a voltage gradient, which were fixed, dried, and autoradiographed. Band compressions were eliminated when necessary by using dITP instead of dGTP. IntelliGenetics software was used for data analysis. Clones trkB.FL, trkB.T1, and trkB.T2 were sequenced by a combination of shotgun strategy and synthetic oligonucleotide-directed sequencing of subclones in M13mp18 or M13mp19. Clone trkB.FL was sequenced in both directions multiple times, as were the unique portions of cDNAs trkB.T1 and trkB.T2. The regions of trkB.T1 and trkB.T2 that are identical with trkB.FL were sequenced completely in at least one direction. The sequences in Fig. 1, 6, and 7, have 13 bp omitted from each end of the cDNA inserts, which include the EcoRI cloning sites.

Northern analyses. Radiolabeled single-strand DNA probes were synthesized from M13 template with the Klenow fragment and with $[\alpha^{-32}P]dCTP$ (Amersham) (14). Enzymatic cleavage in the polylinker region or in the insert sequence at 6-bp recognition sites at the 3' end of the probe was followed by purification on a denaturing polyacrylamide gel. The gel containing the probe was excised and homogenized by centrifugation through 18-gauge needle holes in Eppendorf tubes. The polyacrylamide slurry was added directly to the hybridization solution.

Regions of cDNA trkB.FL subcloned into M13mp19 for DNA sequencing reactions were also used as templates for probe preparation. These antisense probes correspond to the following regions in the nucleotide sequence of cDNA trkB.FL: A, -657 to -298; B, 948 to 1251; C, 2243 to 2433; D, 2461 to 2667. Probes E and F were made by the polymerase chain reaction by using a Perkin-Elmer-Cetus thermal cycler and conditions suggested by Cetus with minor modifications. The antisense oligonucleotide primers contained an 18-nucleotide complementary sequence to the cDNA, an EcoRI restriction site, and a 4-nucleotide GC clamp (a short sequence that may allow better recognition of the ends of the polymerase chain reaction product by restriction enzyme). The sense primer was designed similarly by using a HindIII restriction site. The product of the polymerase chain reaction was purified, digested with EcoRI and HindIII, and ligated into HindIII-EcoRI-digested M13mp19. The final insert sequences were verified by sequencing and corresponded to nucleotides 1363 to 1694 in the trkB.T1 sequence for probe E and nucleotides 1361 to 1566 in the trkB.T2 sequence for probe F.

Total cellular RNA was prepared from adult male Sprague-Dawley rat tissues homogenized in a guanidinium thiocyanate solution (3), and 20 μ g of RNA per lane was resolved on agarose gels containing formaldehyde (23). Northern transfer of the RNA to Nytran (Schleicher & Schuell) membranes was accomplished by using a vacuum transfer apparatus (Pharmacia) and was followed by 3 min of UV cross-linking and 15 min of baking. The methylene blue staining pattern of the 18S and 28S rRNA bands indicated that the tissue Northern blots were loaded uniformly. All other hybridizations were done on replicate electrophoresis lanes of the same RNA preparations, in which the methylene blue staining pattern of the 18S and 28S rRNA bands was uniform. Prehybridization was carried out in $5 \times$ SSPE (1× SSPE is 150 mM NaCl, 10 mM sodium phosphate, and 1 mM EDTA, pH 7.4)-0.2% sodium dodecyl sulfate (SDS)-2× Denhardt solution (1 \times Denhardt solution is 0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin)-100 µg of salmon sperm DNA (sheared by sonication and denatured by boiling) per ml (31). Probes were added for hybridization at 65°C for 18 to 24 h at concentrations of 5×10^6 to 10×10^6 cpm/ml. Low-stringency washes were followed by a final high-stringency wash for 15 or 30 min in $0.2 \times$ SSPE-0.1% SDS preequilibrated to 65°C.

Nucleotide sequence accession numbers. Nucleotide sequence accession numbers assigned by GenBank are M55291, M55292, and M55293 for *trk*B.FL, *trk*B.T1, and *trk*B.T2, respectively.

RESULTS

Cloning trkB cDNAs. A λ ZAP adult rat cerebellar cDNA library (2.5 \times 10⁵ plaques) was screened with the HRD LAAR probe. Among 22 positive clones, nucleotide sequence analysis revealed that 1, trkB.P1, was a new putative receptor PTK and 9 were derived from the rat homolog of the human flg gene (29). The remaining clones did not fit the criteria for a PTK. Subsequently, it was found that flg encodes the bFGF receptor (18, 28, 30).

trkB.P1 contains several sequence motifs characteristic of PTKs, most notably a putative ATP-binding site sequence GXGXXG followed by AXK 20 residues downstream. In addition to the probe target sequence, which turned out to be HRDLATR, trkB.P1 contains a tripeptide motif, DFG, which is conserved in almost all protein kinases. A putative transmembrane region to the N-terminal side of the kinase domain indicated that the clone coded for a transmembrane protein, which, on the basis of precedents, is most likely to be a receptor. trkB.P1 was used to screen the cerebellar library (2 × 10⁶ plaques) at high stringency. Of 100 positive clones, 38 were plaque purified; the nucleotide sequence of one, a 4,757-bp cDNA, trkB.FL, was determined (Fig. 1).

Clone trkB.FL, in addition to containing the entire coding region of a putative growth factor receptor PTK, contains 697 bp of 5' untranslated (UT) region (Fig. 1). There is an in-frame stop codon 192 bp upstream from the first of two potential start codons, lying 10 codons apart, for the single long open reading frame (LORF). The second AUG is likely to be the start codon on the basis of the consensus for vertebrate start codons (17). The sequence of the N-terminal 20 amino acids of the protein initiated at the second AUG has characteristic features of a eucaryotic signal peptide (35). However, if the first AUG codon were used, the resulting 30-amino-acid signal peptide also fits the criteria of a signal peptide. The LORF of 2,430 bp beginning with the second AUG codes for a protein of 810 amino acids. Removal of the predicted signal peptide would convert the product to 790 amino acids with a mass of 88,629 Da. There are 12 potential N-linked glycosylation sites in the extracellular region. The 5' UT region also has two AUG codons out

-631 -526 -421 TGGCCAGGGCCGGCACTGTCCTGCTACCGCAGTTGCTCCCCAGCCCTGAGGTGCGCACCGATATCGATATCCGTGCCGGTTTAGCGGTTCTGCGACCCAAAGAGT -316 CCAGGGAGAGCCACCGAGTGGCGCCTGGCGTATAGGACCATGCAGCCGCCTTGTGGCTTGGAGCAGCGGCCCGTGATGTTCCAGCCACTGTGAACCATTTGGTCA -211 -106 -1 M S P W P R W H G P A M A R L W G L C L L V L G F W R A S L A C P M S C K C S T T R I W C T GAGCCTTCTCCTGGCATCGTGGCATTTCCGAGGTTGGAACCTAACAGCATTGACCCAGAGAACATCACCGAAATTCTCATTGCAAACCAGAAAAGGTTAGAAATC E P S P G I V A F P R L E P N S I D P E N I T E I L I A N Q K R L E I ATCAATGAAGATGATGTCGAAGCTTACGTGGGGCTGAAAAACCTTACAATTGTGGATTCCGGCTTAAAGTTTGTGGCTTACAAGGCGTTTCTGAAGAACGGCAAC NEDDVEAYVGLKNLTIVDSGLKFVAYKAFLKNGN CTGCGGCACATCAATTTCACTCGAAAACAAGCTGACGAGTTTGTCCAGGAGACATTTCCGCCACCTTGACTTGTCTGACCTGATCCTGACGGGTAATCCGTTCACG TRNKLTSLSRRHFRHLDLSDLILTGNP F Ι NF 175 D D S G K Q I S C V A E N L V G E D Q D S V N L T V H F A P T I S D H H W C I P F T V R G N P K P A L Q W F Y N G A I L Ρ V L K R E L<mark>G E G A F G K V</mark> F L A E C Y N L C P E Q D K I L V A ACGCTGAAGGACGCCAGCGACAATGCTCGCAAGGACTTTCATCGCGAAGCCGAGCTGCTGACCAACCTCCAGCACGAGCACATTGTCAAGTTCTACGGTGTCTGT LKDASDNARKDFHREAELLTNLQHEHIVKFYGV GTGGAGGGCGACCCACTCATCATGGTCTTTGAGTACATGAAGCACGGGGACCTCAACAAGTTCCTTAGGGCACACGGGCCAGATGCAGTGCTGATGGCAGAGGGT V E G D P L I M V F E Y M K H G D L N K F L R A H G P D A V L M A E G AACCCGCCCACCGAGCTGGCAGTGGCAGATGCTGCACATCGCTCAGCAAATCGCAGCATGGTCTACCTGGCATCCCAACACTTCGT<u>GCACCGAGACCTG</u> N P P T E L T Q S Q M L H I A Q Q I A A G M V Y L A S Q H F V H R D L <u>GCCACCCGGAA</u>CTGCTTGGTAGGAGAGAAACTGGTGGTGAAAATTGG<u>GGACTTCGG</u>GATGTCCCGGGATGTATACAGCACCGACTACTACCGGGTTGGTGGCCAC A T R N C L V G E N L L V K I G D F G M S R D V Y S T D Y Y R V G G H ACAATGTTGCCCATCCGATGGATGCCTCCAGAGAGGCATCATGTACAGGAAATTCACCACCGAGAGTGACGTCTGGAGCCTGGGAGATTGTGTGGGGAGATCTTC T M L P I R W M P P E S I M Y R K F T T E S D V W S L G V V L W E I F ACCTACGGCAAGCAGCCCTGGTATCAGCTATCAAACAACGAGGTGATAGAATGCATCACCCAGGGCAGAGTCCTTCAGCGGCCTCGCACGTGTCCCCAGGAGGTG G K O P W Y O L S N N E V I E C I T Q G R V L Q R P R T C P Q E 2730 TATTTATTATTGGTCTTCACTGCTTCATGGTCCTCGGCCTCTCTCCTTGACCGATCTGGCTTCTGTACTCCTGTACTGTACATAGACAAAGGCCTTAACAAA CTTCTTGTTGTTGTTGTTCCGGTGGTTTTAGCCTGTGTATGAGAAGGGAAAGTCATGTACAGTCTGGGAAAACTTTATCTGTGGGAAATGGAAACCAGAAGGGGAAAG ATGTTCGGCACTGACCCCCGAGGACCTTTCTGAGGAGGACACAGAATGTTAAACTCTGCATCATGGACACAGTTTCCGATCACAGATACTGGCCTTCAATGGAAA AAAAAAAAAAAAAAAAAACCCAGATAGTTCTTGTGAGACCTGGACAGCACGTCCAACATCCAGACATTGTGGTCGGGCACAGTGACAGAGTTGATGCATTTCTCACGG GTTATTCTACAGAGCTTTTGTCAAGTCCAATGGAAGGAGGTAGATTCTTGTTCAGATATGATTTCGGGAAAAACCGAGTCCTTGACAAAGACAGGAGACACCCTC AACCCACCGGGCTGCACAGGGGACAGGCACAGGCCACCCCTGAGGGACAGGGAAGCTCTCTTGGGATACCACCTGAGTTTACATTCAGTGTGCTCAGGTCAAGTC TCTCGCTCGGGGGCTCTGTTTCGGGGGGGGGAGAATGGTTTCATTCCAACGCACTCATTATCAGGATTCTGTTTTC

FIG. 1. trkB full-length PTK (trkB.FL) nucleotide sequence (4,757 bp) and deduced amino acid sequence (810 amino acids) of the LORF (2,430 bp). In the amino acid sequence, the predicted initiating methionine and signal peptide are given bold underlines, the potential glycosylation sites are overlined, the predicted transmembrane domain is indicated by inverse lettering, the kinase domain is enclosed by overline arrows, and five distinctive sequence motifs conserved among PTKs are boxed. In the nucleotide sequence, two ATTT repeat sequences in the 3' UT region which resemble AUUUA boxes are underlined.

of frame, which are followed by a stop codon upstream of the putative start codons. Although this is unusual, several proto-oncogene mRNAs have upstream AUGs (17). The cDNA also contains a 1,630-bp 3' UT region, which lacks a poly(A) tract or a recognizable poly(A) addition sequence near the 3' end. The cDNA contains two regions rich in ATTT repeats, which resemble AUUUA boxes. AUUUA repeating sequences are found in the 3' UT regions of some mRNAs, such as granulocyte-macrophage colony-stimulating factor mRNA, with short half-lives (33). However, it would be unusual for a growth factor receptor mRNA to have a short half-life.

Analysis of the protein sequence data base indicated that trkB.FL has an amino acid sequence identity of 37% in the extracellular region and 75% in the kinase domain with the human proto-oncogene product trk (Fig. 2) (22). All PTKs exhibit amino acid sequence similarity in their kinase domains. However, only closely related subfamily members exhibit sequence similarity in the extracellular region, typically in the range of 35 to 45% sequence identity, such as c-erbB-2 (HER 2) and c-erbB (4, 38) or the insulin and the insulinlike growth factor-1 receptors (34). Therefore, like Klein et al. (16), we have named the gene trkB, since there is a notable similarity in the amino acid sequences of their respective extracellular regions. Rat trkB and mouse trkB have 99% amino acid sequence identity.

Northern analysis of trkB. Northern hybridization to rat cerebellar or cerebral mRNA with trkB probes indicates a perplexing number of mRNAs apparently expressed from the trkB locus. Although similar patterns of trkB mRNA expression were observed in both cerebral and cerebellar RNA preparations, trkB is expressed at a higher level in the cerebrum (data not shown). To delineate structural differences in the coding sequences of these mRNAs, we performed Northern hybridization of rat cerebral RNA with a variety of trkB probes corresponding to 5' UT, coding, and 3' UT regions (Fig. 3). Probes B and C, which correspond to the extracellular region and the kinase domain, respectively, detect overlapping sets of bands. It is striking that the kinase domain probe C hybridizes only to a subset of the mRNAs to which the extracellular domain probe B hybridizes. It is also evident that some of the mRNAs are not large enough to encode the full-length trkB PTK, which requires 2.4 kb of coding sequence alone. These results suggest that some of the mRNAs encode a C-terminally truncated receptor lacking the kinase domain. mRNAs of 9.0 and 4.8 kb (there may be more than one mRNA of 4.8 kb) are detected with both probes B and C; this indicates that they could encode a full-length receptor PTK. Because probe D, which corresponds to the 3' UT region of trkB.FL, hybridizes to mRNAs of 4.8 and 1.6 kb, it is most probable that cDNA trkB.FL is derived from the 4.8-kb mRNA. On the other hand, prominent bands at 7.5, 7.0, and 2.4 kb are detected with probe B, but not probe C. These mRNAs could encode C-terminally truncated receptors. Probe A, which is complementary to the 5' UT region of trkB.FL, detects bands at 9.0, 7.5, 4.8, and 2.4 kb. The mRNAs of 7.0, 1.6, 0.9, and 0.7 kb were not detected with probe A.

The expression of trkB mRNAs in a variety of tissues was examined (Fig. 4). trkB is expressed predominantly in the brain. There are low levels of the 2.4-kb transcript in other tissues, most notably the spleen. Both brain and testis tissues express a 1.8-kb mRNA. Submaxillary gland tissue expresses only a 0.9-kb mRNA.

In summary, our analysis indicates there are three classes of mRNA (Fig. 5): a group that encodes the full-length receptor PTK (9.0- and 4.8-kb mRNAs), a group that contains the extracellular region but not the kinase domain (7.5-, 7.0-, 2.4-, 0.9-, and 0.7-kb mRNAs), and one that encodes the kinase domain only (1.6-kb mRNA). As the final washes of the Northern blots were at high stringency, it is probable that the mRNAs detected are derived from the *trkB* locus. Also, since most of the mRNAs are also detected by probes from either 5' or 3' UT regions, it seems improbable that there is a very closely related gene expressing mRNAs which cross-hybridize to *trkB* probes.

Cloning of cDNAs for truncated receptors. Northern analyses indicated that trkB expresses mRNAs encoding trkB products that lack the kinase domain, most probably resulting from C-terminal truncations. Because the initial trkB cDNA, trkB.P1, used as a probe to screen the λ ZAP cDNA library contained parts of both the extracellular region and the kinase domain, the 38 clones isolated with this probe could represent mRNAs encoding both full-length and truncated receptors. Southern hybridization with probes B and C were used to find clones which encoded the extracellular region, but not the kinase domain. Several such clones were identified. The complete nucleotide sequence of a 2,249-bp clone, trkB.T1, indicates that it encodes a C-terminally truncated receptor, which lacks the catalytic domain but contains the membrane-spanning region (Fig. 5 and 6). The protein product is predicted to have a complete extracellular region and transmembrane domain with a short (23-aminoacid) cytoplasmic tail in which the final 11 amino acids differ from the corresponding region of the full-length receptor PTK. Southern hybridization with a probe (probe E) derived from the putative alternate 3' exon(s) indicated that 12 of the 38 trkB cDNA clones had this structure. Southern analysis of the 38 trkB cDNAs with probes A to E also indicated that there were other trkB cDNAs which could not be accounted for by the structures represented by trkB.FL or trkB.T1. The nucleotide sequence of one of these cDNAs, trkB.T2, a 3,991-bp clone, indicates that it would also code for a truncated receptor, which is spliced at the same site as trkB.T1 but has a different cytoplasmic tail (Fig. 5 and 7). The predicted cytoplasmic domain of trkB.T2 is 21 amino acids, the last 9 of which differ from the corresponding region in trkB.FL or trkB.T1. Using a probe (probe F) corresponding to the splice junction that gives rise to the short alternate C-terminal tail in cDNA trkB.T2 and part of the adjacent 3' UT region, we screened the original 38 trkB cDNAs. Probe F hybridized to five cDNAs, including trkB.T2, which is a lower frequency than we found with probe E. These frequencies suggest that trkB.T1 is likely to represent the predominant trkB mRNAs that encode truncated receptors. Four of the cDNAs that hybridize to the trkB.T2-specific probe F have different sizes, which clearly indicates independent origin in the cDNA library. It is therefore highly probable that cDNA trkB.T2 is derived from a genuine trkB mRNA.

To assign which mRNAs clone trkB.T1 might be derived from, we carried out Northern hybridization with probe E, which hybridizes to the exon(s) on the 3' side of the splice site (Fig. 3). Analysis of cerebral RNA with probe E indicates that the prominent trkB mRNAs of 2.4, 7.0, and 7.5 kb belong to this class. These mRNAs do not hybridize to probe C or D, which correspond to the kinase domain and 3' UT region of trkB.FL, respectively. Since trkB.T1 is 2,249 bp and does not have a poly(A) tail or poly(A) addition sequence, it could be derived from the 2.4-, 7.0-, or 7.5-kb mRNAs. Probe A, which hybridizes to the 5' UT region of trkB.FL, detects the 9.0-, 7.5-, 4.8-, and 2.4-kb mRNAs, but



FIG. 2. (a) Amino acid sequence alignment of rat trkB and human trk (22). Sequence identity is indicated by inverse lettering. (b) Schematic representation depicting the amino acid sequence identity between different domains of trkB and trk.



FIG. 3. (a) Northern hybridization with various trkB probes of 20 μ g of total cellular RNA isolated from adult rat cerebrum resolved on replicate gels. Sizes were determined by using the 28S and 18S rRNAs as internal markers. (b) Schematic representation of the origins of the probes in the trkB.FL and trkB.Tl cDNAs.

not the 7.0-kb mRNA. This suggests that the 7.0-kb mRNA has a different 5' UT region from that of the other mRNAs. However, trkB.T2 is not derived from any of the prominent mRNAs detected in Northern analysis, as it was not detectable upon Northern hybridization of probe F with either cerebral or cerebellar total RNA preparations. It is likely that this trkB mRNA is expressed in limited regions of the brain.

DISCUSSION

The human trk proto-oncogene and rat trkB gene products do not have any sequence similarity to other known PTKs in the extracellular region. The conserved extracellular cysteines of trk and trkB are dispersed rather than grouped in cysteine boxes, and neither *trk* or *trk*B has immunoglobulinlike repeats. On the basis of sequence homology in the kinase domains, *trk* and *trk*B are members of the insulin receptor subfamily of PTKs and have two vicinal tyrosine residues at the site homologous with the autophosphorylation site in other PTKs (Tyr-416 in pp60^{c-src}). Both *trk* and *trk*B have short (8-amino-acid) C-terminal tails beyond the kinase domain containing a conserved tyrosine.

While our work was in progress, Klein et al. (15, 16) reported that the murine *trkB* locus also expresses multiple mRNAs similar to the multiple rat *trkB* mRNAs in this report. Mouse tissues also contain mRNAs that encode both full-length and C-terminally truncated receptors. Specifically, there are rat mRNAs encoding full-length receptor PTKs of 9.0 and 4.8 kb corresponding to the mouse mRNAs



mRNA Size

FIG. 4. Northern hybridization of 20 μ g of total cellular RNA from various tissues with *trkB* probes B and C simultaneously, which in combination recognize both the extracellular and kinase domains. RNAs were isolated from adult rat tissues, except thymus RNA, which was isolated from juvenile rat tissues. The lanes were loaded with 20 μ g of total cellular RNA isolated from heart (H), skeletal muscle (Sk), brain (B), intestines (I), kidney (K), spleen (Sp), submaxillary gland (Sm), lung (Lu), liver (Li), testes (Te), thymus (Th), and cerebrum (C).

of 9.0 and 5.0 kb, respectively. In rat tissues there are mRNAs encoding C-terminally truncated receptors of 7.5, 7.0, and 2.4 kb, whereas in mouse tissues there are mRNAs encoding C-terminally truncated receptors of 7.2, 7.0, 2.5, and 2.0 kb. Rat *trk*B.FL corresponds to the mouse clone pFRK43, both of which encode a full-length receptor PTK. Rat *trk*B.T1 corresponds to the mouse clone pFRK42, both of which encode a C-terminally truncated receptor. The short cytoplasmic tails of the truncated *trk*B receptors predicted by *trk*B.T1 and pFRK42 are identical in amino acid

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Predicted	Protein	Structure

	A	B	<u>C</u>	D	E	
9.0 kb	+	+	+	-	-	Receptor PTK
7.5 kb	+	+	-	-	+	Truncated Receptor
7.0 kb	-	+	-	-	+	Truncated Receptor
4.8 kb	+	+	+	+	?	Receptor PTK
2.4 kb	+	+	-	-	+	Truncated Receptor
1.8 kb	+	+	-	-	-	Truncated Receptor
1.6 kb	-	-	+	+	-	Truncated Kinase
0.9 kb	-	+	-	-	-	Truncated Receptor
0.7 kb	-	+	-	-	_	Truncated Receptor

Probe

b)



FIG. 5. (a) Summary of Northern hybridization data. (b) Schematic representation of *trkB* cDNAs. An expansion depicting the amino acid sequences of the splice junction region of *trkB*.P1, *trkB*.FL, *trkB*.T1, and *trkB*.T2 is shown.

														(ĊTG	СТС	ссто	GCGO	CTG	GCT/	ACGO	GAG	GCC	CGGG	66G/	AGC	CGC	GCC	GAC	AGT	ссто	CTG	-421
TGGCCAG	GGGCC	GGC	ACTO	TCC	TGC	TAC	CGC	AGT	rgc'	TCC	CCA	GCC	CTG	AGG	TGC	GCA	CCG/	ATA1	rcg/	ATA	TCCC	atec	CGe	STT	AG	Cee.	TTC [.]	TGC	GAC	CCA	AAG/	AGT	-316
CCAGGG/	AGAGC	CAC	CGAG	GTGG	CGC	CTG	GCG	TAT/	AGG/	ACCI	ATG	CAG	CCG	CCT	TGT	GGC	TTG	GAG(CAG	CGGG	CCCG	GTG/	\TG1	TTC	CAG	CCA	CTG.	TGA	ACC	ATT'	tee.	TCA	-211
GCGCCA/	ACCTE	ictc	AGCO	CCA	GCA	CCG	ACA	GGC1	FCA	GCC.	TCT	GGT /	ACG	CTC	CTC	TCG	GCG(GGA(GGC(CAT	CAG	CACO	CAA	GCA	SCA	AGA	GGG	CTC	AGG	GAA	GGC	CTC	-106
CCCCCTC	CCGGC	GGG	GGAC	CGCC	TGG	CTC	AGC	GTAG	GGG	ACA	CGC	ACT	CTG	ACT	GAC	TGG	CAC	FGG	CAG	CTC	GGG/	ATGI	rcg	CCC	rgg(CCG	AGG	TGG	CAT	GGA	CCC	GCC	-1
																					- 1	4 5	5 1	P 1	1	P	R 1	W	H (G I	P	A	
ATGGCGC	CGGC1	CTG	GGGG	CTTA	TGC	TTG	CTG	GTCI	FTG	GGC.	TTC	TGG	AGG	GCT	TCT	CTT	GCC.	TGCO	CCC/	ATG	TCC	rgc/	444	TGC/	AGC	ACC	ACT	AGG	ATT	TGG	TGT	ACC	105
MAF	λL	W	G	L	C	L	ι '	V I	L	GI	F 1	W I	R.	A _:	S	L	<u>A</u> (C 1	PI	M (S (C 1	K (C :	5	T	T I	R	I	W (C '	Т	35
GAGCCT	CTC	TGG	CAT	GTG	GCA	TTT	CCG	AGG	ITG	GAA	CCT	AAC	AGC	ATT(GAC	CCA	<u>G</u> ag/	AAC/	ATC/	ACCO	GAA	ATTO	CTC	ATT	GCA	AAC	CAG	AAA	AGG	TTA	GAA	ATC	210
E P S	5 P	G	I	V	A	F	P	RI	LI	E I	Ρ	N :	S	I	D	P	E 1	N C		T I	E 🛛	I I	L	I	A I	N I	Q	K	R	L	E	I	70
ATCAATO	Gaag/	TGA	TGT	CGAA	GCT	TAC	GTG	GGGG	CTG	AAA	AAC	CTT	ACA	ATT	GTG	GAT	TCC	GGC ⁻	TTA	AAG	TTT	GTG	GCT	TAC	AAG	GCG	ÌΠ	CTG	AAG	AAC	GGC	AAC	315
INE	E D	D	V	Ε	A	Y	V	GΙ	LI	K 1	N	L	T	I	V	D	S (G I	LI	K I	F۱	VI	A '	Y	K	A	F	L	K	N	G	N	105
CTGCGGG	CACAT	CAA	TTT	CACT	CGA	AAC	AAG	CTG/	ACG	AGT	TTG	TCC	AGG	AGA	CAT	TTC	CGC	CAC	CTT	GAC'	TTG	TCTO	GAC	CTG	ATC	CTG	ACG	ĠGT	AAT	CCG	ŤTC	ACG	420
LRH	1 I	N	F	Т	R	N	K	Ľ	T :	S I	L	S I	R	R I	H	F	R I	H 1	LI	DI	L	SI	D	Ĺ	I	Ĺ	T	G	N	P	F	T	140
TGTTCCT	rgtg/	ACAT	CAT	GTGG	icto	AAG	ACT	СТС	CAG	GAG	ACG	AAA	TCC	AGC	CCC	GAC	ACT	CAG	GAT'	TTG	TAT	TGC	CTC	AAT	GAG	ĀGC	ÁGC	ĀAG	AAT	ACC	CCT	CTG	525
C S (C D	Ι	M	W	L	ĸ	Т	L (Q	E	Т	K :	S	S	P	D	Т (QI	D 1	Ľ	Y (CI	L 1	N	E :	5	S	K	N	T	Ρ	L	175
GCGAACO	CTGC/	\GAT	TCC	CAAT	TGT	GGT	CTG	CCG ⁻	İ CT	GCA	CGT	CTG	GCC	GCT	ССТ	AAC	СТС	ÁCGI	GTG	GAG	GAA	GGG/	AAG	TCT	GTG	ACC	ÂTT	TCC	TGC	AGC	GTC	GGG	630
A N L	LQ	Ι	Ρ	N	С	G	L	P :	S.	A I	R	L	A	A	P	N		T۱	V I	E I	E (GI	K	S	V	T	I	S	C	S	V	G	210
GGTGACO	CCGČI	гссс	CAC	CTTG	TAC	TGG	GAC	GTT	GGG	AAT	TTG	GTT	TCC	AAA	CAC	ATG	AAT	GAA	ACA	AGC	CAC	ACA	CAG	GGC	TCC	TTA	AGG	ÂTA	ACA	AAC	ATT	TCA	735
GDI	ΡL	Ρ	Т	L	Y	W	D	V (G	N I	L	V	S	K	H	M	N	E	T :	S I	H '	T (0 (G	S	L	R	I	T	N	I	3	245
TCGGAT	GACA	GTGG	GAA	ACAA	ATC	TCT	TGT	GTG	GCA	GAA	AAC	CTC	GTC	GGA	GAA	GAT	CAA	GAC'	TCT	GTG	AAC	CTC	ÀCT(GTG	CAT	TTT	GCA	CCA	ACC	ATC	ACA	TTT	840
S D I	DS	G	K	Q	I	S	C	V I	A	ΕI	N	L	V	G	E	D	Q	D :	S '	v 1	N	L	T	V	H	F	A	P	T	I	Т	F	280
CTCGAA	TCTC	CAAC	CTC	AĠAC	CAC	CAC	TGG	TGC	ATC	CCA	TTC	ACT	GTG	AGA	GGC	AAC	CCC.	AAG	CCA	GCA	CTT	CAG	TGG	TTC	TAC	AAC	GGA	GCC	ATA	CTG	AAT	GAA	945
LES	S P	T	S	D	Н	H	W	C	I	P	F	T	V	R 🕆	6	N	P	K	P /	A I	L	0 1	W	F	Ŷ	N	G	A	I	L	N	Ē	315
TCCAAG	TACA	ICTG	TAC	CAAA	ATA	ACA(GTC	ACC	AAT	CAC	ACG	GAG	TAC	CAC	GGC	TGC	CTC	CAG	CTG	GAT	AAC	ČCC,	ACT	CAT	ATG	AAT	ĀAT	GGA	ĠAC	:TAC	ACC	CTA .	1050
<u>5</u> K '	ΥI	C	Т	K	I	H	V	TÌ	N	H	T	E	Y	H	G	C	L	0	LI	DÌ	N	P	Т	H	M	N	N	G	D	Y	Т	L	350
ATGGCC	AAGA	ATGA	ATA	TGGG	SAAG	GAC	GAG	AGA	CAG	IATT	TCT	GCT	CAC	TTC	ATG	GGC	CGG	ĊCT	GGA	GTT	GAC	TAT	GAG	ACA	AAC	CCA	AAT	TAC	CCT	GAA	GTC	CTC	1155
MA	K N	Ε	Y	G	Κ	D	Ε	R	Q	I	S	A	H	F	M	G	R	P (G	V	D	ΥI	E	T	N	Ρ	N	Y	Ρ	Ē	V	L	385
TATGAA	GACT	GGAC	CAC	GCC/	AC1	GAC	CATC	GGG	ĠAT	ACT	ACA	AAC	AAA	AGT	AAT	GAG	ATC	CCC	TCC	ACG	GAT	GTT	GCT	GAC	CAA	ACC	AAT	CGG	GAG	GCAT	СТС	CTCG	1260
YEI	DW	T	T	Ρ	T	D	I	G	D	T	T	N	K	5	N	Ε	I	P :	S	T	D	V I	Ā	D	0	T	N	R	E	н	L	S	420
GTCTAT	GCTG	TGGT	GGT	GAT1	rgco	CTCI	IGTG	GTA	GGÁ	TTC	TGC	CTG	CTG	GTG	ATG	CTG	CTT	CTG	СТС	AAG	TTG	GCG	AGA	ĊAT	ÌCC	:AAG	ÎTT	GGC	CĀTE	GAAA	GGT	TTT	1365
V Y	A V	V	V	Ι	A	S	V	V	G	F	С	L	L	V	M	L	_	Ľ	L	K	L	A	R	H	Ś	K	F	G	M	K	G	F	455
GTTTTG	TTTC	ATA/	GAT		CCTO	GGA1	rggg	ITAG	CTG	GAGA	TÂĂ	AGG	AAA	GAC	AAG	igct	GGG	GCT	GTG	CTG	ĊTT	GGT	GCT	TGG	ČGC	222:	GTG	iĀGO	CTG/	AACT	IČCI	GGG	1470
V L	FH	K	Ι	Ρ	L	D	G	*																									465
ACTACT	GTTG	CCT/	TČC	CAGO	SAAG	STG	CTGC	TTA	TTT	GGGG	GGT	GTC	TGG	TGG	AAA	TGG	GTG	TTC	TCC	AAA	GAT	GTC	CGC	AGC	CTG	стт	GTT	GTG	GAGO	CTGT	GAC	CTGG	1575
GGAACC	CCAA	GGC/	GAG	GCA	GGGG	GTC/	AGGC	AGC	TGA	GAA	GCA	GCA	GAA	GAA	CAC	ACT	TAG	ATT	CĂŤ	CTT	CTG	TTC	TTA	CAA	TAG	CTC		TAT	FAG/	AATO	AA.	AGTG	1680
AAATCT	CATT	GGAT	TGT	GCC	ГСТО	CTA	ATGA	AAA	ATG	TGC	TGT	TTG	ACT	ATA	TGG	GAA	ATG	TGC	TGA	CAT	TAA	TTG	СТТ	CTG	TTT	ATT		GGI	TGA				1775
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FIG. 6. trkB.T1 nucleotide sequence (2,249 bp) and deduced amino acid sequence (465 amino acids). In the amino acid sequence, the predicted initiating methionine and signal peptide are given bold underlines, the potential glycosylation sites are overlined, the predicted transmembrane domain is indicated by inverse lettering, and the short cytoplasmic tail unique to trkB.T1 is underlined.

sequence, although there is one base change. Klein et al. (15) observed that the murine 8.2- and 2.5-kb mRNAs have a similar 5' UT region, whereas 8.0- and 2.0-kb mRNAs have a different 5' UT region. We found that probe A hybridizes to the 7.5- and 2.4-kb mRNAs, but not the 7.0-kb mRNA. These observations hint that *trkB* may have two promoters that would generate two different 5' UT regions. However, one striking difference between the mouse and the rat is that the small rat mRNAs (1.6, 0.9, and 0.7 kb) detected with the extracellular domain probe B were not detected in mouse mRNA.

Because the *trk*B.T1 and *trk*B.T2 cDNAs diverge from *trk*B.FL at an identical site in their respective sequences, it is likely that they arise from alternative splicing of *trk*B mRNAs. There are at least two products of c-*src* generated by neural specific alternative splicing. In addition to $pp60^{c-src}$, there is another product of c-*src* with a 6-amino-acid insert in the N-terminal region (20, 25). There is yet another c-*src* mRNA with an additional 33-bp exon that is used in conjunction with the 18-bp alternative exon, which gives rise to a c-*src* product with a 17-amino-acid insert (26). The function of these inserts in c-*src* products is unknown.

trk mRNA is expressed in restricted regions of mouse brain, specifically trigeminal, superior, jugular, and dorsal root ganglia (21). Klein et al. (15) have found that the mouse trkB mRNA encoding a full-length PTK is expressed in the cerebral cortex and the pyramidal cell layer of the hippocampus. trkB mRNA encoding a truncated receptor was observed in the ependymal linings of the cerebral ventricles and the choroid plexus. Expression of trkB is detected in the central and peripheral nervous systems during embryogenesis (16). The trk subfamily of PTKs must have roles restricted to signal transduction in the nervous system.

A striking feature of trkB is the plethora of mRNAs expressed. This paper has presented evidence for both a full-length receptor PTK and two C-terminally truncated receptors that contain the putative ligand-binding domain, the transmembrane domain, and different short cytoplasmic tails. Klein et al. (15) have identified in mouse brain both a full-length receptor trkB protein that has PTK activity and a truncated receptor that, as expected, lacks PTK activity. In rat tissue, there is also a 1.6-kb mRNA that could encode a trkB protein lacking the extracellular domain. As this mRNA hybridizes with a 3' UT probe (D) derived from the cDNA encoding the full-length receptor PTK, it is likely to be a trkB transcript as opposed to an mRNA derived from a related gene. On the basis of its size, it seems likely that this 1.6-kb mRNA encodes either a soluble PTK or a membraneassociated PTK similar to the src family kinases. There are two small mRNAs (0.7 and 0.9 kb) that hybridize to an extracellular region probe. However, since these mRNAs did not hybridize with any of the 5' or 3' UT probes described in this paper, it is possible that they are derived from a gene closely related to trkB. Nonetheless, as these mRNAs are just large enough to encode most of the extracellular region of the trkB product, a key question is whether

CAGCACCAAGCAGCAAGAGGGCTCAGGGAAGGCCTC -106

CCCCCTCCGGCGGGGGACGCCTGGCTCAGCGTAGGGACACGCACTCTGACTGGCACTGGCAGCGCGGGATGTCGCCCTGGCCGAGGTGGCATGGACCCGCC -1 M S P W P R W H G P A

105 M A R L W G L C L L V L G F W R A S L A C P M S C K C S T T R I W C T GAGCCTTCTCCTGGCATCGTGGCATTTCCGAGGTTGGAACCTAACAGCATTGACCCAGAGAACATCACCGAAAATTCTCATTGCAAACCAGAAAAGGTTAGAAATC 35 210 E P S P G I V A F P R L E P N S I D P E N I T E I L I A N Q K R L E I ATCAATGAAGATGATGTCGAAGCTTACGTGGGGCTGAAAAAACCTTACAATTGTGGATTCCGGCTTAAAGTTTGTGGCTTACAAGGCGTTTCTGAAGAACGGCAAC 70 315 I N E D D V E A Y V G L K N L T I V D S G L K F V A Y K A F L K N G N CTGCGGCACATCAATTTCACTCGAAAACAAGCTGACGAGTTTGTCCAGGAGACATTTCCGCCACCTTGACTTGTCTGACCTGATCCTGACCGGATAATCCGTTCACG 105 420 L R H I N F T R N K L T S L S R R H F R H L D L S D L I L T G N P F T TGTTCCTGTGACATCATGTGGCTCAAGACTCTCCAGGAGACGAAATCCAGCCCCGACACTCAGGATTTGTATTGCCTCAATGAGAGGCAGCAAGAATACCCCTCTG 140 525 175 C S C D I M W L K T L Q E T K S S P D T Q D L Y C L N E S S K N T P L GCGAACCTGCAGATTCCCAATTGTGGTCTGCCGTCTGCACGTCTGGCCGCTCCT<u>AACCTCA</u>CGGTGGAGGAAGGGAAGTCTGTGACCATTTCCTGCAGCGTCGGG 630 O I P N C G L P S A R L A A P N L T V E E G K S V T I S C S 210 735 G D P L P T L Y W D V G N L V S K H M N E T S H T Q G S L R I T N I S TCGGATGACAGTGGGAAACAAATCTCTTGTGTGGCAGAAAACCTCGTCGAGAAGAATCAAGACTCTGTG<u>AACCTCA</u>CTGTGCATTTTGCACCAACCATCACATTT 245 840 280 SGKQISC V A E N L V G E D Q D S V N L **T** V H F A P DD 945 L E S P T S D H H W C I P F T V R G N P K P A L Q W F Y N G A I L N E TCCAAGTACATCTGTACCAAAATACACGTCACCACAACAGGAGTACCACGGCTGCCTCCAGCTGGATAACCCCACTCATATGAATAATGGAGACTACACCCCA 315 1050 KYICTKIHVT NHTEYHGCLQLD NPTHMNNGDYT 350 2 1155 NEYGKDERQISAHFMGRPGVDYETNPNYPEVL 385 1260 420 1365 455 1470 Q K C A Y F A S * 463 GAAGGCTAAAAAAAAAAAGCAGCAAGGATATGATACCACAAAACCAAGACAGAATAAACTCAGGATTACTCAGCCTTGCCCACTTTCCCGTGAGCGCGGGTTGTCCCA 1575 1680 GGAGGCACGGGCTGGTTTCTCCAGTGTGGACTGGCTCAGCAGCGAGGGCCTGGGGCAGTGCTTGAGTGGTCCTGTGGCAATCATGGGCAGGTAAGGCACAGGGGC AAGGATATGTTATGGCCGTGCGCATGCGTGCTCTTTTCTGCTGGACGTGGAGATTCTAAGTCTCTTTCCTGCTTTTTTTGCTGTTACTTTTCTGTATTCTCACC 1785 TTTTGAAAATTATCCAACCATCACTGTCAGAAGCAGAACAAGAAAAAGCCACCTTGGCACACTGAGGCTGTACGCGCATGCAGATAGGTTTTTATTGAAGTGTCTC 1890 1995 CTCTTATATAAAAAAAGAAGCGTTCCGGGGACATTGAAGCGTTTGCTGCGACGTTTAAGCTATTACGTATCAGCTATTACGAGGTCACTGTATTTTGCATGGTCT 2100 CTGACAGCTCGACACTGTAAATGCCTAGAGGGGAAGACTCTAAGCAGGCTTGGTGGTGACCTGTGTGGAGGATCGGGTCTGTGGGGTGCCTTTATCTGTAATGTAA 2205 2310 AGTCAGAGGTGACAGGATAGAACTCTTCCGGTCTTGTCTGGCTGCCTCTGAGCCTGTGCACCTCCTTAGGAGAAAGGCTCCTTGGCTCTAGTAAGACCCCGAGAGA 2415 AGGAGTGTACCCAGTGACTGAAGTCATTGTGAAGTGGCTTCTCTAACTGAGGGCCAAAGACCCACAGGGAAGCAAGGATTGCAGAGCTTCCCCCAGAATATGTGT 2520 GGTTCCAGAAATTGGCATCTGAACAGTTTCCAGGCGTGGAAACTGCCACTCATGCCACTGTTACCCAAGTCCTGAGAATCACGACTCCTTTGTCGTGAGTATGGC 2625 CTGCCACACCTTTCCCCTACTGTGCATGGTGGGGTTGCCATGCAAACCCAAACTCCGCCATTTCTCCCCCAAGCCAGGCTGGACTAGCTGTCTGCTCACCGTGC 2730 GCTGGGTACCTACCATGGACATCTTCGTGCCTTAGCCTCGTCAGGTGCTTTATTTCCTTGGGGACATTTTGAGCATGTGTCTTGTCCCCAAGCACAGTTTGGACT 2835 GGAGAATATCAGGTTCGTGAGACTGAATTTCCTGTCTTGAAGGGTTTATCTTGAGACTTTTCATCTGTGGATTCTCCTGAGAAAGGCTACGTTCAGGCCCCTCCA 2940 GGAGTTCACTAGAGGCGAAAGTCTAGGAAGCCTTCTTGTTACGGTCCAGGGATGATAGGTGGACAGAGGAAGTGCCTATAAGTGGCAATCTCCCCAGTGGTGCAGT 3045 GGAAGGGAAACCAAAAGGTAGACATTGTTTCCAAGGCTGCATCCTGCATGGAACCCTCAGACCTCACGCAGTTATTTTAAGTGAACAACAGACTATAACCACCCTA 3150 GCCCCTGCGTGTTCATTCTTAATGAACCGGAAGAAGTCGCTTAGCCTTGAGTGTGGCAGCCAATGACTCTTCCAGGCAGTAACGACTGTGGTGTGTTTTTCTGTC 3255 TTAGTCCTAAACATAAATAAAACTTACATCTGCAATGCTGTCTCAGAGACGCGTGCTCTCGTCCGGAGCAGATGGGCTCCGTTTTCTTTGTTTTCAGCCGCGTGT 3360 TAGTTAGTCTGTGGCGTGGTCTGTGCCCGGTTGACCCCCTGACCCCGAGGTTATCACTTAACAAGTCAGATGCTGAGTCCACCATCATCTTTGGGGATCTAGAAATT 3465 AGGATGGGGCTCATGGATTAAGCACAAAACAAAAACCGAACAAAAGGCGCTGTTTAAAAATTAATGTGAGTTAATGCACTTTCAGTGCCATTAGAGCAAGGGTCGCG 3570 CTCAAAATGTATGCCCTAAAACGGGAAGACTGTGCAAACATTATACTCCTGAAATAGACGGTGCGCCAGTCAGAGTTTCTCAAGAGCAATAGAAGAAACCGCGCA 3675 3780 TGACTAAACGGGGGCTGGGGATTTAGCTCAGTGGTAGAGCGCCTTACCTAGGAAGCGCAAGGCCCGGGGTT 3850

FIG. 7. trkB.T2 nucleotide sequence (3,991 bp) and deduced amino acid sequence (463 amino acids) of the LORF (1,389 bp). In the amino acid sequence, the predicted initiating methionine and signal peptide are given bold underlines, the potential glycosylation sites are overlined, the predicted transmembrane domain is indicated by inverse lettering, and the short cytoplasmic tail unique to trkB.T2 is underlined.

they have a transmembrane domain. If the proteins encoded by these small mRNAs lack a membrane anchor, they could be secreted receptors. It is intriguing that the 0.9-kb trkBmRNA is expressed in submaxillary gland, since both nerve growth factor and epidermal growth factor were originally isolated from this tissue.

trkB is one of a growing set of receptors that are expressed in alternative forms. In embryonic stem cells, the predominant mRNA from the platelet-derived growth factor β receptor locus is a 4.2-kb mRNA rather than the 5.3-kb mRNA expressed in fibroblasts and other cell types (36). This mRNA lacks the first five exons present in the 5.3-kb mRNA, thus encoding an N-terminally truncated protein still containing both a transmembrane domain and a kinase domain. It is possible that a constitutively active truncated receptor is expressed early in development that is not responsive to platelet-derived growth factor, whereas later in development, a platelet-derived growth factor-regulated receptor is expressed. This model is based on the precedent that the oncogenically active v-*erbB* is an N-terminally truncated form of the epidermal growth factor receptor. In the case of the epidermal growth factor receptor, a 2.7-kb mRNA expressed in rat liver is predicted to encode a C-terminally truncated protein containing the extracellular region but lacking both the transmembrane and kinase domains (24). The predicted 95-kDa protein is secreted by a continuous line of rat hepatic epithelial cells, WB. The bFGF receptor is expressed in two forms in developing mouse brain (28). The alternate form found in the nervous system differs by a deletion of 89 amino acids in the extracellular region of the FGF receptor. In the case of the atrial natriuretic peptide, there are two distinct receptor genes. There is a high-molecular-weight receptor with atrial natriuretic peptide-activated guanylate cyclase activity (2) and a low-molecular-weight atrial natriuretic peptide receptor that may be a clearance receptor (8). Cultured Schwann cells shed a truncated receptor for nerve growth factor receptor can be detected in rat urine. Levels of the soluble nerve growth factor receptor, which may arise from proteolytic cleavage of the receptor, are developmentally regulated.

What roles might C-terminally truncated trkB receptors play? A truncated receptor could be a clearance receptor. trkB.T2 encodes a protein with a tyrosine in the cytoplasmic C tail, which could be involved in internalization of the receptor. Complex roles for the truncated receptors could be postulated involving regulation of PTK activity based on formation of heterodimers between the truncated and the full-length receptors or buffered diffusion of the trkB receptor ligand. However, the simplest possibility is that the truncated receptors could be cellular adhesion molecules. The ligand could be immobilized in the extracellular matrix or could be another membrane protein.

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