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*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, *trk*B, 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 *trk*B gene is expressed predominantly in the brain and that *trk*B 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 *trk*B 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 protein-tyrosine 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 D*abl* 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 D*abl* and *fas*I display a defect in growth cone guidance, although mutants with single mutations in either D*abl* or *fas*I 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 time-dependent 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 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.

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-^{32}P]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 (5×10^4 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-

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ization with the degenerate probe reaffirmed a positive cDNA. The insert was purified and digested with *Sau*3A and then ligated into *Bam*HI-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 [α-^{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 *trk*B.FL, *trk*B.T1, and *trk*B.T2 were sequenced by a combination of shotgun strategy and synthetic oligonucleotide-directed sequencing of subclones in M13mp18 or M13mp19. Clone *trk*B.FL was sequenced in both directions multiple times, as were the unique portions of cDNAs *trk*B.T1 and *trk*B.T2. The regions of *trk*B.T1 and *trk*B.T2 that are identical with *trk*B.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 *Eco*RI cloning sites.

Northern analyses. Radiolabeled single-strand DNA probes were synthesized from M13 template with the Klenow fragment and with [α-^{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 *trk*B.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 *trk*B.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 *Eco*RI 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 *Hin*dIII restriction site. The product of the polymerase chain reaction was purified, digested with *Eco*RI and *Hin*dIII, and ligated into *Hin*dIII-*Eco*RI-digested M13mp19. The final insert sequences were verified by sequencing and corresponded to nucleotides 1363 to 1694 in the *trk*B.T1 sequence for probe E and nucleotides 1361 to 1566 in the *trk*B.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 μg 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× 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× 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× 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 *trk*B cDNAs. A λ ZAP adult rat cerebellar cDNA library (2.5×10^5 plaques) was screened with the HRD LAAR probe. Among 22 positive clones, nucleotide sequence analysis revealed that 1, *trk*B.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).

*trk*B.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, *trk*B.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. *trk*B.P1 was used to screen the cerebellar library (2×10^6 plaques) at high stringency. Of 100 positive clones, 38 were plaque purified; the nucleotide sequence of one, a 4,757-bp cDNA, *trk*B.FL, was determined (Fig. 1).

Clone *trk*B.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

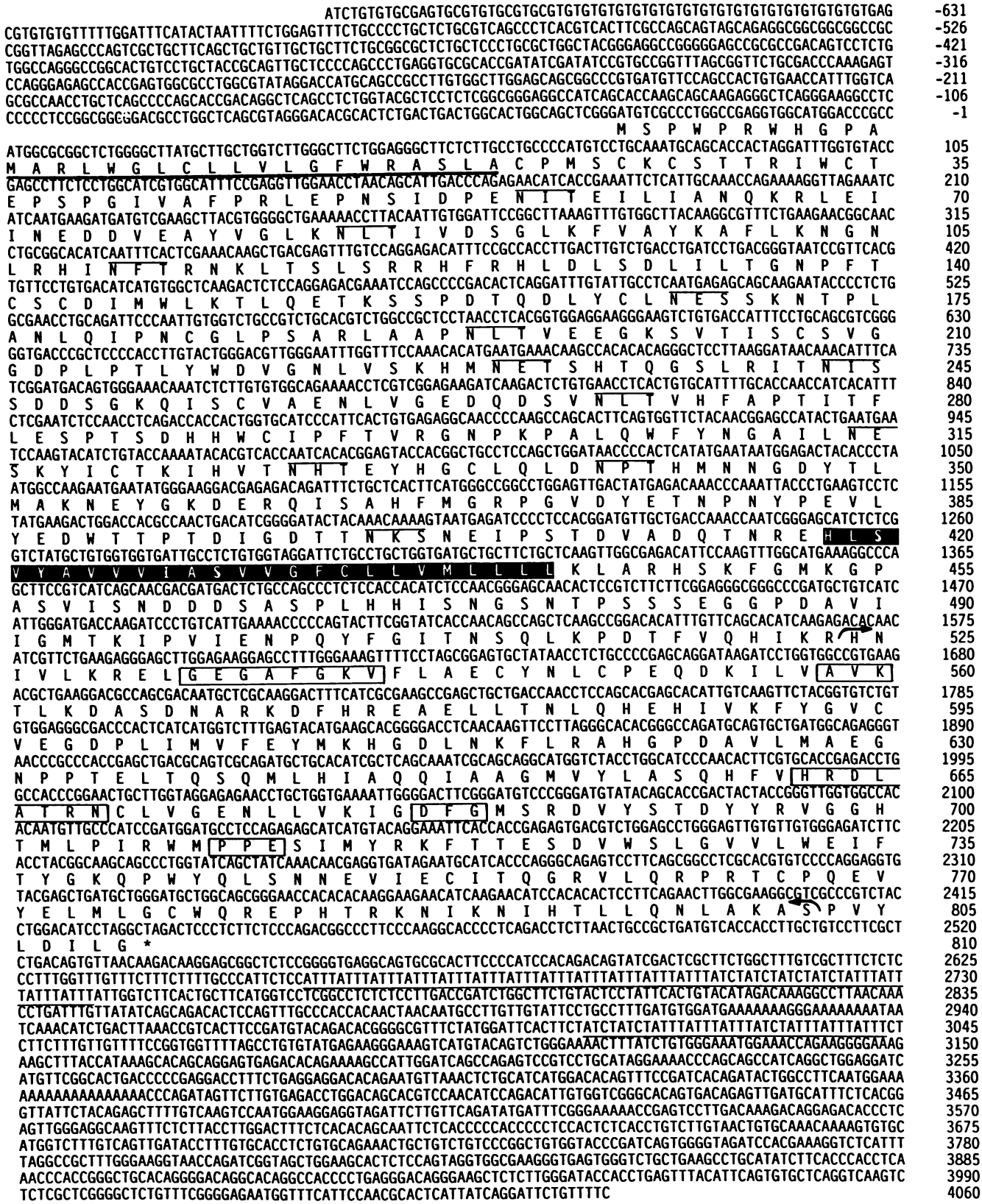

FIG. 1. *trk*B full-length PTK (*trk*B.FL) nucleotide sequence (4,757 bp) and deduced amino acid sequence (810 amino acids) of the LORF (2,430 bp). In the amino acid sequence, the predicted initiating methionine and signal peptide are given bold underlines, the potential glycosylation sites are overlined, 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 *trk*B.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-*erb*B-2 (HER 2) and c-*erb*B (4, 38) or the insulin and the insulinlike growth factor-1 receptors (34). Therefore, like Klein et al. (16), we have named the gene *trk*B, since there is a notable similarity in the amino acid sequences of their respective extracellular regions. Rat *trk*B and mouse *trk*B have 99% amino acid sequence identity.

Northern analysis of *trk*B. Northern hybridization to rat cerebellar or cerebral mRNA with *trk*B probes indicates a perplexing number of mRNAs apparently expressed from the *trk*B locus. Although similar patterns of *trk*B mRNA expression were observed in both cerebral and cerebellar RNA preparations, *trk*B 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 *trk*B 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 *trk*B 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 *trk*B.FL, hybridizes to mRNAs of 4.8 and 1.6 kb, it is most probable that cDNA *trk*B.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 *trk*B.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 *trk*B mRNAs in a variety of tissues was examined (Fig. 4). *trk*B 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 *trk*B 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 *trk*B probes.

Cloning of cDNAs for truncated receptors. Northern analyses indicated that *trk*B expresses mRNAs encoding *trk*B products that lack the kinase domain, most probably resulting from C-terminal truncations. Because the initial *trk*B cDNA, *trk*B.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, *trk*B.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-amino-acid) 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 *trk*B cDNA clones had this structure. Southern analysis of the 38 *trk*B cDNAs with probes A to E also indicated that there were other *trk*B cDNAs which could not be accounted for by the structures represented by *trk*B.FL or *trk*B.T1. The nucleotide sequence of one of these cDNAs, *trk*B.T2, a 3,991-bp clone, indicates that it would also code for a truncated receptor, which is spliced at the same site as *trk*B.T1 but has a different cytoplasmic tail (Fig. 5 and 7). The predicted cytoplasmic domain of *trk*B.T2 is 21 amino acids, the last 9 of which differ from the corresponding region in *trk*B.FL or *trk*B.T1. Using a probe (probe F) corresponding to the splice junction that gives rise to the short alternate C-terminal tail in cDNA *trk*B.T2 and part of the adjacent 3′ UT region, we screened the original 38 *trk*B cDNAs. Probe F hybridized to five cDNAs, including *trk*B.T2, which is a lower frequency than we found with probe E. These frequencies suggest that *trk*B.T1 is likely to represent the predominant *trk*B mRNAs that encode truncated receptors. Four of the cDNAs that hybridize to the *trk*B.T2-specific probe F have different sizes, which clearly indicates independent origin in the cDNA library. It is therefore highly probable that cDNA *trk*B.T2 is derived from a genuine *trk*B mRNA.

To assign which mRNAs clone *trk*B.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 *trk*B 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 *trk*B.FL, respectively. Since *trk*B.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 *trk*B.FL, detects the 9.0-, 7.5-, 4.8-, and 2.4-kb mRNAs, but

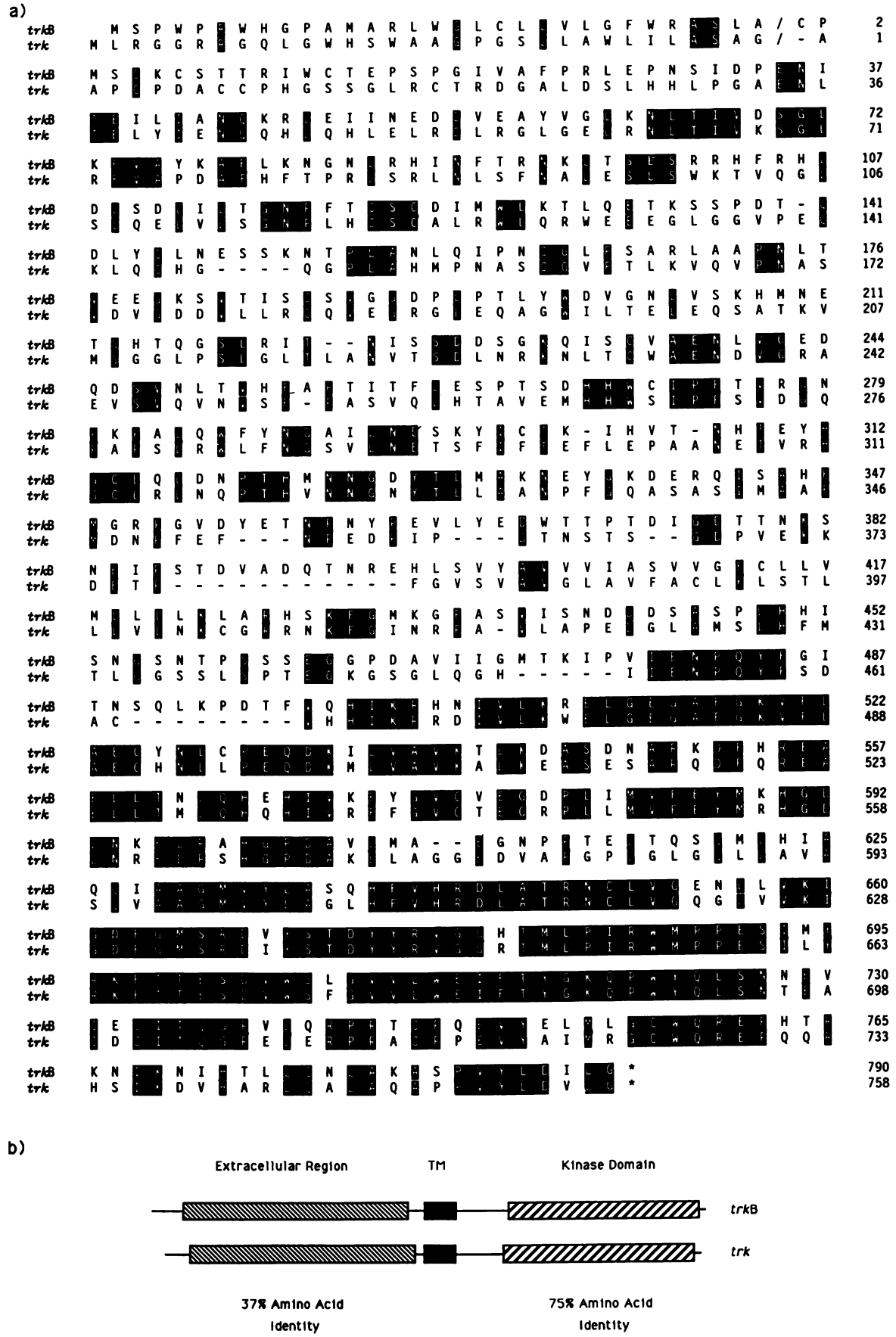

FIG. 2. (a) Amino acid sequence alignment of rat *trk*B and human *trk* (22). Sequence identity is indicated by inverse lettering. (b) Schematic representation depicting the amino acid sequence identity between different domains of *trk*B and *trk*.

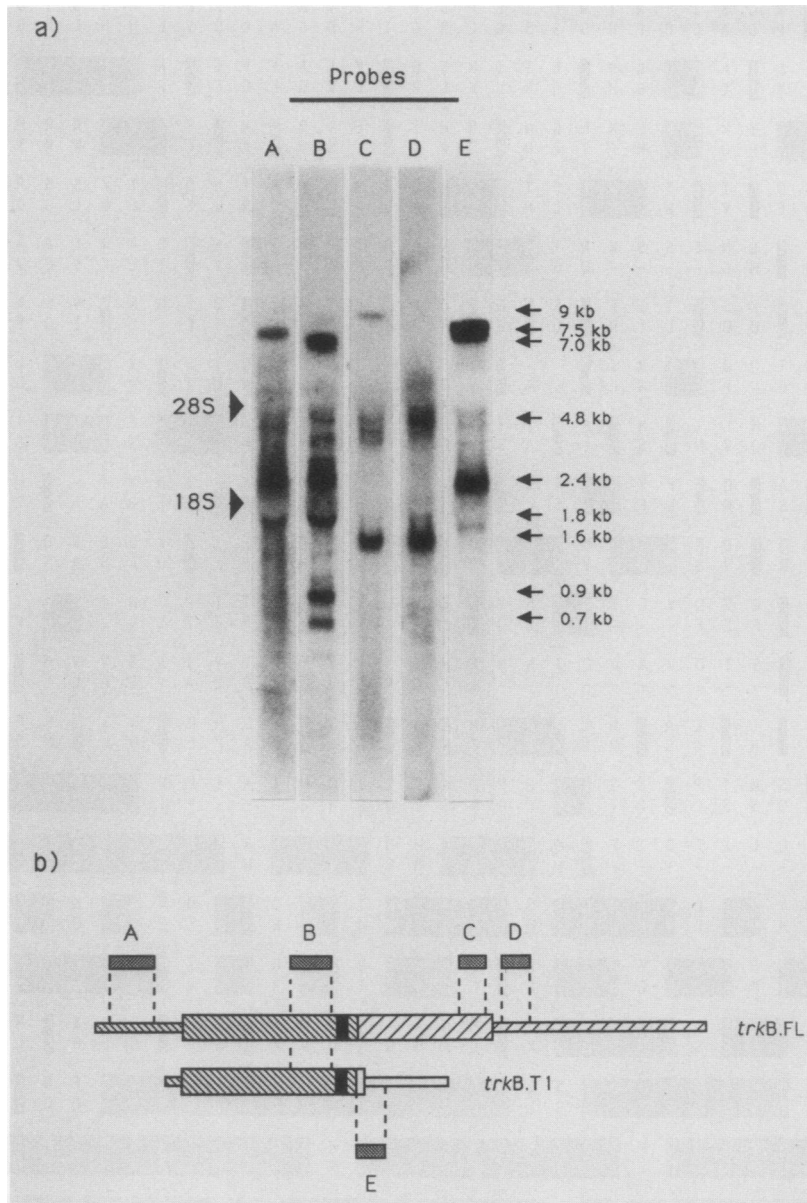

FIG. 3. (a) Northern hybridization with various *trk*B probes of 20 μg of total cellular RNA isolated from adult rat cerebrum resolved on replicate gels. Sizes were determined by using the 28S and 18S rRNAs as internal markers. (b) Schematic representation of the origins of the probes in the *trk*B.FL and *trk*B.T1 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, *trk*B.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 *trk*B mRNA is expressed in limited regions of the brain.

DISCUSSION

The human *trk* proto-oncogene and rat *trk*B gene products do not have any sequence similarity to other known PTKs in the extracellular region. The conserved extracellular cysteines of *trk* and *trk*B are dispersed rather than grouped in cysteine boxes, and neither *trk* or *trk*B has immunoglobulin-like 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 $pp60^{c\text{-}src}$). 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 *trk*B locus also expresses multiple mRNAs similar to the multiple rat *trk*B 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

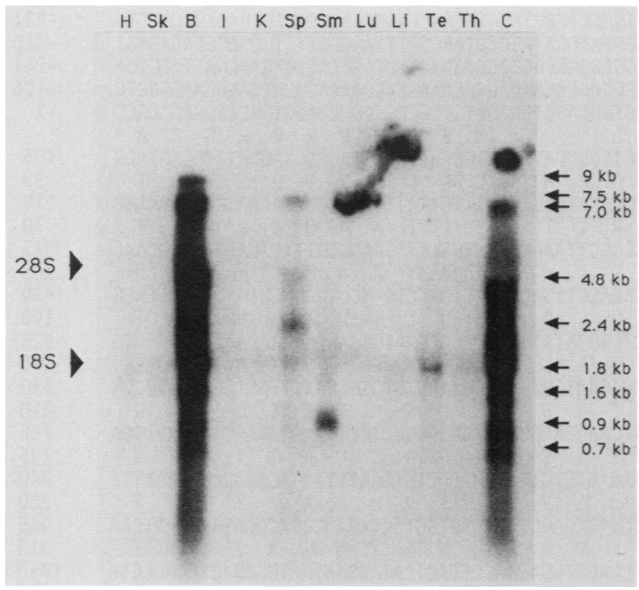

FIG. 4. Northern hybridization of 20 μg of total cellular RNA from various tissues with *trk*B probes B and C simultaneously, which in combination recognize both the extracellular and kinase domains. RNAs were isolated from adult rat tissues, except thymus RNA, which was isolated from juvenile rat tissues. The lanes were loaded with 20 μg 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

a)

mRNA Size	Probe					Predicted Protein Structure
	A	B	C	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

b)

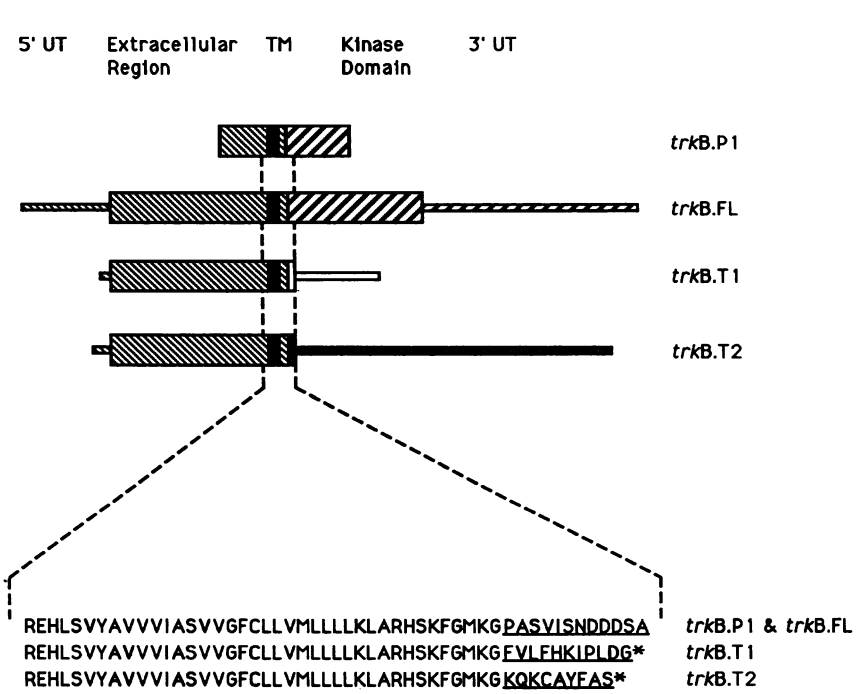

FIG. 5. (a) Summary of Northern hybridization data. (b) Schematic representation of *trk*B cDNAs. An expansion depicting the amino acid sequences of the splice junction region of *trk*B.P1, *trk*B.FL, *trk*B.T1, and *trk*B.T2 is shown.

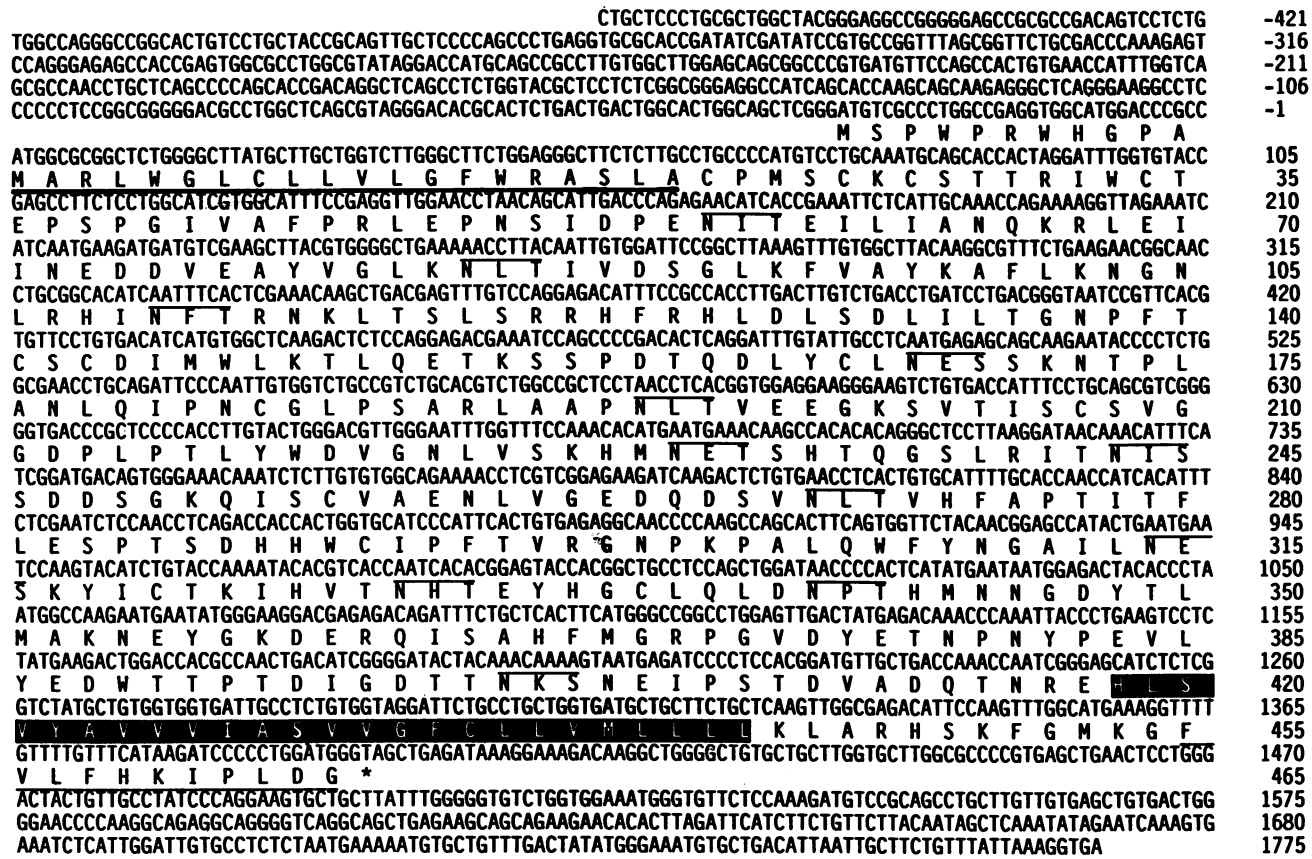

FIG. 6. *trk*B.T1 nucleotide sequence (2,249 bp) and deduced amino acid sequence (465 amino acids). In the amino acid sequence, the predicted initiating methionine and signal peptide are given bold underlines, the potential glycosylation sites are overlined, the predicted transmembrane domain is indicated by inverse lettering, and the short cytoplasmic tail unique to *trk*B.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 *trk*B 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 $pp60^{c\text{-}src}$, 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 *trk*B mRNA encoding a full-length PTK is expressed in the cerebral cortex and the pyramidal cell layer of the hippocampus. *trk*B mRNA encoding a truncated receptor was observed in the ependymal linings of the cerebral ventricles and the choroid plexus. Expression of *trk*B 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 *trk*B 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 *trk*B 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 *trk*B 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 *trk*B 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 membrane-associated 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 *trk*B. Nonetheless, as these mRNAs are just large enough to encode most of the extracellular region of the *trk*B product, a key question is whether

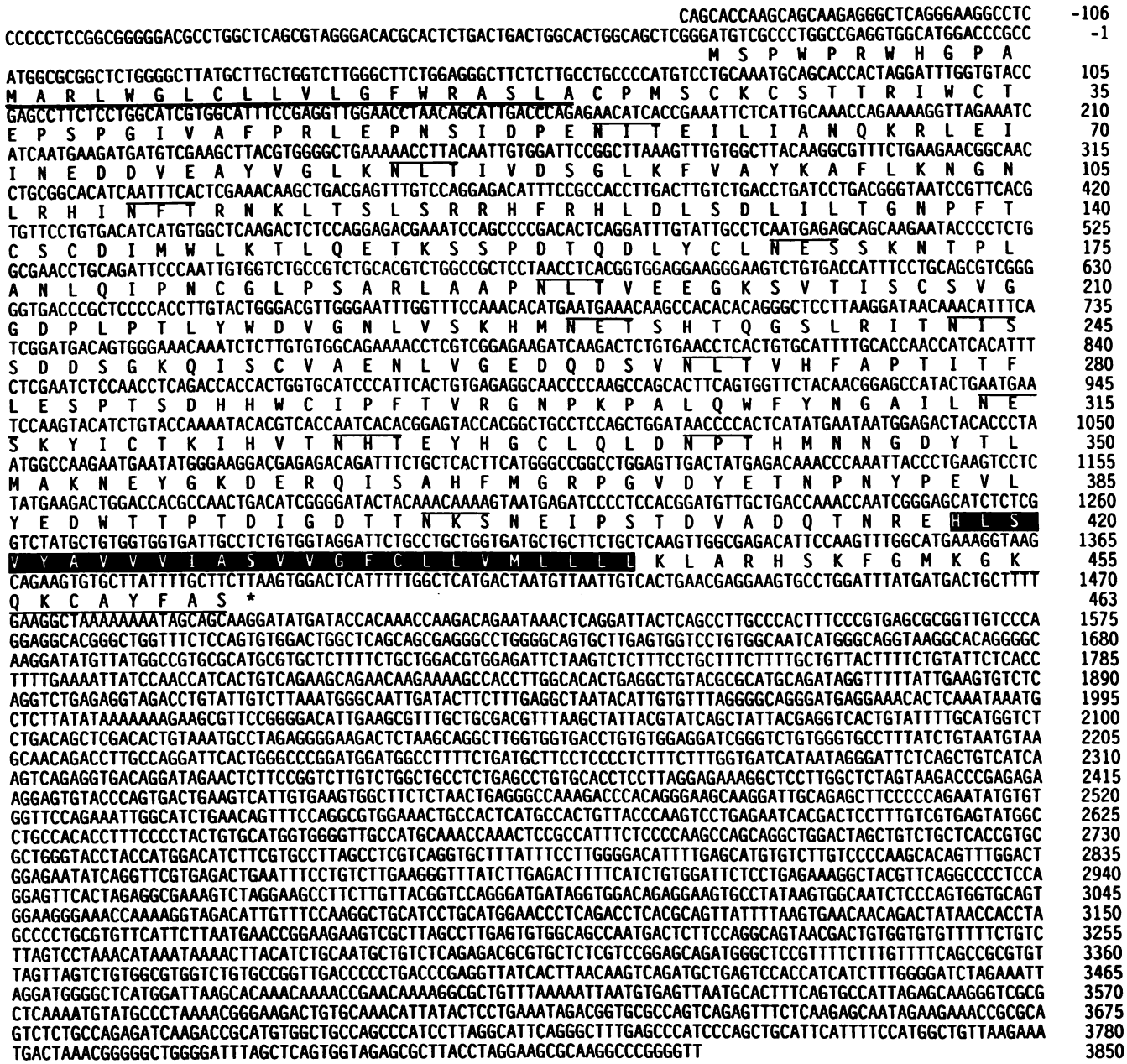

FIG. 7. trkB.T2 nucleotide sequence (3,991 bp) and deduced amino acid sequence (463 amino acids) of the LORF (1,389 bp). In the amino acid sequence, the predicted initiating methionine and signal peptide are given bold underlines, the potential glycosylation sites are overlined, the predicted transmembrane 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 trkB mRNA 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 into the medium (5). In vivo, this truncated 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 *trk*B receptors play? A truncated receptor could be a clearance receptor. *trk*B.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 *trk*B 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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