THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 268, No. 32, Issue of November 15, pp. 24290-24295, 1993
Printed in U.S.A.

Molecular Cloning of *trk*E, a Novel *trk*-related Putative Tyrosine Kinase Receptor Isolated from Normal Human Keratinocytes and Widely Expressed by Normal Human Tissues*

(Received for publication, August 20, 1993)

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We have identified and cloned a new member of the *trk* gene family, termed *trk*E, which generates a 3.9-kilobase (kb) transcript in normal human keratinocytes and in a variety of normal human tissues, but not in liver. Albeit at low level, *trk*E transcript is expressed also by PC12 cells. The open reading frame codes for a polypeptide of 876 amino acids exhibiting the classic features of cell surface tyrosine protein kinases. *trk*E catalytic domain is 41% identical to *trk*A and shows several features unique to the *trk* gene family. Its extracellular domain does not show significant homology to any known proteins. *trk*E is the first member of this gene family found abundantly and widely expressed in normal human tissues. Several lines of evidence suggest that NGF is also the ligand for *trk*E; (i) normal human keratinocytes bind NGF with high affinity, (ii) NGF stimulates keratinocyte growth in an autocrine fashion, (iii) NGF exerts its biological effect on keratinocytes through the stimulation of a *trk*-specific tyrosine kinase, and (iv) keratinocytes lack *trk*A but do express large amount of *trk*E. *trk*E might also be the NGF receptor by other human peripheral tissues, such as pancreatic islets, and might represent a non-neuronal receptor for this ligand.

Nerve growth factor (NGF)[1] is the prototype of a family of related molecules, including brain-derived neurotrophic factor and neurotrophins 3, 4, and 5 (NT-3, -4, and -5), related to maintenance, development, and differentiation of neural crest-derived cell types and central cholinergic neurons (Levi-Montalcini, 1987; Thoenen, 1991). NGF also evokes biological responses in non-neuronal cells; it affects growth and histamine release from mast cells (Levi-Montalcini, 1987), modulates human B lymphocyte differentiated functions (Otten *et al.*, 1989), regulates the onset of meiosis in rat seminiferous epithelium (Parvinen *et al.*, 1992), and induces neuron-like differentiation of insulin-secreting pancreatic cells (Polak *et al.*, 1993). Neurotrophins bind to both the p75 NGF receptor ($p75^{NGFR}$) and to products of the *trk* protooncogene family. The human *trk* locus was first identified as a highly transforming gene formed by a subset of *trk* sequences fused to a non-muscle tropomyosin gene in a colon carcinoma patient (Martin-Zanca *et al.*, 1986). Additional *trk* oncogenes, formed by the fusion of *tpr* and *trk* sequences, were identified in human papillary thyroid carcinomas (Greco *et al.*, 1992). The *trk* protooncogene (*trk*A) was then isolated from cDNA clones derived from human leukemia cells (K562) (Martin-Zanca *et al.*, 1989), leading to the discovery of additional members of the mammalian *trk* gene family (*trk*B and *trk*C), which share common and characteristic features in their cytoplasmic catalytic domains (Klein *et al.*, 1989; Lamballe *et al.*, 1991). Additional members of the *trk* gene family were identified in *Drosophila* (Pulido *et al.* 1992) and in *Torpedo californica* (Jennings *et al.*, 1993). *trk* gene products show high homology in their catalytic domain but lower homology in the extracellular region. For example, D*trk* encodes a neuronal cell adhesion molecule (Pulido *et al.*, 1992).

Contrasting data exist in the literature concerning NGF (and neurotrophin) high affinity binding and signal transduction. High affinity binding and specificity appear to be conferred by a heterodimeric complex of the $p75^{NGFR}$ and the products of the *trk* gene family (Johnson *et al.*, 1986; Klein *et al.*, 1991; Squinto *et al.*, 1991; Soppet *et al.*, 1991; Lamballe *et al.*, 1991; Bothwell, 1991; Hempstead *et al.*, 1991). Alternatively, *trk* monomers might bind NGF with low affinity (Kaplan *et al.*, 1991), while *trk* dimerization (Jing *et al.*, 1992) or ligand-mediated endocytosis (Eveleth and Bradshaw, 1988, 1992; Kahle and Hertel, 1992) could confer high affinity binding properties. It has been reported that stimulation of the $p140^{trkA}$ is necessary and sufficient to elicit a full biological response (Cordon-Cardo *et al.*, 1991; Loeb *et al.*, 1991; Ibanez *et al.*, 1992). Other reports, instead, highlight the crucial role of the association of $p140^{trkA}$ and $p75^{NGFR}$ in regulating NGF biological activities (Hempstead *et al.*, 1990, 1991; Lee *et al.*, 1992) (for a recent review, see Saffioni *et al.* (1993)). The sum of these data, however, clearly demonstrates that the expression of $p140^{trkA}$ is necessary for high affinity NGF binding and NGF signal transduction.

We have shown that normal human keratinocytes, cultured in conditions allowing reconstitution of transplantable sheets of normal epithelium (Rheinwald and Green, 1975; Green *et al.*, 1979; De Luca *et al.*, 1988, 1990a, 1990b; Marchisio *et al.*, 1991; Romagnoli *et al.*, 1990), synthesize and secrete biologically active NGF (Di Marco *et al.*, 1991), which potently stimulates keratinocyte proliferation in an autocrine fashion (Di Marco *et al.*, 1993). Keratinocytes have approximately 1000 high affinity NGF receptors/cell (Di Marco *et al.*, 1993); the NGF-dependent stimulation of keratinocyte proliferation is completely abolished by monoclonal antibodies highly specific for NGF and by nanomolar concentrations of the natural alkaloid K252a (Di Marco *et al.*, 1993), a selective inhibitor of the *trk* tyrosine kinase activity (Tapley *et al.*, 1992; Berg *et al.*, 1992; Knusel and Hefti, 1992). However, normal human keratinocytes do not express $p140^{trkA}$, as demonstrated also by polymerase chain reaction (Di Marco *et al.* 1993). Instead, Northern blot analysis

* This work was supported by P. F. "Biotecnologie e Biostrumentazione" and "Applicazioni Cliniche della Ricerca Oncologica" of the Consiglio Nazionale delle Ricerche and by Associazione Italiana per la Ricerca sul Cancro. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X74979.

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[1] The abbreviations used are: NGF, nerve growth factor; NT, neurotrophin; bp, base pair(s); kb, kilobase(s).

performed in relaxed hybridization conditions, showed that, in human keratinocytes, the human *trk*A-specific cDNA probe hybridizes to multiple bands ranging from 1.8 to 3.9 kb. Moreover, *in vivo* studies in mice have shown that *trk*A expression is restricted to sensory cranial and spinal ganglia of neural crest origin, thus being a specific marker of neural crest-derived sensory neurons (Martin-Zanca *et al.*, 1990).

This prompted us to investigate the presence of additional members of the *trk* gene family in normal human keratinocytes. By screening three independent cDNA libraries (two from different strains of keratinocytes and one from human fetal brain) with the *Bam*HI-*Eco*RI fragment of the plasmid pDM-17, corresponding to the entire cytoplasmic portion of the human $p140^{trkA}$, we have identified a new member of the *trk* family of tyrosine kinases (which we have termed *trk*E). *trk*E generates a 3.9-kb transcript, which is expressed in human keratinocytes, as well as in a wide variety of normal human tissues.

EXPERIMENTAL PROCEDURES

3T3-J2 cells were a gift from Dr. Howard Green, Harvard Medical School, Boston, MA. PC12 cells were a gift from Dr. Ralph A. Bradshaw, University of California, Irvine, CA. The fetal human brain cDNA library was from Stratagene. The *Bam*HI-*Eco*RI fragment of the plasmid pDM-17 was a gift from Dr. Mariano Barbacid, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ. The *Eco*RI 0.8-kilobase fragment of human $p75^{NGFR}$ cDNA was a gift from Dr. Moses Chao, Cornell University Medical College, New York, NY. Adult and fetal human multiple tissue Northern (MTN) blots were purchased from Clontech.

Human epidermal keratinocytes were obtained from skin biopsies of healthy volunteers and cultivated as described (Di Marco *et al.*, 1993).

cDNA was synthesized by oligo(dT) priming (Stratagene Kit) using 5 μg of poly(A^+) RNA isolated from subconfluent secondary keratinocyte cultures. The cDNA library was prepared in λUni-Zap II vector (Stratagene cloning system); bacteriophages (10^6) were plated on a layer of *Escherichia coli* XL1-Blue cells, incubated 6 h at 37 °C, and transferred onto Hybond N^+ filters (Amersham Corp.). Membranes were incubated 7 min in 1.5 M NaCl, 0.5 M NaOH; 3 min in 1.5 M NaCl, 0.5 M Tris/HCl, pH 7.2, 1 mM EDTA; and washed in 2 × SSC. DNAs were prehybridized and hybridized under stringent conditions at 65 °C for 1 and 12 h, respectively, in 5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, 200 μg/ml freshly denatured sheared salmon sperm DNA with a ^{32}P-labeled probe (2×10^6 cpm/ml) corresponding to the 1.2-kb *Bal*I-*Eco*RI insert of pDM 17 *trk* oncogene. After hybridization filters were washed in 2 × SSPE, 0.1% SDS at room temperature for 10 min; in 2 × SSPE, 0.1% SDS at 65 °C for 30 min; in 0.75 × SSPE, 0.1% SDS at 65 °C for 20 min; and in 0.1 × SSPE, 0.1% SDS at 65 °C for 30 min. Positive λ clones were plaque-purified and converted to Bluescript plasmid clones by automatic *in vivo* excision (Stratagene).

cDNA clones containing the 5′ *trk*E sequence were isolated following two strategies. (i) A cDNA library from human fetal brain (17–18 weeks gestation) was screened under stringent conditions using the 400-bp *Eco*RI-*Pvu*II ^{32}P-labeled fragment derived from the 5′ end of pBS21-1, a partial cDNA clone containing the entire *trk*E tyrosine kinase domain. (ii) A second cDNA synthesis was performed using P18, an oligonucleotide primer (5′-TGGGCACTGAGGAACTGGTTGAGGTCGC-3′, complementary to nucleotides 372 and 400 of the pBS21-1 *trk*E partial clone) and 8 μg of poly(A^+) RNA derived from normal human keratinocytes. A cDNA library prepared in λZap II vector (Stratagene Cloning System) was screened using the 140-bp ^{32}P-labeled *Eco*RI-*Sal*I fragment derived from the 5′ of the pBS21-1 partial clone. Sequencing reactions were carried out with Sequenase 2.0 (U. S. Biochemical Corp.). Sequence analysis was performed with the Gene Works 2.2 software package (IntelliGenetics, Mountain View, Ca).

Total cellular RNA and poly(A^+) mRNA extraction and RNA blotting were performed as described (Di Marco *et al.*, 1993).

RESULTS AND DISCUSSION

Molecular Cloning of trkE cDNA Clones—A cDNA library from human keratinocytes in secondary culture was screened with a probe corresponding to the entire catalytic domain of the human $p140^{trkA}$.

Two phages containing an identical 1274-bp insert were isolated and converted to Bluescript plasmid clones designated pBS12-1 and pBS19-1. A pBS21-1 plasmid containing a 5′ longer insert of 1873 bp was identified by rescreening the above cDNA library with a ^{32}P-labeled *Eco*RI-*Xho*I fragment of the pBS12-1 clone. Sequence analysis of this clone revealed the presence of a kinase catalytic domain displaying high homology with members of the *trk* gene family (see below). Northern blot analysis performed in high stringency conditions on keratinocyte-derived poly(A^+) mRNA showed the presence of a 3.9-kb transcript (Di Marco *et al.*, 1993). The same transcript was also present in human fetal brain (see Fig. 3). Therefore, we decided to isolate and sequence this gene from both keratinocyte and human fetal brain libraries.

A cDNA library from human fetal brain was screened, under stringent conditions, with the 400-bp *Eco*RI-*Pvu*II ^{32}P-labeled fragment derived from the 5′ end of the pBS21-1 plasmid. Three phages, designated pBS10B, pBS34B, and pBS7B, contained inserts of 3553, 2916, and 1947 bp, respectively, which cross-hybridized with the insert derived from pBS21-1 (not shown); a second cDNA human keratinocyte library was obtained using a P18 oligonucleotide primer. A 140-bp ^{32}P-labeled *Eco*RI-*Sal*I fragment from the 5′ end of the pBS21-1 partial clone was used to screen 106 bacteriophages. One positive recombinant phage was characterized. This plasmid clone, designated pBS1, contained an insert of 1040 bp and overlapped the 5′ insert of pBS21-1 for 400 bp. The two overlapped clones were assembled into a single clone, and its nucleotide sequence showed complete identity with that of pBS10B clone, the only exception being four point mutations. Three of them were in the coding region but did not change the amino acid sequence. The pBS10B clone contained the entire coding sequence of this new gene (Fig. 1). Based on the high homology of its kinase catalytic domain with other members of the *trk* gene family (see below), this gene was called *trk*E.

Nucleotide, Deduced Amino Acid Sequences, and Homology—Fig. 1 shows the nucleotide and the deduced amino acid sequences of *trk*E. The open reading frame consists of 2628 nucleotides flanked by a 881-nucleotide 3′-noncoding region containing a polyadenylation site (AATAAA) located 15 nucleotides before the poly(A) tail.

The open reading frame is capable of coding for a polypeptide of 876 amino acid residues, which exhibits classic features of cell surface tyrosine protein kinases (Hanks *et al.*, 1988). The putative signal peptide (positions 1–16; von Hejne (1986)) is followed by 401 amino acids forming an extracellular domain displaying 5 consensus *N*-glycosylation sites (Asn-*X*-Ser/Thr) (13 in *trk*A), and 7 cysteine residues (11 in *trk*A). One of these cysteines (position 177) is conserved *trk*A/B/C. A single transmembrane domain (positions 418–438) is followed by a cytoplasmic region (positions 439–876), which includes a kinase catalytic domain (positions 598–861). An ATP binding site has been identified at position 609. Its consensus sequence (GEGQFG) was 24 residues upstream (positions 580–585).

Sequence comparison between the tyrosine kinase domain of *trk*E and the catalytic domain of other members of the *trk* gene family shows that *trk*E is 39% identical to *trk*A/B/C (Fig. 2). The tyrosine kinase domain of *trk*E is 41% identical to *trk*A, but it shows only 8% homology to a wide variety of other tyrosine kinases (not shown). More importantly, several features unique to the *trk* gene family (Martin-Zanca *et al.*, 1989; Klein *et al.*, 1989; Lamballe *et al.*, 1991) are maintained: (i) a single amino acid gap between residues 623 and 624 (542 and 543 in *trk*A), (ii) a threonine (position 732) instead of the alanine present in all other tyrosine kinases, (iii) a phenylalanine (position 808) instead of the tyrosine present in all other tyrosine kinases, (iv) the putative autophosphorylation site (position 759; Fig. 1,

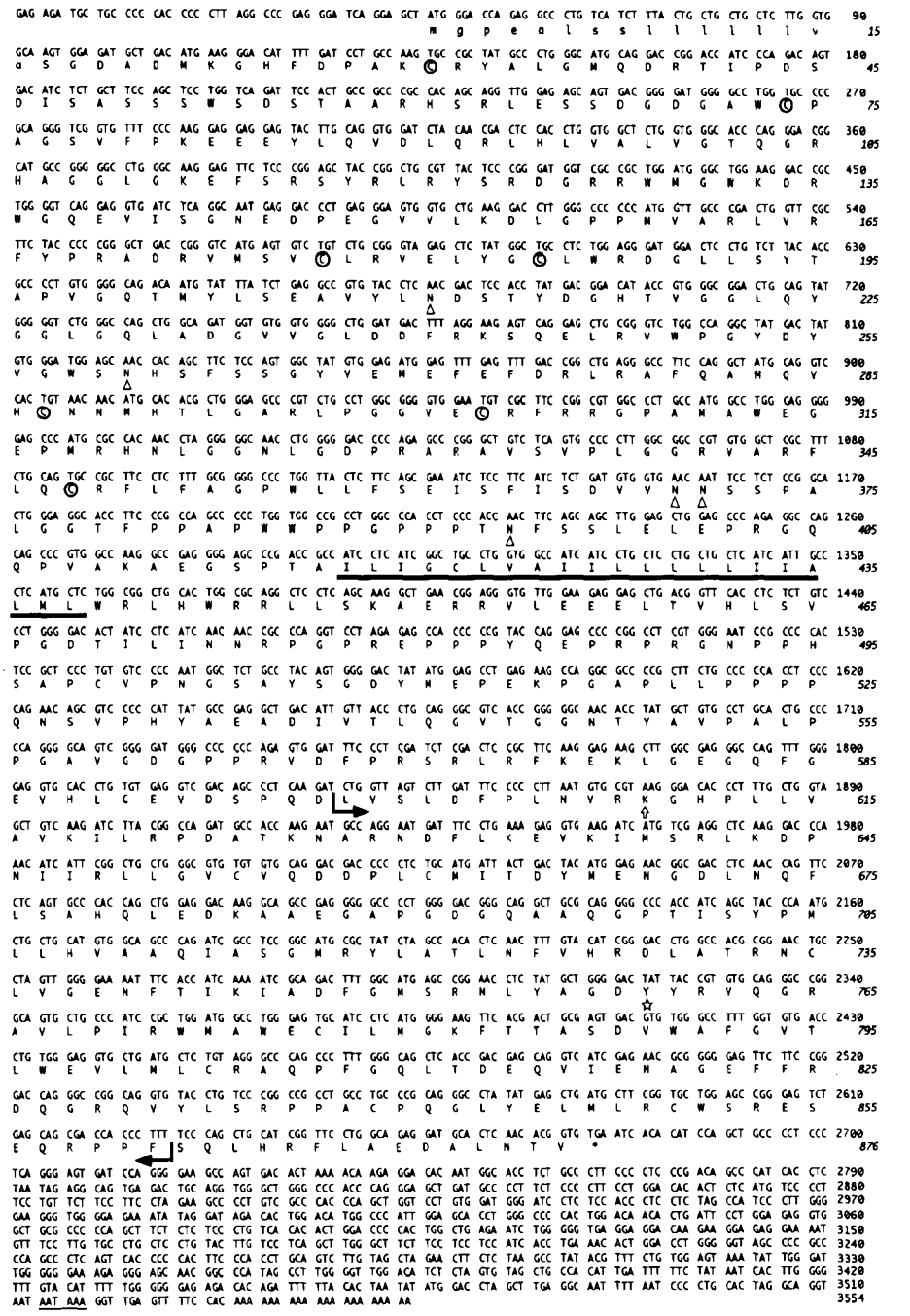

FIG. 1. ***trk*E nucleotide and amino acid sequence.** The putative signal peptide (*lowercase letters*) is between residues 1–16. The consensus *N*-glycosylation sites are indicated by *open triangles*. The cysteine residues in the extracellular domain are *circled*. The putative transmembrane domain is *underlined* by a *solid bar*. The tyrosine kinase catalytic domain is flanked by *horizontal arrows*. The putative ATP binding site is indicated by an *open arrow*. The *star* shows the the putative autophosphorylation site. The in-frame terminator codon TAG is indicated by an *asterisk*.

star) that is followed by a second tyrosine (a feature sheared also with the insulin receptor subfamily), (v) 11 extra residues in the region corresponding to the long intervening domain characteristic of the c-Kit protein and the platelet-derived growth factor and colony-stimulating factor-1 receptor, (vi) 3 out of 5 additional residues, Leu-*X*-Ala-Val-*X* (707–711), representing a characteristic insert of the central region of the *trk* catalytic domain. Finally, *trk*E ends with a very short C-terminal domain (15 amino acid), a feature so far characteristic of the mammalian *trk* kinases (Pulido *et al.*, 1992). The sum of these data allows *trk*E inclusion in the *trk* gene family. The *trk*E kinase domain is 158 residues from the putative transmembrane domain (76 residues in *trk*A); this region has 5 tyrosine residues as compared the single tyrosine present in *trk*A. Interestingly, *trk*E C-terminal domain is not homologous to the one present in *trk*A/B/C and does not possess the tyrosine residue reported as a selective interaction site for phospholipase Cγ in *trk*A/B/C (Obermeier *et al.*, 1993). This feature is shared with D*trk*. Activation of phospholipase Cγ generates inositol trisphosphate and diacylglycerol (Rhee *et al.*, 1989), two messengers playing roles in triggering a mitogenic response by activating the serine/threonine-specific protein kinase C and by increasing intracellular calcium levels (Nishizuka, 1992). *trk*E has the 15-amino acid C-terminal region but lacks the tyrosine residue, suggesting either that phospholipase Cγ does not interact with *trk*E or that some other tyrosines are involved. Therefore, the tyrosine-rich region between the putative transmembrane domain and the kinase domain is of potential importance for tyrosine phosphorylation processes, leading to the creation of potential binding sites for SH2-SH3-containing signaling proteins (see Pawson and Gish (1992)), able to act as downstream mediators of different signaling pathways (Boulton *et al.*, 1991; Fanti *et al.*, 1992; Valius and Kazlauskas, 1993).

*trk*E, as with pp60 $^{v\text{-}src}$ and other tyrosine kinases, maintains the alanine residue (position 774) instead of the proline present

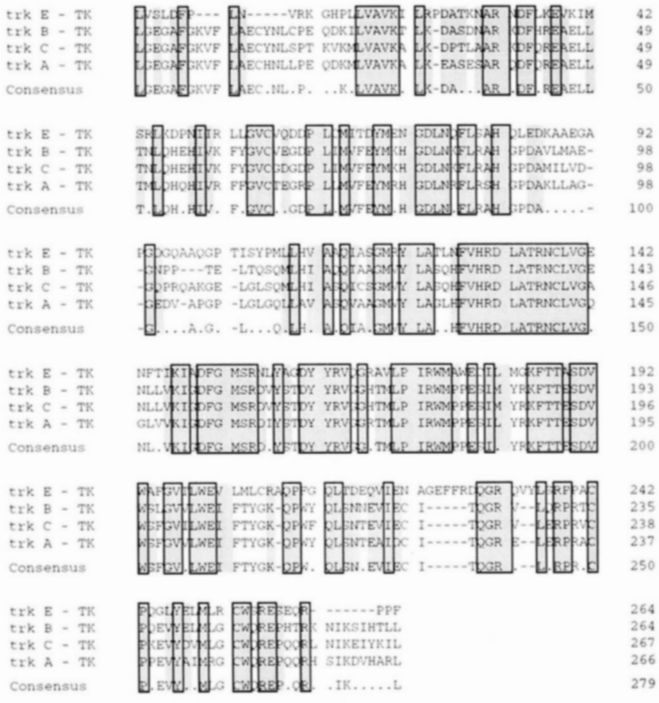

FIG. 2. **Tyrosine kinase amino acid sequences.** Comparison of the deduced amino acid sequences of the tyrosine kinase domains encoded by human *trk*E, mouse *trk*B, porcine *trk*C, and human *trk*A. Residues shared by at least two (but less than four) of these genes are *shaded*. Residues shared by all members are *shaded* and *boxed*. *Dots* indicate gaps introduced to maximize alignment.

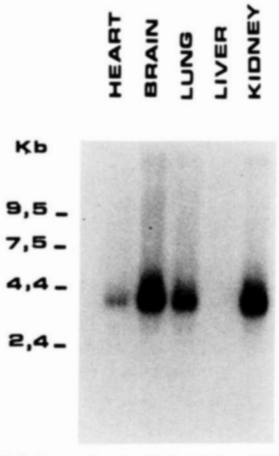

FIG. 3. **Northern blot analysis.** Poly(A⁺) mRNA obtained from several normal human fetal tissues were hybridized with a ^{32}P-labeled cDNA probe corresponding to a 400-bp *Eco*RI-*Pvu*II fragment derived from the 5′ end of the pBS-21 clone. The 3.9-kb *trk*E transcript was detected in all tissues, except in liver.

in the *trk* gene family. In addition, as in other tyrosine kinases and in contrast to other *trk* members, the helix-breaking proline (position 859), is conserved. Highly conserved amino acids, important for the tyrosine kinase catalytic activity, such as Arg-Asp-Leu (positions 728–730) and Asp-Phe-Gly (positions 747–749), are present within the *trk*E catalytic domain.

Poly(A⁺) mRNA, obtained from several normal human fetal and adult tissues, were hybridized with a ^{32}P-labeled cDNA probe corresponding to a 400-bp *Eco*RI-*Pvu*II fragment derived from the 5′ end of the pBS21 clone. As shown in Fig. 3, a 3.9-kilobase transcript was detected at high levels in brain, lung, and kidney and, at lower levels, in fetal human heart tissues. In adult normal human tissues, *trk*E levels were low in heart and lung, whereas highest levels were detected in kidney

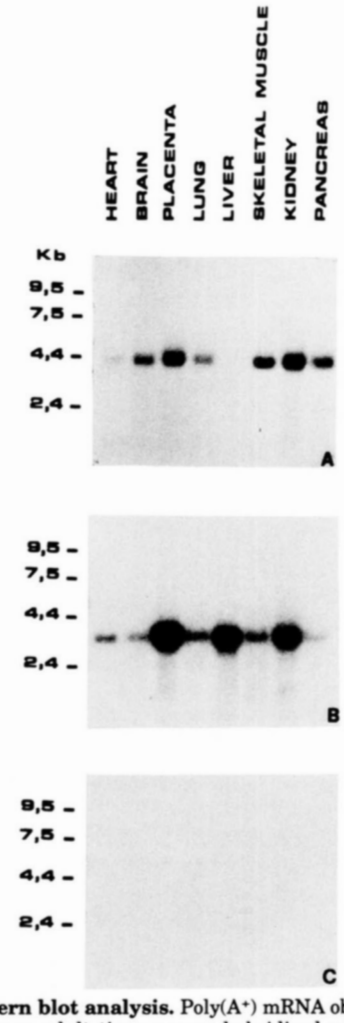

FIG. 4. **Northern blot analysis.** Poly(A⁺) mRNA obtained from several normal human adult tissues were hybridized with a ^{32}P-labeled cDNA probe as in Fig. 3 (*panel A*), a human p140^{trkA} cDNA probe (*panel C*), or he *Eco*RI 0.8-kilobase fragment of human p75^{NGFR} cDNA (*panel B*). The 3.2-kb *trk*A transcript was not detected. The 3.8-kb p75^{NGFR} transcript was present in all tissues. The level of expression of the 3.9-kb *trk*E transcript was very low in heart and lung, whereas highest levels were detected in kidney and placenta. *trk*E was undetectable in liver.

and placenta (Fig. 4, *panel A*). Interestingly, *trk*E was undetectable in both fetal and adult liver (Figs. 3 and Fig. 4*A*). The same blot was hybridized (in high stringency conditions) with a human p140^{trkA} cDNA probe and with the *Eco*RI 0.8-kilobase fragment of human p75^{NGFR} cDNA. The 3.2-kilobase *trk*A transcript, was undetectable in normal human tissues (Fig. 4, *panel C*), whereas all tissues examined expressed the 3.8-kb p75^{NGFR} transcript (Fig. 4, *panel B*). Poly(A⁺) mRNA from normal human keratinocytes and PC12 cells were hybridized (in high stringency conditions) with both the *trk*A- and *trk*E-specific ^{32}P-labeled cDNA probes. As shown in Fig. 5, the 3.2-kilobase *trk*A transcript was present in PC12 cells and absent in keratinocytes (*panel B*). The *trk*E mRNA was abundantly expressed in keratinocytes and, albeit at lower levels, also in PC12 cells (*panel A*).

In mammals, the expression of the *trk* protooncogene products is restricted to specific areas of the brain (Martin-Zanca *et al.*, 1990; Lamballe *et al.*, 1991), thus representing a specific marker of neural crest-derived sensory neurons and spinal ganglia (Martin-Zanca *et al.*, 1990), which is consistent with the commonly accepted neuronal activity of NGF. However, very recently, polymerase chain reaction and RNase protection experiments have suggested the presence of *trk*A or its alterna-

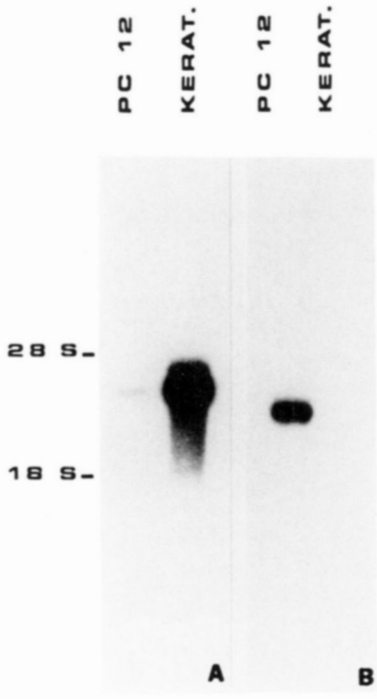

FIG. 5. **Northern blot analysis.** Five μg of poly(A$^+$) mRNA extracted from normal human keratinocytes and PC12 cells were hybridized with both the *trk*A- and *trk*E-specific ^{32}P-labeled cDNA probes (as in Fig. 4). *trk*A mRNA was present in PC12 cells and absent in normal human keratinocytes (*panel B*). *trk*E mRNA was abundantly expressed in normal human keratinocytes and, albeit at lower levels, also in PC12 cells (*panel A*).

tive spliced isoform *trk*AI (lacking a 6-amino acid insert in its extracellular domain) both in human monocytes (Ehrhard *et al.*, 1993) and in human kidney (Barker *et al.*, 1993). Our expression data are of particular interest, since *trk*E, to our knowledge, is the first member of this gene family found abundantly expressed in a wide variety of normal human tissues. Interestingly, liver, although it expresses the p75^{NGFR}, does not express *trk*E, suggesting the possibility of an additional *trk* member present in this tissue. Although we have not defined the cell types expressing *trk*E in the brain, it would be consistent with other observations that they will be glial in origin. Albeit at low levels, *trk*E is also expressed in PC12 cells, raising interesting questions concerning its role in these cells.

The putative *trk*E extracellular region (residues 17–417) does not show homology to any known proteins, with the exception of *trk*A (16% identity).

Binding studies have demonstrated that the mammalian *trk* genes bind neurotrophins. *trk*A, albeit with different affinities, binds NGF and NT-3 (Cordon-Cardo *et al.*, 1991; Hempstead *et al.*, 1991; Klein *et al.*, 1991; Kaplan *et al.*, 1991); *trk*B serves as a receptor for brain-derived neurotrophic factor and NT-3 but not for NGF (Squinto *et al.*, 1991; Soppet *et al.*, 1991); *trk*C encodes a primary receptor for NT-3 (Lamballe *et al.*, 1991). Detailed comparative sequences (Schneider and Schweiger, 1991) have recently demonstrated that *trk*A and *trk*B, in addition to their role as receptors and signal transducers for neurotrophins, combine, in their extracellular parts, functional domains that strongly indicate their potential role as cell adhesion molecules, important during neural development. These motifs, corresponding to the cysteine-rich conserved regions (not present in *trk*E), suggest that, in mammals, the *trk*s so far identified might serve as receptor tyrosine kinases as well as adhesion molecules to either extracellular matrix proteins or certain target cells (Schneider and Schweiger, 1991).

We cannot at present identify the *trk*E ligand, but several lines of indirect evidence (Di Marco *et al.*, 1993) suggest that it is NGF (and/or other neurotrophins). (i) Normal human keratinocytes bind NGF with high affinity. (ii) NGF stimulates keratinocyte growth in an autocrine fashion. (iii) NGF exerts its biological effect on keratinocytes through the stimulation of a *trk*-specific tyrosine kinase. (iv) Normal human keratinocytes lack *trk*A but express large amounts of *trk*E. Thus, it is reasonable to speculate that the *trk*E gene product represent the NGF receptor expressed by normal human keratinocytes, as well as by other human peripheral tissues, and that the lack of the cysteine-rich regions, present in the other *trk* members, might suggest that, at least in keratinocytes, *trk*E functions solely as a growth-mediating tyrosine kinase receptor and not as a potential adhesion molecule. This agrees well with the observation that the *trk*B and *trk*C genes encode alternative neurogenic receptors lacking the catalytic kinase domain and thus potentially act only as a cell adhesion receptor in the brain (Klein *et al.*, 1990; Schneider and Schweiger, 1991; Valenzuela *et al.*, 1993; Tsoulfas *et al.*, 1993). Binding studies and transfection experiments with mutated forms will answer these questions shortly.

The identification of *trk*E as a potential non-neuronal receptor for NGF greatly broadens the possible functional role for this hormone. It will be of interest to compare the catalytic specificities of the *trk*A and *trk*E kinase domains, particularly as they function in a definite paradigm, such as the PC12 cells.

Acknowledgments—We thank Dr. Ralph A. Bradshaw for criticism and help in editing the manuscript. We thank Drs. Patrizio Castagnola and Roberto Biassoni for sequence analysis.

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