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Characterization of the 1B Promoter of Fibroblast Growth Factor 1 and Its Expression in the Adult and Developing Mouse Brain*

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The present study elucidates the molecular structure of a murine fibroblast growth factor 1 (FGF-1) promoter and describes its distribution in the adult and developing mouse brain. A cDNA clone coding for FGF-1 was isolated from a mouse brain cDNA library. Nucleotide sequence analysis revealed that the clone contained, in addition to the protein coding region, an untranslated exon (FGF-1B) 34 base pairs upstream of the translation start codon ATG. The mouse cDNA clone corresponded to the sole FGF-1 transcript in the brain. An RNase protection assay was used to map the transcription start site of the 1B promoter. The sequences upstream from the major transcription initiation site lacked consensus TATA or CAAT boxes. *In situ* hybridization with cRNA probes specific for the 1B transcript showed the message to be restricted largely to sensory and motor nuclei in the brainstem, and to the ventral spinal cord and cerebellum. Although occasional brainstem nuclei were labeled at low levels by embryonic day 18, the majority of nuclei became detectable autoradiographically during postnatal weeks 1 and 2, and adult levels of grain density were reached during the 3rd and 4th postnatal weeks. FGF-1B mRNA was expressed in phylogenetically older brain regions, which are involved primarily in processing information from exteroceptive sensory mechanoreceptors and in motor control. The relatively late developmental expression suggests a role for FGF-1 in neuronal maturation, rather than in neurogenesis.

Acidic fibroblast growth factor, also known as fibroblast growth factor 1 (FGF-1),[1] is one of the nine members of the FGF family (1–3). All proteins in this family have 30–50% amino acid sequence similarity and a similar exon/intron structure in the protein coding region of the gene (4, 5). FGF-1 is expressed predominantly in neural tissues, including brain and retina (4, 6, 7); it exerts its effect through high affinity cell surface receptors, which are distributed densely throughout the nervous system (8–10). In peripheral nervous system cultures, FGF-1 has been shown to promote neuronal regeneration (11) and survival (12). Within the central nervous system, FGF-1 has been shown to stimulate glial mitogenesis (13, 14) and to have trophic effects (15). The trophic effects of FGF-1 also have been demonstrated in primary neuronal cultures and in neuronal cell lines (5, 7, 12, 16–20). Furthermore, FGF-1 has been shown to promote mitogenesis in neuroblasts (21), to enhance both neurite initiation and elongation in retinal ganglion cells (7), and to stimulate neuronal differentiation and neurite outgrowth in PC12 cells (19). FGF-1 also has been found to be released into the wound cavity following brain lesions (22).

The human FGF-1 gene is over 100 kb long and contains three protein-coding exons and a long 3′-untranslated region. It also contains at least four unique upstream untranslated exons, designated -1A, -1B, -1C, and -1D, which are alternatively spliced to the first protein-coding exon (23, 24). The different transcripts code for the same single chain polypeptide with a molecular mass of 17.5 kDa. The expression of the four mRNA's generated by alternative splicing is regulated in a tissue-specific manner and relies on the use of different promoters (23, 25). Thus, FGF-1A transcript predominates in the human kidney (23), and FGF-1C and -1D transcripts predominate in vascular smooth muscle cells and fibroblasts (26). Within the human brain (23) and retina (27), the FGF-1B transcript predominates. Both FGF-1 mRNA and its protein have been anatomically localized within the central nervous system of adult rodents (28–32). In the present studies, the FGF-1B promoter that is active in the murine brain was isolated, its structure was defined, and its distribution in the adult and developing mouse brain was determined.

MATERIALS AND METHODS

cDNA Library Screening—An oligo(dT) and random primed mouse (BALB/c) brain cDNA library in a λgt11 vector was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). Double-stranded cDNAs were inserted into the *Eco*RI site of the λgt11 vector. A ^{32}P-labeled mouse cDNA probe (33) was used to screen 3×10^6 recombinant phages. Hybridization was performed in 6 × SSC, 1 × Denhardt's solution, 0.5% SDS, 100 μg/ml herring sperm DNA at 42 °C with 1×10^6 cpm/ml ^{32}P-labeled DNA probe. Filters were washed twice in 2 × SSC, 0.5% SDS at room temperature for 15 min each, and once at 60 °C for 30 min in 0.1 × SSC, 0.1% SDS. Thirty-three positive clones were isolated, 10 of which were purified to homogeneity.

Mouse FGF-1 Genomic Clone—A P1 clone (designated 980) containing the mouse FGF-1 gene was isolated by polymerase chain reaction (PCR) screening (Genome Systems, Inc., St. Louis, MO). The mouse genomic DNA from C129 fibroblasts was partially digested with *Sau*3AI and cloned into the P1 cloning vector, pAd10sacB11 (34, 35), at the *Bam*HI site.

Southern Blotting and Hybridization—Genomic DNA from the C129SVJ mouse was prepared by homogenizing the liver in digestion buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA (pH 8.0), and 0.5% SDS. The homogenate was incubated for 18 h at 55 °C in digestion buffer containing 0.1 mg/ml proteinase K. The high

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U67609 and U67610.

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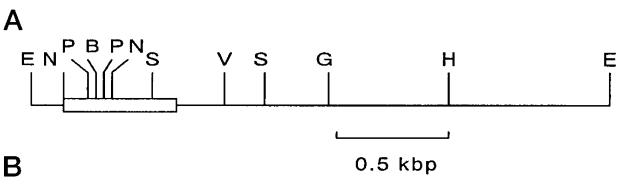
[1] The abbreviations used are: FGF, fibroblast growth factor; kb, kilobase pair(s); bp, base pair(s); PCR, polymerase chain reaction; nt, nucleotide; E, embryonic day; P, postnatal day; Pipes, 1,4-piperazinediethanesulfonic acid.



B

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GAATTCCGGCAGCTTCAGCCCAGGCACCCTGTGACAGCGCAAAGGCTGCCCAGCGGACTT      60
CATTCCCGTCTTGTGATAAAGTGGAGTGAAGAGAGCCCCCCAGCCTGCCAGTTCTTCAGT     120
GCTGAGCCTACCACCGCTGCTTGCTGCCGAGCCATGGCTGAAGGGGAGATCACAACCTTC     180
                                  M  A  E  G  E  I  T  T  F
GCAGCCCTGACCGAGAGGTTCAACCTGCCTCTAGGAAACTACAAAAAGCCCAAACTGCTC     240
A  A  L  T  E  R  F  N  L  P  L  G  N  Y  K  K  P  K  L  L
TACTGCAGCAAACGGGGGCCACTTCTTGAGGATCCTTCCTGATGGCACCGTGGATGGGACA     300
Y  C  S  N  G  G  H  F  L  R  I  L  P  D  G  T  V  D  G  T
AGGGACAGGAGCGACCAGCACATTCAGCTGCAGCTCAGTGCGGAAAGTGCGGGCGAAGTG     360
R  D  R  S  D  Q  H  I  Q  L  Q  L  S  A  E  S  A  G  E  V
TATATAAAGGGTACGGAGACCGGCCAGTACTTGGCCATGGACACCGAAGGGCTTTTATAC     420
Y  I  K  G  T  E  T  G  Q  Y  L  A  M  D  T  E  G  L  L  Y
GGCTCGCAGACACCAAATGAGGAATGTCTGTTCCTGGAAAGGCTGGAAGAAAACCATTAT     480
G  S  Q  T  P  N  E  E  C  L  F  L  E  R  L  E  E  N  H  Y
AACACTTACACCTCCAAGAAGCATGCGGAGAAGAACTGGTTTGTGGGCCTCAAGAAGAAC     540
N  T  Y  T  S  K  K  H  A  E  K  N  W  F  V  G  L  K  K  N
GGGAGCTGTAAGCGCGGTCCTCGGACTCACTATGGCCAGAAGCCATCTTGTTTCTGCCC     600
G  S  C  K  R  G  P  R  T  H  Y  G  Q  K  A  I  L  F  L  P
CTCCCGGTGTCTTCTGACTAGAGGAGTCTGTTCTGAGTGTTCTCTATTTTGGTTGACCCT     660
L  P  V  S  S  D  Trm
ACCATGTTCCCTTGACCATTGGCTGCGCTAACCCTCAGGCCACAGAGCCTGAATTTGTAA     720
GCAGTGCTTCTAAAATAGCCAGTTCACTTTTCTGCTGAGCCCTTCCCCACCACCCCAGCA     780
CAATTTGGAACACAGGGACCAAATTGCTTCAAGAGGACAACTGGCTGGCCAGTCTAGGTC     840
TGGGTTTGGATATCCGGATGCCTCAGGGCACCTGCTACAAGCTGGACCTGGTGATGCAAA     900
GTTGGGGGCCATACGAAGCATTCAAAGGGGTCTAAGAAGTGGGTTCTTAGAAAGATACCC     960
ACTGCATGCTCTTTCCCCTTCTGAGCAATCCCTGGTTTTAAGCACCCCAGGCAATTAGCA    1020
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AGTAAGAAGGCAAGAGTCAGCCACAGGGAGTCAGGATGAGCCCACAGCCCAGCAGTTATC    1260
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TCTTCAGCCGTGGATGTTAGCCTCTAAAAAGGAAGATGTTAGACGGGAGGAGGAGGAGAG    1440
AAAAAGTAATATCACGTCACTGTGTGCTTGGCCACCACGAAACTGGGGTTGTGGGGGTCC    1500
CAGTACGATGGCCCCAAATAGCCCATACATTCAGATCATCTGTATTCAGATTCATTTGTA    1560
TGACTGTGAAACCTGATTTGGTGGGGCTGGTGTGGGGGAGGTCTGTGTCTTGAAAGGCCC    1620
GCAAGTCCCATAGATGCTGCTGGCTTCCCCCACCATGCTTCAAGAGCAGCCCCAGAGAATG    1680
CTGTTCAAATGAAGGTTGCTCAGACTTCACCCTCTTTTACCTCCAGTGCCCGGGTTTTCC    1740
TGAGTATCCATCCAGGCAGTGTCCATAGTTAGTGAAGCATGGTTTCAAGGCACCTTGGCC    1800
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GCTTCCTCCTTAAAGTAAACCTGGCCCAAGTGCCCAGCAAATTAGAAACACTATTCATCT    1980
CTGACATAGGACACAAACGCCATGAGAAGGCCTTTCATACGCCAATTTAATATACTGTGA    2040
GACTTATCTTCTCGTTGCTTCTTATATAGCTTGAAGGCATTAAATAGATTCACAATCAGC    2100
CACAGAGGATAATAACAGCCTAGAATGTAAAAGCTACTTAGTTATCTAACATGCTACATT    2160
CATTCATGAGGCCTTTCCTCTTTGTCTTCCACTGTGCTTTAAGAATGGGTATCTAGGGGC    2220
TGGTGAGATGGGACAGGAGTTCAGTTCCTAGCATTCACAGTGTGATGCTCAGACCCAATT    2280
CTAACTCCAACTCCAGGGGTACTGCATACACATGTACAAACCCACACTTTTCAGAAATA    2360
TAAACCAATAATATGGTTGTGGATTAAAACTGATGACTGTGTTCATTTGTTTGACAGGGA    2400
TCTAAAGTCATTATTTGCTGGAAAAAAATCCTTATAGCAACACTGTCATATCTTAGCACA    2460
GAAGTCAGATGACACTCTTGAGAGTTCAGAGTGCTAATGCCGAATTC                 2507
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FIG. 1. *Panel A*, restriction enzyme map of mouse FGF-1 cDNA. The *open box* represents the coding sequence for mouse FGF-1 cDNA. *B*, *Bam*HI; *E*, *Eco*RI; *G*, *Bgl*II; *H*, *Hin*dIII; *N*, *Nco*I; *P*, *Pst*I; *S*, *Sph*I; *V*, *Eco*RV. *Panel B*, nucleotide and deduced amino acid sequences of mouse FGF-1 cDNA. The plasmid DNA containing the cDNA insert was sequenced by the chain termination method. The 5′-end of the first protein coding exon starts at nucleotide 120. The upstream untranslated sequence of the cDNA, which is derived from exon -1B, is *underlined*. The *inverted triangles* represent the exon junctions. The GenBank™ accession number is U67610.

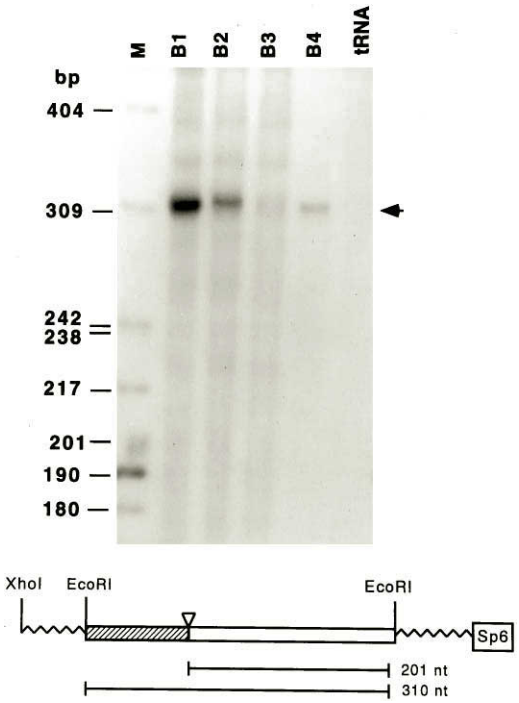

FIG. 2. **RNase protection analysis using an FGF-1B cDNA-derived riboprobe.** Plasmid FGF-1B cDNA digested with *Xho*I was used as the riboprobe template. The *open box* represents the sequence derived from the first coding exon. The *open triangle* denotes the 5′ boundary of this exon. The *cross-hatched box* represents sequence derived from exon -1B and is unique to the FGF-1B cDNA. Total RNA from four mouse brains was used in RNA-RNA hybridization reactions. *Arrow* indicates the protected fragment representing FGF-1B transcript. Lane abbreviations are *M*, pBR322 DNA digested with *Msp*I; *B1*, 40 μg; *B2*, 30 μg; *B3*, 25 μg; *B4*, 20 μg of total RNA from four mouse brains.

molecular weight DNA was collected by spooling with a glass rod. Twenty μg of genomic DNA was digested with appropriate restriction enzymes, separated on a 0.8% agarose gel, and transferred to a nylon filter (Hybond-N, Amersham). The filters were prehybridized in 40% formamide, 6 × SSPE, 3% SDS, 0.5% powdered milk for 12 h at 42 °C. Hybridization (48 h) with 1×10^6 cpm/ml probe in prehybridization buffer was carried out at 42 °C. DNA from the P1 clone was prepared by the standard plasmid DNA preparation method (36) and digested with appropriate restriction enzymes, electrophoresed on 0.8% agarose gels, and blotted onto nylon filters according to standard procedures (37). The DNA was hybridized with the 109-bp -1B untranslated exon probe.

RNase Protection Analysis—RNA was isolated by the guanidinium thiocyanate method, with a cesium chloride centrifugation step (38). RNase protection analysis was performed according to Ausubel *et al.* (39) with minor modifications. Probe sequences were cloned into either pBluescript II KS(+) (Stratagene, La Jolla, CA) or PCR II vector (Invitrogen, San Diego, CA). The ^{32}P-labeled antisense riboprobes were generated by *in vitro* transcription using an RNA transcription kit (Stratagene, La Jolla, CA). Twenty μg of total RNA and the antisense riboprobe (5×10^5 cpm) were denatured at 85 °C for 5 min and hybridized overnight at 42 °C in a buffer containing 40 mM Pipes (pH 6.4), 400 mM NaCl, 1 mM EDTA (pH 8.0) and 80% formamide. Following hybridization, the reaction product was treated with RNase A and T1 for 60 min at 37 °C to remove free probe. The protected fragments generated by annealing FGF-1 mRNA with the complementary probe were analyzed on a 6% denaturing polyacrylamide gel.

Subcloning, Nucleotide Sequencing, and Sequence Comparison—The longest FGF-1 cDNA obtained from screening the brain cDNA library was subcloned into the PCR II vector at the TA cloning site. The 7.0-kb *Eco*RI fragment from P1 DNA, which hybridized to the 109-bp untranslated exon -1B probe, was cloned into the pBluescript II KS(+) vector. DNA was sequenced by the dideoxy chain termination method of Sanger *et al.* (40) using double-stranded DNA as the template. The

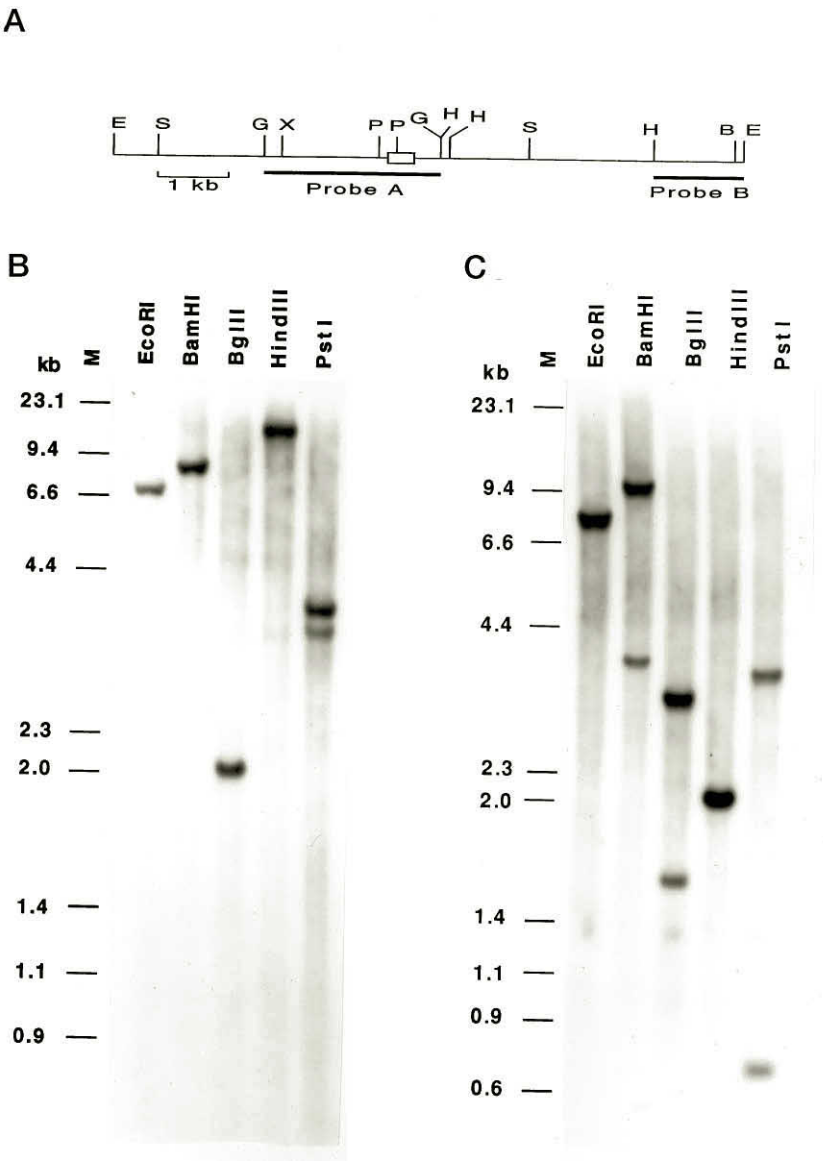

FIG. 3. *Panel A*, restriction enzyme map of 1B promoter containing the untranslated exon -1B of the FGF-1 gene. The 7.0-kb *Eco*RI fragment from P1 clone 980, which hybridizes to the 109-bp upstream untranslated sequence of mouse FGF-1B cDNA, was subcloned into pBluescript II KS(+). The *box* represents the FGF-1B upstream untranslated exon present in FGF-1 cDNA. *B*, *Bam*HI; *E*, *Eco*RI; *G*, *Bgl*II; *H*, *Hin*dIII; *P*, *Pst*I; *S*, *Sac*I; *X*, *Xba*I. *Panels B* and *C*, hybridization of mouse genomic DNA with the mouse FGF-1 gene probe. Genomic DNA from mouse liver was digested with indicated restriction enzymes. After electrophoresis, the DNA was transferred to a nylon filter and hybridized to a 2.0-kb *Bgl*II fragment (probe A, *panel B*) and a 0.9-kb *Hin*dIII-*Eco*RI fragment (probe B, *panel C*) obtained from the 7.0-kb *Eco*RI fragment. A mixture of λ DNA digested with *Hin*dIII and φX DNA digested with *Hae*III was used as a molecular size marker.

ALIGN and DOTPLOT programs of DNAstar (Madison, WI) were used to compare the mouse and human exon -1B sequences.

Riboprobe Preparation for in Situ Hybridization—Antisense and sense RNA probes for FGF-1 cDNA and FGF-1B transcript were generated by PCR. For FGF-1, a 150-bp *Eco*RI-*Bam*HI fragment containing the first and second protein-coding exons, but excluding any 5′-untranslated exons, was cloned into pBluescript II KS(+). The resulting plasmid was linearized with *Eco*RI and *Bam*HI for the subsequent production of antisense and sense RNA probes, respectively. The sequences of the upstream and downstream primers for the FGF-1B probes were 5′-AGCTTCAGCCCAGGCACC-3′, and 3′-CTGAAGAACTGGCAGGCT-5′, respectively. The 109-bp DNA fragment was amplified using the P1 clone 980 DNA as template and was ligated into a PCR II vector. Plasmids were linearized with *Hin*dIII and *Xba*I for the subsequent production of antisense and sense probes, respectively. The linearized templates were purified and transcribed as described (41); the specific activity of each riboprobe was approximately 1–1.5 × 10^9 dpm/μg.

In Situ Hybridization—Adult and developing C57BL/6 mice, bred from stocks obtained from Jackson Laboratories (Bar Harbor, ME), were decapitated after inhalation anesthesia (Metofane, Pitman-Moore, NJ). The brain and cervical spinal cord were removed rapidly, frozen on dry ice, and stored at −70 °C. Coronal and sagittal cryostat sections, 20 μm thick, were thaw-mounted onto subbed (300 bloom gelatin and chrome alum) slides and stored at −70 °C. Slide-mounted sections were brought to room temperature and hybridized with ^{35}S-labeled probes as described (42). After incubation, the sections were washed in 2 × SSC at room temperature for 10 min, followed by incubation in RNase A solution (20 μg/ml RNase A in 10 mM Tris-HCl, 500 mM NaCl, 1 mM EDTA (pH 8.0)) for 30 min at 37 °C. RNase-treated sections were washed at increasing stringency, as follows: 2 × SSC (10 min), 1 × SSC (10 min), 0.5 × SSC (10 min), 0.25 × SSC (10 min), and 0.25 × SSC (60 min) at 70 °C. After final washes in 0.25 × SSC (10 min) at room temperature, sections were dehydrated (1 min each) through 70%, 80%, 90% and 100% EtOH. All washing solutions, except RNase A solution, contained 10 mM 2-mercaptoethanol to prevent nonspecific binding. The nonspecific hybridization signal was determined by exposing adjacent sections to sense RNA probes.

Autoradiograms were generated as follows. Acid-washed coverslips (no. 0, Corning Glass, Corning, NY), previously coated with a uniform layer of photographic emulsion (NTB-2, Eastman Kodak Co.) were apposed to the slide-mounted sections and exposed for 6 days, at 4 °C. Coverslips containing the autoradiographs were developed in Kodak Dektol developer (diluted 1:1 with distilled water) for 2 min at 17 °C, fixed for 3 min in Kodak Rapid-Fix, washed in distilled water for 30 min, and mounted onto microscope slides. After the coverslips were developed, the slides containing the sections were dipped in Kodak NTB-2 photographic emulsion at 38 °C, dried overnight, exposed at 4 °C, and developed as above. Emulsion covered sections were counterstained with cresyl fast violet and used for the cellular localization of grain density.

RESULTS

Cloning, Sequencing, and Characterization of Mouse FGF-1B cDNA—A mouse brain cDNA library was screened with a 530-bp mouse cDNA probe (33) to obtain the 5′-extended se-

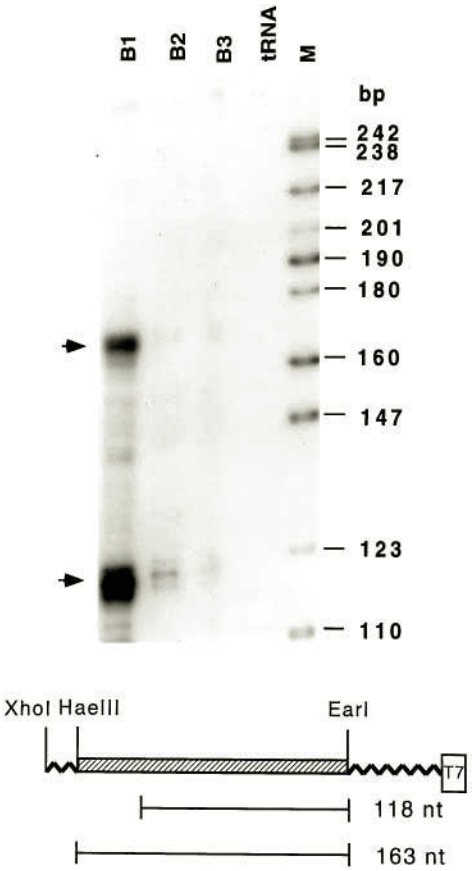

FIG. 4. **Determination of the transcription start site of brain-specific FGF-1B mRNA by RNase protection analysis.** The *Hae*III-*Ear*I fragment of the 1B promoter (nucleotides 1030–1092 in Fig. 5) was cloned into pBluescript II KS(+) and linearized with *Xho*I; the antisense RNA was transcribed using T7 RNA polymerase. The RNase protection results show two protected fragments, indicated by *arrows*. The most abundant fragment is at 118 nucleotides, and the less predominant fragment is at 163 nucleotides. RNAs from three different mouse brains (60, 20, and 10 μg in *lanes B1*, *B2*, and *B3*) were used in the hybridization reactions. *Lane M*, pBR322 DNA digested with *Msp*I.

quences of the murine FGF-1 mRNA. Thirty-three positive clones were isolated, and 10 were plaque-purified to homogeneity. Of these 10 clones, the one containing the longest cDNA insert (pC7, 2.5 kb) was chosen for further analysis. The insert from the phage clone was amplified by PCR using λ forward and reverse primers. Subsequently, it was cloned into PCR II vector and subjected to restriction enzyme mapping analysis (Fig. 1*A*). The complete nucleotide sequence of the FGF-1 cDNA was determined by the chain termination method (Fig. 1*B*). Sequence analysis revealed that the FGF-1 cDNA insert had a 153-bp untranslated sequence at the 5′-end, an open reading frame that encodes the 155 amino acid residues of the FGF-1 protein, and an 1889-bp 3′-untranslated region. The 3′-end of the cDNA clone contained multiple polyadenylation signals (AATAA) spanning nucleotides 2111–2115 and 2367–2371. In addition, a polyadenylation sequence (ATAAA) characteristic of rat FGF-1 cDNA (43) was present at nucleotides 2360–2364.

The 109-bp sequence of the cDNA derived from the upstream untranslated exon -1B was amplified from mouse genomic DNA by PCR, using primers spanning nucleotides 11–28 and 119–102 of upstream and downstream regions, respectively (Fig. 1*B*). The DNA insert was sequenced and compared to the untranslated -1B exon of the human FGF-1 gene. There was 77% sequence similarity between the two regions, suggesting that the upstream untranslated nucleotide sequence spliced to the first protein coding exon of the mouse cDNA clone was the

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GGAGAGAGACCTCAGGTACACTCCCAGTTCCCCTAGGTCCACGTGCCTCATCTCTATCTG   60
AACTATCAGGTGGGCAGGCTGGACCACACCACCGCACACACACACGCACACGCACACGCA  120
CACACACACACACACACACACACGCTCGTCGCTCGCTCACTCATGCCTGACCCAGGCT    180
GAAACGCCTGTGGAAGGGAGTCCTCTTCACCAACGATCGGAAACAACTAATCATGGTTTC  240
AGTTGCTTGCTTTGATCCATACCGCCTGAGTCCACTTCATTCCCTACCATAAGCCAGACT  300
TATTTGTTCAGATGGCAGCCTTCCCCCTCTGTGGTAAGAAGATGGGCATGCAGGGCCGAC  360
TGAGGAGGTCCCAGGCTCACCTGCGGACTCTCCTAGTCTAGCAGGGATGGGTGGCCTATG  420
GTGCAGAATGCTGGGGACCTCCCTCTGCCGTCCCTCCCTAGCTCTAACCAGGTGGTCACT  480
ACCTTGCCTAGCTCTGTAGAACACACTTGGTAGGGCTTGGCTCTGGGAATGGTGTAGATG  540
CATAGACAGACACTGCCACACACAGACACCTACACCCTGCATGGGGACAGATGACAGAAC  600
AGAGAAGGGCTCCCTTTTACCAGCAGTGTGATTTTAGAGGAGTGTCTTTCAACACAACCA  660
GTTGTTTCCCTGGTAACAGAGAGGCCTCAAAATAAACAGGACTCTGCTCAGACATTAGTC  720
CACTGGGCTCAGACTTCTGCCCCAAGACAAACCGTGCTAAAATAACACCCAAGGTAGTTG  780
CTGCCCCTGTCTGCCTCTCTGCAGTCCCAGGTCTGCTGCAGACTGTGAAGAGCTAGAGGC  840
ACTTAAGAGTTTGTTGTGCACTGATGTGGTAGGGTGGGGCTGTGGGGTGGTCTGCAGGCA  900
GGGGAGGGGAGCCCCTCTGCTGATGAGCAAGGGCCAAGGGCAGACCTGGAGGCCAGCGCT  960
CTCTGCTCCCTGCACCCGCCTCCCTGCTTCCCACACAGCCTCTGGACTGGCATGGTGTCT 1020
                HaeIII ↓                          ♦
GGAGGCGGGCCAGCAACCTGATGTGCATGCCACAGCCCGTCCCTCTCCCCACACAGAGCT 1080
GCAGAAATCCTGAGGCTCAGAGAGCGCTGGAGAGGCAGCTTCAGCCCAGGCACCCTGTGA 1140
                                          ↓ EarI
CAGCGCAAAGGCTGCCCAGCGGACTTCATTCCCGTCTTGTGATAAAGTGGAGTGAAGAGA 1200
                    ▽
GCCCCCCAGCCTGCCAGTTCTTCAGGTAAGAATTAGGGGTGTGTTCATTCTATCCCGAGC 1260
TGGATTTGGCTGTTTGTACAAAGCTAGTAGGAAGGGAAGAGAAGAGGAACCTGTAAGGTA 1320
GAGAAGTGTT                                                    1330
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FIG. 5. **Nucleotide sequence of the FGF-1B promoter, exon -1B and their flanking regions.** The *underlined* sequences were present in the cDNA clone. The *open triangle* indicates the 3′-end of the -1B exon. The *closed diamond* represents the major transcription start site of the FGF-1B mRNA. *Shaded box* represents the sequence corresponding to the RR2 regulatory region in the human 1B promoter. Sequences with 80% or higher sequence similarity to the human 1B promoter are *double underlined*. Potential AP-1, SP1, and AP2 binding sites are indicated with a *dashed line*. The *downward arrows* indicate the cleavage sites for the indicated restriction enzymes. The GenBank™ accession number is U67609.

-1B exon of the mouse FGF-1 gene. Expression of the FGF-1B transcript was examined by RNase protection assay using RNA isolated from C129SVJ mouse brain. A 310 bp riboprobe, containing 109 bp of the 5′-untranslated exon spliced to 201 bp of exon 1 (Fig. 2), protected a single fragment of 310 nucleotides (nt) (Fig. 2). Densitometric scanning showed that greater than 99% of the FGF-1 transcript was FGF-1B. The presence of exon 1 in the probe, in addition to the -1B sequence, permitted any non-1B transcripts to be recognized. No 201-nt protected fragments were observed (Fig. 2). Taken together, these results suggest that mouse brain expresses the FGF-1B transcript, exclusively.

Isolation and Characterization of an FGF-1B Genomic DNA Clone—To map the mouse FGF-1 gene more extensively, a 7.0-kb *Eco*RI DNA fragment of the P1 980 phage clone, which hybridized to the 109-bp mouse -1B untranslated exon probe, was subcloned and characterized by restriction enzyme map-

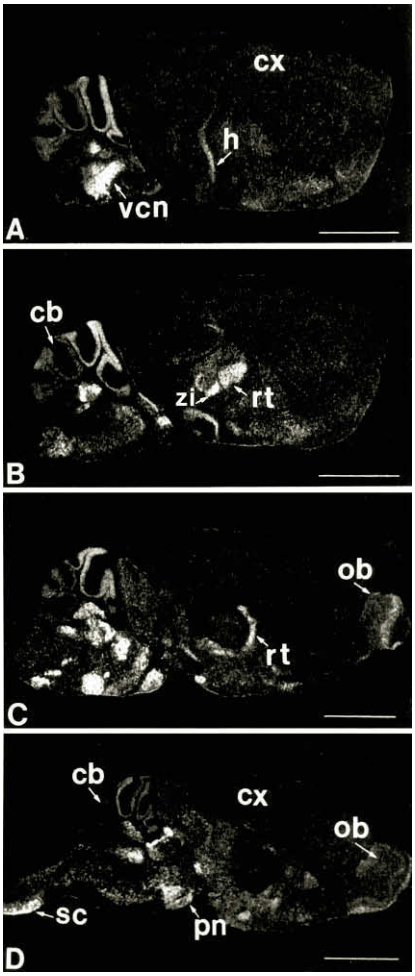

FIG. 6. **Darkfield autoradiographs showing the lateral to medial (*A–D*) distribution of FGF-1 mRNA in sagittal sections through the adult (P60) mouse brain.** The ^{35}S-labeled cRNA probe was designed to recognize the protein coding region of the FGF-1 gene and hybridized, therefore, to all splice variants of FGF-1 transcript. *cb*, cerebellum; *cx*, cortex; *h*, hippocampus; *ob*, olfactory bulb; *pn*, pontine nucleus; *rt*, reticular thalamic nucleus; *sc*, spinal cord; *vcn*, ventral cochlear nucleus; *zi*, zona incerta. *Scale bars*, 2 mm.

TABLE I

The distribution of FGF-1B mRNA in the adult (P60) mouse brain

Subjective grain density values are indicated by +, with +++++ being the highest, and + (just above background) the lowest. Asterisks (*) indicate labeled cells scattered within the region. All other central nervous system regions were unlabeled. A virtually identical distribution was observed for FGF-1 mRNA.

Location in brain	Regional grain density
Cervical spinal cord	
Supraspinal nucleus	+++++
Dorsomedial nucleus	+++++
Medulla	
Lateral reticular nucleus*	++++
Ventral reticular nucleus*	++++
Nucleus ambiguus	+++++
Hypoglossal nucleus	+++++
Prepositus hypoglossal nucleus	++
Roller nucleus	++++
Dorsal motor nucleus of the vagus	+++
Lateral cuneate nucleus	++
Medial, lateral and superior vestibular nuclei*	+++
Facial nucleus	+++++
Accessory facial nucleus	+++++
Dorsal cochlear nucleus	++
Ventral cochlear nucleus	++++
Cerebellum	
Granule cell layer of the anterior lobe	+++++
Granule cell layer of the posterior lobe	++
Deep cerebellar nuclei	+++
Midbrain and Forebrain	
Abducens nucleus	++++
Trigeminal motor nucleus	+++++
Mesencephalic trigeminal nucleus	+++++
Locus coeruleus	++
Nucleus of the trapezoid body	++++
Reticular tegmental pontine nucleus	+++
Pontine nucleus	+++
Central oral pontine nucleus*	+
Central caudal pontine nucleus*	+
Reticular caudal pontine nucleus*	++++
Occulomotor nucleus	+++++
Ruber nucleus	+
Interpeduncular nucleus	++
Lateral mammilary nucleus	++
Substantia nigra, pars compacta	+
Zona incerta, pars ventralis	++
Hippocampal pyramidal layer (Ca3 region)	++
Ventral region of the medial geniculate nucleus	+
Medial habenular nucleus	+++++
Anterodorsal thalamic nucleus	+++
Reticular thalamic nucleus	+++
Glomerular layer of the olfactory bulb	++

ping (Fig. 3*A*) and sequencing. To ascertain the presence of the -1B untranslated exon in the mouse genome, C129SVJ genomic DNA was digested with restriction enzymes and hybridized to probes A (Fig. 3*B*) and B (Fig. 3*C*). Probe A was a 2.0-kb *Bgl*II fragment containing the -1B exon, and probe B was a 0.9-kb *Hin*dIII-*Eco*RI fragment obtained from the 3′-end of the 7.0-kb *Eco*RI fragment. As seen in Fig. 3 (*B* and *C*), the 7.0-kb *Eco*RI fragment hybridized to both probes, thus confirming the presence of the -1B exon in the mouse genome, and further indicating that FGF-1 is a single copy gene.

An RNase protection assay was performed to position the FGF-1B mRNA start site within the genomic clone containing the -1B exon and the 5′-flanking sequences. Using a riboprobe of 442 bp in length, which extended 383 bp upstream from the 3′-end of exon -1B, a major protected fragment of 151 nt was detected. Additional minor bands with sizes greater than 250 nt also were observed (data not shown). To map the major transcription start site more precisely, a shorter riboprobe was derived from a plasmid containing a 163-bp *Hae*III-*Ear*I fragment of the 1B promoter cloned into pBluescript II KS(+) by linearization with *Xho*I (Fig. 4). The results show protection of a predominant fragment of 118 nt (67% of total), which is consistent with the results obtained with the longer probe, and confirms the major start site. A minor fragment of 163 nt (33% of total), corresponding to the truncated product of longer transcripts from minor start sites, also was observed. This suggests the existence of multiple transcription start sites for the FGF-1B transcript. This result also indicates that the major transcription start site of the 1B transcript resides 151 nt upstream from the 3′-end of the -1B exon.

Sequence analysis of the 5′- and 3′-flanking ends of the -1B exon showed that the region upstream of the transcription initiation site did not contain consensus CAAT or TATA boxes (Fig. 5). However, there were potential SP1 transcription factor binding sites (CCCGCC or GGCGGG) located at nucleotide positions 975 and 1024. Furthermore, putative AP-2 DNA regulatory elements were located at nucleotide positions 872, 892, and 933. There was, in addition, a potential AP-1 transcription factor binding site (TGAC(T/A)(C/G)A), residing at nucleotide position 592. An additional feature was the presence of an RR2 enhancer element (640–680) similar to that found in the human FGF-1 gene, which seems to be important for the expression of FGF-1B transcript in human brain (25). The sequences surrounding the RR2 region (Fig. 5) has high similarity (80% or more) with the corresponding region in the human sequence,

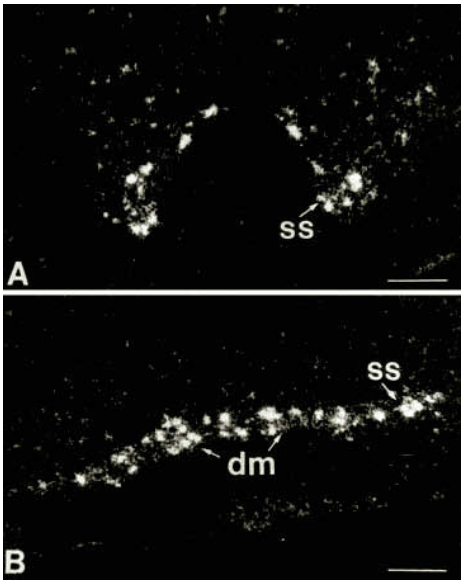

FIG. 7. **Darkfield autoradiographs of coronal (*A*) and sagittal (*B*) sections showing the distribution of FGF-1B mRNA in the adult (P60) cervical spinal cord.** The ^{35}S-labeled cRNA probe (FGF-1B) was designed to recognize the exon -1B region of the FGF-1 gene, and hybridized exclusively to the FGF-1B transcript. *dm*, dorsomedial nucleus; *ss*, supraspinal nucleus. *Scale bars*, 250 μm.

suggesting biological significance of this region. DOTPLOT analysis of the mouse 1B sequence showed that a stretch of 1330 bp of the fragment, which contained exon -1B and its 5′- and 3′-flanking sequences, has approximately 70% similarity with the corresponding region in the human FGF-1 gene.

Expression of FGF-1B Transcript in Adult and Developing Brain—The expression of FGF-1 (Fig. 6) and FGF-1B (Figs. 7–9) mRNAs was examined in the brains of adult C57BL/6 mice using *in situ* hybridization with ^{35}S-labeled cRNA probes. Consistent with our RNase protection results, the distribution of the two messages was identical (compare Fig. 6 with Figs. 7–9). Each message was restricted largely to sensory and motor nuclei in the midbrain, brainstem, and spinal cord, and to the granule cell layer and deep nuclei of the cerebellum. The general expression pattern, and relative intensity of the hybridization signal within labeled regions, are shown in Table I. The regions labeled at the highest grain density included the supraspinal (Fig. 7, *A* and *B*), ventral cochlear, ambiguus, hypoglossal, facial, accessory facial, motor trigeminal, mesencephalic trigeminal, occulomotor, and medial habenular nuclei (Figs. 8 and 9). The granule cells of the anterior cerebellar cortex also were densely labeled (Fig. 6, *A–D*). Lower levels of expression were observed in other brainstem and midbrain regions (Table I), and the message was very low, or absent, in forebrain structures.

In developing mice, the hybridization signal was absent

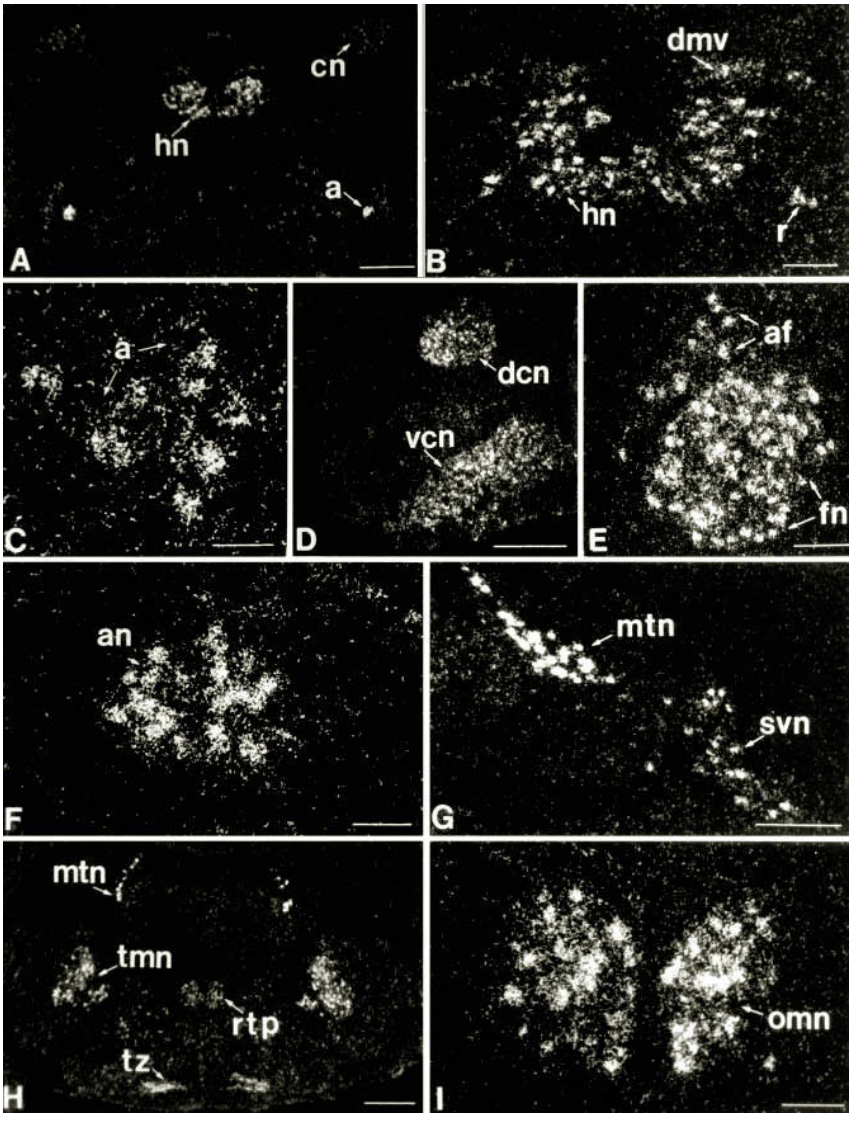

FIG. 8. **Darkfield autoradiographs showing the distribution of FGF-1B mRNA in selected sensory and motor nuclei in the adult (P60) brainstem and midbrain (*panels A–I*).** All brains were sectioned in the coronal plane, except *D*, which was sectioned in the sagittal plane. *a*, nucleus ambiguus; *an*, abducens nucleus; *cn*, cuneate nucleus; *hn*, hypoglossal nucleus; *dmv*, dorsal motor nucleus of the vagus; *r*, roller nucleus; *vcn*, ventral cochlear nucleus; *dcn*, deep cerebellar nuclei; *fn*, facial nucleus; *af*, accessory facial nucleus; *mtn*, mesencephalic trigeminal nucleus; *rtp*, reticular tegmental pontine nucleus; *svn*, superior vestibular nucleus; *tmn*, trigeminal motor nucleus; *tz*, trapezoid nucleus; *omn*, occulomotor nucleus. *Scale bars*: *A*, 500 μm; *B* and *F*, 250 μm; *C* and *H*, 50 μm; *D*, 2 mm; *E*, 200 μm; *G*, 1 mm; *I*, 100 μm.

throughout the CNS at embryonic day (E) 13. By E16, moderate levels of FGF-1B mRNA were present in the trigeminal and superior cervical ganglia (Fig. 10, *A* and *B*). Within the brain, by E18, very low levels of autoradiographic grain density were detectable over the supraspinal, facial, hypoglossal, trapezoid, occulomotor, mesencephalic trigeminal, and trigeminal motor nuclei (Fig. 10*D*). Moderate grain density also was detectable in the pia-arachnoid membrane lining the mesencephalic flexure (Fig. 10*C*). During postnatal week 1, both the intensity of the autoradiographic signal and the number of clearly labeled brainstem nuclei increased (Fig. 11). Between postnatal days (P) 10 and 20, FGF-1B mRNA expression increased in all labeled nuclei, most notably in the pontine, medial habenular and deep cerebellar nuclei, while labeling in the forebrain was retained at low levels. Although only weakly labeled at earlier time points, by P40, FGF-1B mRNA expression increased markedly in granule cells of the anterior cerebellar cortex (lobule 1 through the dorsal aspect of 6), while much lower expression levels were retained in posterior lobules (Fig. 6, *A–D*).

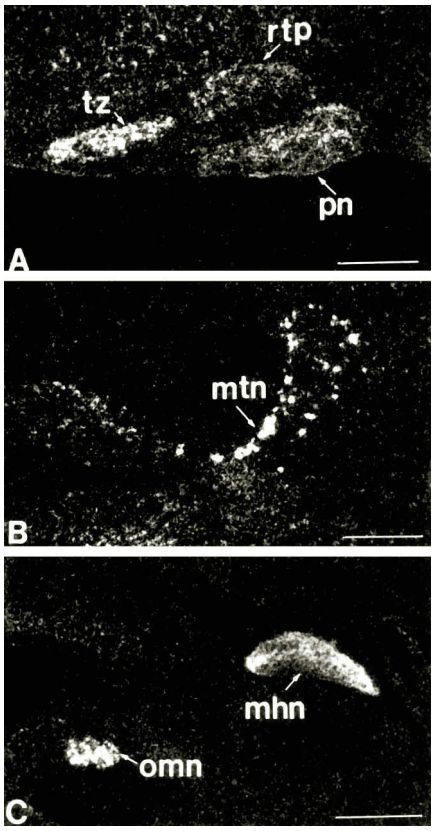

FIG. 9. **The distribution of FGF-1B mRNA in sagittal sections through the adult (P60) midbrain.** *A*, pontine (*pn*), trapezoid (*tz*), and reticular tegmental pontine (*rtp*) nuclei; *B*, mesencephalic trigeminal nucleus (*mtn*); *C*, occulomotor (*omn*) and medial habenula nucleus (*mhn*). Scale bars: *A* and *B*, 500 μm; *C*, 1 mm.

DISCUSSION

We report here the isolation of a full-length murine brain FGF-1 cDNA containing a 5′-untranslated exon -1B spliced to the first protein-coding exon. The mouse FGF-1 cDNA had a single open reading frame of 465 bp. RNase protection analysis showed that the FGF-1B transcript is the sole FGF-1 transcript in the mouse brain. The transcription start site of the mouse upstream untranslated exon -1B was mapped by RNase protection analysis. Potential regulatory sequences, including an AP1 element and SP1 binding sites, were identified in the promoter region. Expression in the adult and developing mouse brain was examined using *in situ* hybridization with ^{35}S-labeled probes specific for the protein coding (FGF-1) and brain-specific (FGF-1B) regions. The first probe was designed to hybridize to all splice variants of FGF-1 transcripts, whereas the second probe hybridized only to the FGF-1 transcript that contained the 5′-untranslated sequence derived from exon -1B. The two signals became clearly detectable during postnatal week 1. Adult levels of grain density were reached during the 3rd and 4th postnatal weeks as the signal became restricted largely to sensory and motor nuclei in the brainstem and spinal cord, and to the anterior granule cell layer and deep nuclei of the cerebellum.

Comparison of the mouse FGF-1 cDNA with that of the human and bovine sequences revealed considerable similarity in the 5′-untranslated region. In previous studies, FGF-1 cDNA clones containing the -1B untranslated exon were isolated from human brainstem (44) and bovine retinal (45, 46) cDNA libraries. FGF-1B mRNA has been shown to be the predominant FGF-1 transcript in the human brain (23) and retina (27). In the present study, we showed that the mouse 1B sequence had over 77% similarity to the corresponding region in the human, and 68% to the bovine sequence. In each of the three species, the -1B untranslated exon was located 34 bp upstream of the

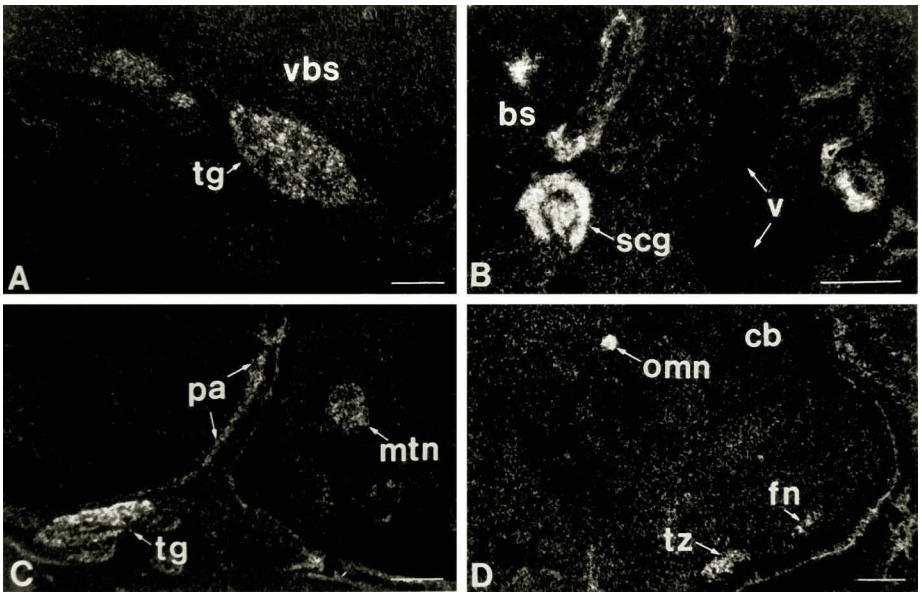

FIG. 10. **Darkfield autoradiographs showing FGF-1B mRNA expression in sagittal sections through the embryonic mouse brain.** *A*, the trigeminal ganglion at E16; *B*, the superior cervical ganglia at E18; *C*, the pial membrane lining the mesencephalic flexture at E18; and *D*, brainstem and midbrain nuclei at E18. *bs*, brainstem; *fn*, facial nucleus; *pa*, pia-arachnoid; *scg*, superior cervical ganglion; *tg*, trigeminal ganglion; *tmn*, trigeminal motor nucleus; *tz*, trapezoid nucleus; *v*, unlabeled vertebra; *vbs*, ventral brainstem. *Scale bars*: *A*, 250 μm; *B–D*, 500 μm.

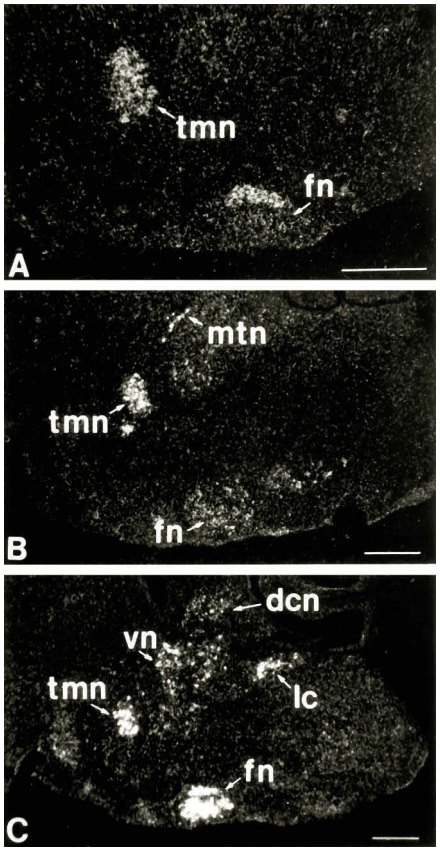

FIG. 11. **FGF-1B mRNA in sagittal sections through the postnatal mouse brain at P3 (*A*), P8 (*B*), and P14 (*C*).** *fn*, facial nucleus; *lc*, lateral cuneate nucleus; *mtn*, mesencephalic trigeminal nucleus; *tmn*, trigeminal motor nucleus; *vn*, superior and lateral vestibular nuclei. *Scale bars*, 250 μm.

ATG start codon.

RNase protection analysis of the 5′-untranslated region identified the presence of multiple RNA transcription start sites with the major site residing at 151 nt upstream from the 3′-end of exon -1B. This is in agreement with earlier studies showing that the human 1B promoter has similar start sites located at positions −110, −135, and −140 (23). The biological significance of multiple transcription start sites is still undetermined; however, they are often associated with genes containing non-TATA box promoters. Although the human FGF-1A promoter contains consensus TATA and CAAT variant sequences, both the human (23) and murine 1B promoter lack these regulatory regions. The murine 1B promoter region did contain multiple GC box sequence motifs. Only one such GC box was present in the corresponding region (−229) in the human gene (25). In addition, the consensus AP-1 core DNA sequence, TGAC(T/A)(C/G)A, was located at position 592 in the mouse FGF-1B promoter region. A similar TGAC(T/A)GA sequence has been found in the human 1B promoter region at position −553. It is of interest that RR2 enhancer elements, present in the human FGF-1B promoter and thought to be involved with the regulation of 1B gene expression (25), also spanned positions 640–680 of the murine 1B promoter. This region has been shown to contain the binding site for a tissue-specific 37-kDa nuclear protein; mutation of the RR2 region results in the loss of nuclear protein binding and a decrease in reporter gene expression (25).[2] Protein binding at this site may be required for the correct assembly of an oligomeric complex that can interact with the initiation complex at the start site. Current evidence indicates that the formation of a stereospecific nuclear protein complex may be the underlying mechanism responsible for a high level of gene activation and specificity (47).

Some degree of cross-species variability has been detected in the unusually long 3′-untranslated region of the FGF-1 gene, a region thought to be involved in the regulation of gene expression through mRNA destabilization (44). This region in the murine sequence has high (85%) similarity with rat (43), and somewhat less with human (67%) and bovine (58%) genes. As in human and bovine cDNA, the 3′-untranslated sequence of the murine cDNA has at least two polyadenylation signals (AATAA) at positions 2111–2115 and 2367–2371, indicative of transcript termination sites. However, unlike the human and bovine cDNAs, the murine cDNA contained an additional ATAAA polyadenylation sequence at position 2360–2364, which corresponds to the only polyadenylation signal sequence present in rat FGF-1 cDNA (43). This suggests divergence of DNA sequences during evolution, or preferential usage of the polyadenylation sequence by a particular species.

Regional analysis of the 1B hybridization pattern in the adult murine CNS revealed that it was identical to that of the FGF-1 cDNA probe, which detected all splice variants of the FGF-1 transcript. These studies, together with the findings of the RNase protection assay, indicate that in the murine CNS, the FGF-1 distribution is accounted for entirely by the FGF-1B transcript. Our *in situ* hybridization data are in general agreement with earlier studies, in which FGF-1 was localized in neurons in the adult (28, 30, 31) and developing (30, 48) rat brain. In these studies, both FGF-1 mRNA (28) and its protein (28, 30, 31) were present in subcortical regions, with only very limited expression in the forebrain (28). Our *in situ* hybridization studies also are in general agreement with an earlier study in the mouse brain (29), in which several nuclei in the brainstem and spinal cord were stained by an antibody to the FGF-1 protein. However, in that study, cerebellar Purkinje cells were found to be immunoreactive. This contrasts with the present studies, in which Purkinje cells contained neither FGF-1B nor any other FGF-1 messages. Importantly, in our studies on the mouse, the granule cells of the anterior cerebellar cortex showed a distinct, highly compartmentalized hybridization signal. This differs from earlier studies on the mouse and rat, in which the cerebellar granule cell layer appears to lack the FGF-1 signal. Neither were our findings of highly localized and discreet FGF-1 and FGF-1B expression patterns in agreement with studies by Wilcox and Unnerstall (32), who found FGF-1 mRNA to be distributed somewhat diffusely throughout the adult and developing rat brain. Part of our developmental data do concur, however, with those of Oellig *et al.* (49), who reported that FGF-1 was expressed in the dorsal root ganglia at E18. There is a general consensus in the studies mentioned above that the cellular localization of the FGF-1 message and its protein appears to be largely neuronal, with little or no glial component. This is of interest because high levels of FGF-1B transcript occur in both glioblastomas and in glioblastoma-derived cell lines. In these studies, expression increased with the malignancy of the tumor (27). The lack of glial labeling at any of the time points examined in our studies, as well as the absence of FGF-1 gene expression in a normal fetal glial cell line (27), suggests that in normal glia, FGF-1 expression may be repressed by some mechanism that is impaired in abnormal glioblastoma cells.

In conclusion, the present studies characterize the brain-specific 1B promoter region in the mouse. The existence of a brain-specific FGF-1 promoter and the finding that it is the only splice form utilized in the brain are significant because they suggest that the regulation of FGF-1 expression in the

[2] S. Ray and I.-M. Chiu, unpublished observations.

brain is carried out by mechanisms distinct from those in other tissues. The transcript is expressed in phylogenetically older brain regions, which are involved primarily in processing information from exteroceptive sensory mechanoreceptors and in motor control. The message is absent in telencephalic regions traditionally thought to be involved in processing complex information. As has been suggested previously (30), the relatively late appearance of the FGF-1B message suggests a role in the regulation of maturation, maintenance, and repair, rather than in neurogenesis. Studies using 1B promoter knockout mice generated by homologous recombination will determine the functional significance of the upstream untranslated -1B exon.

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