We isolated the cDNA encoding a novel member of the fibroblast growth factor (FGF) family from rat embryos by homology-based polymerase chain reaction. The FGF-related cDNA encodes a protein of 215 amino acids (24 kDa), which has a conserved 120-amino acid core with 30–60% amino acid sequence identity with the FGF family. This protein with a hydrophobic amino terminus appears to be a secreted protein. The cDNA was translated in a coupled in vitro transcription-translation system. The molecular mass of the translation product was observed to be 26 kDa. The expression of the FGF-related mRNA in the rat embryo and adult tissues was determined by Northern analysis and in situ hybridization. The mRNA was expressed in several discrete regions of the embryo. In adult tissues, the mRNA was preferentially expressed in the lung. The expression profile of the FGF-related mRNA was different from those of other FGF family mRNAs. As this protein is the 10th documented protein related to FGFs, we tentatively term this protein FGF-10.

Two fibroblast growth factors (FGFs), aFGF and bFGF, were isolated as mitogens for fibroblasts from the brain and pituitary and have been well characterized. These molecules are widely expressed in developing and adult tissues (1, 2). The FGF family now consists of at least nine members, FGF-1 (aFGF), FGF-2 (bFGF), FGF-3 (int-2), FGF-4 (hst/KGF), FGF-5, FGF-6, and FGF-7 (KGF), FGF-8 (AIGF), and FGF-9 (GAF) (1–9). The genes for FGF-3, FGF-4, FGF-5, and FGF-6 were first isolated as oncogenes (5–8); FGF-7 was isolated as an androgen-induced growth factor from mouse mammary carcinoma cells (3), and FGF-9 was isolated as a glia-activating factor from human glioma cells (4). These FGFs are expressed predominantly during embryonic development and are also expressed in restricted adult tissues.

aFGF and bFGF are polypeptides with multiple biological activities in vivo and in vitro, including roles in angiogenesis, mitogenesis, cellular differentiation, and repair of tissue injury (1, 2). Although the physiological significance of most FGFs remains to be elucidated, FGFs appear to play important roles as peptide growth factors in both developing and adult tissues. Although members of the FGF family are of different sizes, they have a conserved 120-amino acid residue core with 30–70% amino acid sequence identity (1–9). To isolate the cDNA encoding a novel FGF, we examined rat embryo cDNA amplified by the polymerase chain reaction with primers specific for conserved amino acids in the FGF family. Recently, we isolated the cDNA encoding a novel member of the FGF family from rat embryos by homology-based polymerase chain reaction. The nucleotide sequence of the cDNA revealed the complete amino acid sequence of a novel member of the FGF family. Here, we report the structure of this novel FGF and the expression of the mRNA encoding the FGF.

**EXPERIMENTAL PROCEDURES**

Preparation of RNA from Rat Embryos and Adult Rat Tissues—RNA was prepared from Wistar rat embryos (embryonic day 14, E14) and adult rat tissues by the acid guanidinium thiocyanate/phenol/chloroform extraction method (10). Poly(A) RNA was prepared using oligo(dT)-cellulose (Collaborative Research Inc., type 2).

Isolation and Analysis of Rat FGF Family cDNAs—For cDNA synthesis, rat embryo poly(A) RNA (5 μg) was incubated for 60 min at 37 °C in a reaction mixture (20 μl) containing 300 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.), 15 units of human placenta RNAase inhibitor (Wako Pure Chemicals, Japan), and 0.5 μg of random hexadeoxynucleotide primer. To amplify the FGF family cDNAs, the polymerase chain reaction (PCR) was performed for 30 cycles in a reaction mixture (25 μl) containing an aliquot of the above cDNA solution, 0.05 unit/μl Taq DNA polymerase (Wako Pure Chemicals, Japan), and 5 pmol/μl each of the sense and antisense degenerate primers representing all possible codons corresponding to the consensus amino acid sequences of mouse FGF-3 and FGF-7, YL—AMNK and YNYTYS, respectively (11, 12). The reaction product was fractionated by electrophoresis on an 8% polyacrylamide gel. The amplified DNA of the expected size (~110 base pairs) was eluted from the gel by electrophoresis and was cloned into the pGEM-T DNA vector (Promega Co.). The nucleotide sequence of the cloned DNA was determined with a DNA sequencer model 373A (Applied Biosystems, Inc.). To determine the entire coding region of the cDNA, the cDNA was analyzed by the rapid amplification of cDNA ends method (13).

cDNA-directed Protein Synthesis—The poly(A) region (58 base pairs) of the pSP64 poly(A) vector DNA (Promega) was added at the 3′ end of the FGF-related cDNA containing the entire coding region (645 base pairs) and 3′-noncoding region (49 base pairs). The cDNA containing the poly(A) region was inserted at the site downstream of the 5′ leader sequence of pSPUTK vector DNA (Stratagene). cDNA-directed protein synthesis was performed in a coupled in vitro transcription-translation system (TNT SP6 Coupled Reticulocyte Lysate System, Promega) using the pSPUTK containing the cDNA as a template and [35S]-methionine (37 TBq/mmol) (ICN Biomedicals) in the presence or absence of canine pancreatic microsomal membranes (Promega). The translation products were resolved by sodium dodecyl sulfate (SDS)-polyacrylamide (15%) gel electrophoresis, transferred onto a nitrocellulose membrane, and then detected with a radiograming analyzer (BAS 2000, Fuji Photo Film Co., Japan).

Northern Analysis—Aliquots of poly(A) RNA (each 12 μg) from rat embryos and adult rat tissues were resolved on a denaturing agarose gel (1%) containing formaldehyde and transferred to a nitrocellulose
RESULTS AND DISCUSSION

Isolation of FGF-related cDNA from Rat Embryos—Members of the FGF family have conserved a 120-amino-acid residue core with 30–70% amino acid sequence identity. The amino acid sequences corresponding to amino acids 96–101 (YLMNK) and 120–126 (Q) by the method of von Heijne (14) (Fig. 1). Two

FGFs are abundantly present in embryos (5–9). Therefore, cDNA synthesized from rat embryo (E14) poly(A)+ RNA was amplified by PCR using these degenerate oligonucleotide primers. DNA of the expected size (9110 base pairs), which was the major amplified product (data not shown), was cloned. Thirty-six clones were isolated, and their nucleotide sequences were determined. Thirty-six clones had sequences homologous to those of the FGF family. Among the FGF-related cDNA clones, 11 clones were isolated, and their nucleotide sequences were determined. Thirty-six clones had sequences homologous to those of the nine known members of the FGF family, suggesting that these clones encode a novel member of the FGF family. To determine the entire coding region of the novel FGF-related cDNA, the cDNA clone covering the entire coding region was isolated by the rapid amplification of cDNA ends method (13).

Structure of the FGF-related Protein—The nucleotide sequence of the coding region of the novel FGF-related cDNA is shown in Fig. 1. The translation initiation site was assigned to the translation initiation codon, ATG, at nucleotides 1–3, because the translation termination codon, TAA, was found at a site (nucleotides 108–110) upstream from the initiation codon in the same reading frame (data not shown). The translation termination site was assigned to the termination codon, TAG (nucleotides 646–648). Thus, the cDNA was found to contain a coding region of 645 nucleotides.

The nucleotide sequence of the coding region allowed elucidation of the complete amino acid sequence (215 amino acids) of a protein with a conserved 30–70% amino acid sequence identity with the FGF family (1–9) (Fig. 1). As this protein is the 10th documented protein related to FGFs, we propose the tentative designation FGF-10. The highest sequence identity (60%) was observed with FGF-3 and FGF-7 (Fig. 2). FGF-1, FGF-2, and FGF-9 have no typical signal sequence in their amino termini (1, 2, 4), and so the pathway of release and degradation of these proteins remains to be determined. Another 25 clones had sequences highly homologous to those of the mouse FGF-7 or FGF-3 cDNA (11, 12), indicating that they were rat homologues of these genes. Another 25 clones had an identical sequence that was similar to but distinct from those of the nine known members of the FGF family, suggesting that these clones encode a novel member of the FGF family. To determine the entire coding region of the novel FGF-related cDNA, the cDNA clone covering the entire coding region was isolated by the rapid amplification of cDNA ends method (13).

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cysteine residues are well conserved in the FGF family, and these amino acids correspond to residues 91 and 157 in the FGF-10 sequence. Although a cysteine residue was found at position 157, a serine instead of a cysteine residue was found at position 91. The same substitution was also observed in the FGF-8 sequence (3). The most characteristic feature in the FGF-10 sequence was a serine-rich domain (amino acid positions 51–69) in the amino-terminal region. A similar domain was also found in the amino-terminal region of the FGF-5 sequence (7), although the significance of the domain remains to be elucidated.

**FGF-10 cDNA-directed Protein Synthesis**—FGF-10 cDNA was translated in a coupled in vitro transcription-translation system. The translation products were resolved by SDS-polyacrylamide gel electrophoresis. A major translation product of $26 \, \text{kDa}$ was detected (Fig. 3). We assumed that the translation product was FGF-10, as the molecular mass of FGF-10 was calculated to be $24 \, \text{kDa}$ by the above assignment. However, the translation efficiency of the cDNA was greatly reduced in the presence of canine pancreatic microsomal membranes. Then, we could not detect any translation products processed by the membranes (data not shown).

Expression of FGF-10 mRNA in Embryonic and Adult Rat Tissues—To examine the expression of FGF-10 in the rat embryo (E14), its mRNA was identified in the embryo poly(A)$^+$ RNA by Northern analysis using a $^{32}$P-labeled FGF-10 cRNA probe. The integrity of RNA was confirmed by electrophoresis on a denaturing agarose gel containing formaldehyde (data not shown). The labeled probe mainly hybridized to an mRNA of approximately $4.5 \, \text{kilobases}$ which is large enough to encode FGF-10 (Fig. 4).

To examine the region-specific expression of FGF-10 mRNA in the embryo, sagittal sections of the embryo were analyzed by in situ hybridization with an antisense $^{35}$S-labeled FGF-10 cRNA probe, followed by microautoradiography. With the

**Fig. 2.** Amino acid sequence comparison of rat FGF-10 with mouse FGF-3 and FGF-7. Numbers above amino acids refer the amino acid sequence of FGF-10. Amino acid residues shown by boldface letters indicate the amino acid residues identical with those of FGF-10 in the conserved core region (amino acids 82–173 and 184–212).

**Fig. 3.** Detection of the translation product from the rat FGF-10 cDNA in a coupled in vitro transcription-translation system. The rat FGF-10 cDNA was translated in a coupled in vitro transcription-translation system (25 $\mu\text{l}$) with $[^{35}\text{S}]$L-methionine. The translation product was resolved by SDS-polyacrylamide gel electrophoresis. Lane 1, without the cDNA; lane 2, with the cDNA. Prestained protein marker broad range (New England Biolabs) was used as molecular mass-standard proteins.

**Fig. 4.** Expression of FGF-10 mRNA in the rat embryo and adult tissues. Poly(A)$^+$ RNA (12 $\mu$g each) was electrophoresed on a denaturing agarose gel (1%) containing formaldehyde. RNA was transferred onto a nitrocellulose membrane, and hybridization was performed with a $^{32}$P-labeled rat FGF-10 cRNA probe. The positions of 28 S and 18 S RNAs are indicated as 28 S and 18 S. Lanes Em, Br, He, Lu, Ki, and In indicate poly(A)$^+$ RNA from the embryo (E14) and the adult, brain, heart, lung, kidney, and intestine, respectively.

**Fig. 5.** Localization of FGF-10 mRNA in a sagittal section of the rat embryo (E14). A sagittal section of the embryo was hybridized with a $^{35}$S-labeled FGF-10 cRNA probe. The labeled section was counterstained with hematoxylin and eosin. Bright-field (A) and dark-field (B) photomicrographs are shown. Pi, the posterior pituitary; Cv, the first cervical vertebra; Du, the duodenum; Lu, the lung; Sp, sacral and coccygeal segments of the spinal cord. Scale bar $= 0.25 \, \text{cm}$. 

**FIG. 2.** Amino acid sequence comparison of rat FGF-10 with mouse FGF-3 and FGF-7. Numbers above amino acids refer the amino acid sequence of FGF-10. Amino acid residues shown by boldface letters indicate the amino acid residues identical with those of FGF-10 in the conserved core region (amino acids 82–173 and 184–212).
probe, discrete labeling was observed in several regions of the embryo, including the posterior pituitary, the first cervical vertebra, sacral and coccygeal segments of the spinal cord, the duodenum, and the lung (Fig. 5). However, only diffused labeling was observed with a sense 35S-labeled FGF-10 cRNA probe as a control (data not shown).

To examine the expression of FGF-10 mRNA in the adult rat tissues, poly(A) \(^+\) RNA from tissues including the brain, heart, lung, liver, kidney, and small intestine was also examined by Northern analysis using the 32P-labeled FGF-10 cRNA probe. The integrity of RNA was also confirmed by electrophoresis on a denaturing agarose gel containing formaldehyde as described (15). The labeled probe strongly hybridized to an mRNA of ~4.5 kb in the lung (Fig. 4). The size of the hybridizing mRNA in the lung was similar to that in the embryo. The probe also weakly hybridized to an mRNA of the same size in the heart but not in the brain, liver, kidney, or small intestine. We also examined the expression of FGF-10 in other tissues including the thymus, stomach, pancreas, spleen, testis, and muscle by PCR with specific primers for the mRNA. However, no FGF-10 mRNA was detected in these tissues (data not shown).

FGF-1 and FGF-2 are widely expressed in developing and adult tissues (1, 2). In contrast, most other FGFs are predominantly expressed in the embryo and show restricted expression patterns in the adult tissues as described below (3-9). FGF-3 is expressed in the embryo and in the adult brain and testis. FGF-4 is expressed in the embryo but not in the adult tissues. FGF-5 is expressed in the embryo and in the adult brain, FGF-6 is expressed in the embryo and in the adult heart, testis and muscle. FGF-7 is expressed in the embryo and the adult kidney and gastrointestinal tract. FGF-8 is expressed in the embryo and in the adult testis and ovaries. FGF-9 is expressed in the embryo and in the adult kidney and brain. FGF-10 is predominantly expressed in the embryo and the adult lung. The expression profile of FGF-10 is quite different from those of other members of the FGF family. Although the physiological significance of FGF-10 remains to be elucidated, FGF-10 appears to be a novel FGF which presumably has a unique physiological role.

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