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# Characterization of a bovine acidic FGF cDNA clone and its expression in brain and retina

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A cDNA encoding the acidic eye-derived growth factor (EDGF II) similar to the acidic fibroblast growth factor (aFGF), a potent cell mitogen, has been isolated from a bovine retinal cDNA library. The cDNA, 4.1 kb in size, has a sequence coding for the 155 amino acids of bovine aFGF, and shows similarity with human aFGF (87% identity). The coding sequence is flanked by a 5'-untranslated region of 0.8 kb and a 3'-untranslated end of 3.0 kb. Northern blot analysis of bovine brain and retina poly($A^+$) RNAs showed the existence of four aFGF mRNA species. Two of these species are 9.9 and 6.0 kb in size, not abundant and could represent premessengers. The other two species, 4.2 and 2.5 kb, are abundant.

Fibroblast growth factor; Retina; mRNA; Nucleotide sequence; (Bovine, Brain)

## 1. INTRODUCTION

Growth factor activity was first identified in adult bovine retina in 1978 [1]. This mitogenic activity is also present in other ocular tissues such as iris, vitreous body and pigmented epithelium from bovine and chick embryo [2,3]. It was designated as eye-derived growth factor (EDGF). Affinity chromatography on heparin-Sepharose has allowed the purification of two retinal growth factors (EDGF I, EDGF II) to homogeneity. Biochemical and immunological techniques have shown that EDGF I and EDGF II are similar to the basic and acidic fibroblast growth factors (bFGF and aFGF), respectively [4]. bFGF originally purified from bovine brain has 146 amino acid residues and aFGF 140 [5,6]. The acidic and basic FGFs are closely related: 55% of their amino acid sequence is identical. These two FGFs as well as the retinal aFGF and bFGF forms are potent mitogens for a wide variety of cells of both mesodermal and ectodermal embryonic origin (for a review see [7]).

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The biological activity of aFGF is potentiated by the glycosaminoglycan heparin [8,9].

Previous results have indicated that FGFs are involved in eye development and in vision. Basic and, to a lesser extent, acidic FGFs specifically bind to basement membranes of the eye [10]. We have recently reported that EDGF II is present in the dark-adapted rod outer segments (ROS) from bovine retina [11]. In newt, the addition of EDGF II to the dorsal iris in culture stimulates lens regeneration [12]. These data suggest that aFGF could be involved in the photoreceptor cell biology. Most tissues so far investigated contain several forms of bFGF differing in their amino-terminal ends [7,13,14]. Similarly, an amino-terminally truncated aFGF which lacks six terminal residues has been purified from bovine brain [15]. More recently an amino-terminally blocked form of aFGF with 155 residues has been isolated from bovine brain [16]. Using specific cDNA probes, a 7.0 kb and a 3.7 kb mRNA coding for bFGF [17] have been detected in the bovine hypothalamus, while a cDNA clone coding for human aFGF hybridized to a 4.8 kb mRNA in the human brain stem and glioma cells [18,19].

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However, it is not clear if the different forms of bFGF and aFGF have different biological effects. In order to investigate the effect of aFGFs in retina, we isolated a cDNA clone coding for the retinal form of aFGF. This probe specifically hybridizes to four different mRNAs from bovine brain and retina.

## 2. MATERIALS AND METHODS

### 2.1. *RNA isolation*

RNA containing aFGF messengers was isolated from bovine brain and retina, and from a confluent murine neuroblastoma cell line (N 115) grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum. Total RNA was isolated with 4 M guanidinium isothiocyanate and 1 M 2-mercaptoethanol according to standard procedures [20,21]. Poly($A^+$) RNA was prepared by two oligo(dT)-cellulose affinity chromatographies (Collaborative Research) [22].

### 2.2. *Synthesis of cDNA library*

The poly($A^+$) RNA isolated from bovine retina was used to construct a cDNA library [23]. Briefly, the first strand cDNA was primed with oligo(dT) and synthesized using reverse transcriptase. The second strand was synthesized with a combination of *E. coli* RNase H and DNA polymerase I. After addition of *Eco*RI linkers, the double-stranded cDNAs were ligated into the vector λgt11. The cDNA library ($7 \times 10^6$ plaques) was established in *E. coli* Y1090 [24].

### 2.3. *Screening of the library*

A 42-mer oligonucleotide probe was derived from the aFGF genomic clone as previously described [17]. It corresponds to the residues 24–37 of the amino acid sequence. This $^{32}P$-labelled probe was used to screen $2 \times 10^6$ recombinant phages. Hybridization was performed in 6 × SSC, 50% formamide, 10 × Denhardt's solution, 0.1% SDS and 100 μg/ml salmon sperm DNA at 42°C. Filters were washed two times 20 min in 0.1 × SSC, 0.1% SDS at 60°C. Six clones giving a positive signal were further characterized by restriction mapping and Southern analysis. This finally led to the selection of one clone (11.32.12).

### 2.4. *Subcloning and sequencing*

The insert of clone 11.32.12 was excised with *Eco*RI from λgt11 vector and restricted with *Xba*I. The fragments *Eco*RI-*Xba*I were made blunt ended with the Klenow fragment of the DNA polymerase I and inserted in both directions in M13 mp18 and mp19 vectors. Exonuclease III digestion [25] was used to introduce progressive deletions into the cDNA sequence. The sequencing was carried out by the dideoxy chain termination method [26].

### 2.5. *Northern hybridization*

5–20 μg of poly($A^+$) RNA purified from brain and retina was electrophoresed on a horizontal formaldehyde agarose gel (1%), transferred to a nitrocellulose membrane [27], and hybridized to nick-translated, $^{32}P$-labelled DNA fragments corresponding to different regions of the cDNA insert. Hybridizations were carried out as described above, the filters were washed in 0.1 × SSC, 0.1% SDS at 50°C and autoradiographed for different lengths of time. Ribosomal RNA from brain and retina were used as controls.

### 2.6. *Probes*

A 250 bp bovine genomic clone coding for the first 56 amino acids of aFGF (PCB), and a 1.4 kb cDNA specific for bovine bFGF [17] were obtained from J.C. Fiddes and J. Abraham.

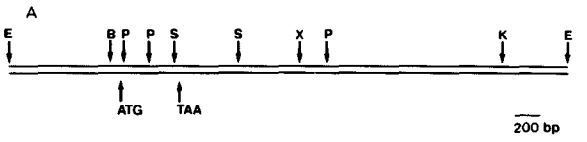
## 3. RESULTS

### 3.1. *Screening of the cDNA library*

A cDNA library constructed from bovine retina poly($A^+$) RNA in λgt11 vector was screened with a synthetic 42-mer oligonucleotide probe. From $2 \times 10^6$ clones only one cDNA clone (11.32.12) was characterized. Digestion of this clone with *Eco*RI resulted in a 4.1 kb insert. The cDNA contained 0.8 and 3 kb untranslated regions at the 3′- and 5′-ends and had a single open reading frame coding for the 155 residues of bovine aFGF. The partial restriction map and the sequence of the 465 nucleotides of the coding region are shown (fig.1A,B). The protein sequence of the aFGF deduced from this sequence coincides well with that of the human aFGF. The comparison of the nucleotides from human and bovine coding sequences of aFGF shows a 87% sequence similarity. As described for human aFGF, the 155 amino acids coding for the bovine aFGF are flanked by stop codons indicating that aFGF is not synthesized as a precursor.

### 3.2. *RNA analysis by Northern hybridization*

To examine the expression of aFGF and bFGF mRNAs in bovine brain and retina, we hybridized 10–20 μg poly($A^+$) RNAs to acidic (fig.2A) and basic (fig.2B) cDNAs. The two tissues contained four aFGF mRNA species (fig.2A). The 9.9 and the 6.0 kb transcripts were only detectable in 20 μg poly($A^+$) RNA. In both tissues the 4.2 kb and 2.5 kb transcripts were more abundant than expected since the specific oligonucleotide probe yielded only one positive clone out of $2 \times 10^6$ plaques which were screened. In the two tissues, the 4.2 kb mRNA was the most abundant of the four transcripts. No aFGF mRNAs were visualized in 10 μg poly($A^+$) RNA isolated from the neuroblastoma N 115 cell line. When using a bFGF




B

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GGATCCTCTTTCCCTTCTACTGGAGAGGAAAAGCCCTCAGCCTGCAAGCTGTTCAGCCTTGAAACAGCCACA    72

ACCAGCAGCTGCTGAGCC  ATG GCT GAA GGA GAA ACC ACG ACC TTC ACG GCC CTG ACT    129
                    Met Ala Glu Gly Glu Thr Thr Thr Phe Thr Ala Leu Thr

GAG AAG TTT AAC CTG CCT CTA GGC AAT TAC AAG AAG CCC AAG CTC CTC TAC TGC    183
Glu Lys Phe Asn Leu Pro Leu Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys

AGC AAC GGG GGC TAC TTC CTG AGA ATC CTC CCA GAT GGC ACA GTG GAT GGG ACG    237
Ser Asn Gly Gly Tyr Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr

AAG GAC AGG AGC GAC CAG CAC ATT CAG CTG CAG CTC TGT GCG GAA AGC ATA GGG    291
Lys Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Cys Ala Glu Ser Ile Gly

GAG GTG TAT ATT AAG AGT ACG GAG ACT GGC CAG TTC TTG GCC ATG GAC ACC GAC    345
Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Phe Leu Ala Met Asp Thr Asp

GGG CTT TTG TAC GGC TCA CAG ACA CCC AAT GAG GAA TGT TTG TTC CTG GAA AGG    399
Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu Cys Leu Phe Leu Glu Arg

TTG GAG GAA AAC CAT TAC AAC ACC TAC ATA TCC AAG AAG CAT GCA GAG AAG CAT    453
Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile Ser Lys Lys His Ala Glu Lys His

TGG TTC GTT GGT CTC AAG AAG AAC GGA AGG TCT AAA CTC GGT CCT CGG ACT CAC    507
Trp Phe Val Gly Leu Lys Lys Asn Gly Arg Ser Lys Leu Gly Pro Arg Thr His

TTC GGC CAG AAA GCC ATC TTG TTT CTC CCC CTG CCA GTC TCC TCT GAT     TAAAG  560
Phe Gly Gln Lys Ala Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp

AAATCTGTTGTGGGTGCTGAGCCACTCCAGAGGAATCTGAAGGGGTCCTCACCTGGCTGACCCCAGATTGTA  632

CCCTTTACCATTGGCCGTGCTAACCCCTGGCCCACA                                    668
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Fig.1. (A) Partial restriction map of the 4100 bp cDNA coding for bovine acidic FGF. E, *Eco*RI; B, *Bam*HI; P, *Pst*I; S, *Sph*I; X, *Xba*I; K, *Kpn*I. Positions of the initiator (ATG) and terminator (TAA) codons are indicated. bp, base pairs. Some *Xba* additional sites may have been protected by methylation and may not have been detected. (B) Nucleotide sequence and deduced amino acids of the bovine acidic FGF. The 2.7 kb restriction fragment *Eco*RI-*Xba*I was subcloned into M13 mp19 in both directions. The subclones were treated with exonuclease III for different periods of time, and the bovine acidic FGF open reading frame and the 5′- and 3′-flanking regions were sequenced by the chain termination method as described in section 2. The solid box corresponds to the sequenced region.

probe, a 7.0 kb and a 3.7 kb mRNAs were detected in retina and in brain (fig.2B). With similar hybridization conditions, the intensity of the signal obtained with the bFGF probe even after 7 days of exposure is less intense than the one obtained after 2 days of exposure with the aFGF probe, suggesting that in these tissues the bFGF is less abundant than aFGF.

We probed the same RNA blots with DNA fragments containing the 800 nucleotides of the 5′-untranslated region (*Eco*RI-*Bam*HI fragment) or the 600 nucleotides of the 3′-untranslated region (*Eco*RI-*Kpn*I fragment, see fig.1A). A genomic clone (PCB) containing the region coding for the first 56 amino acids of bovine aFGF [17] was also used in Northern blot analysis.

As seen in fig.3, the 4.2 kb mRNA hybridized with all three probes. The 2.5 kb mRNA hybridized to the full-length probe and to the probe containing the 3′-untranslated region in both tissues (brain, retina). It is worth noting that in contrast to the hybridization profile corresponding to the full-length cDNA probe, the 2.5 kb mRNA was more intensely labelled than the 4.2 kb mRNA when probed with the 3′-end DNA fragment. This mRNA species was not detected by the

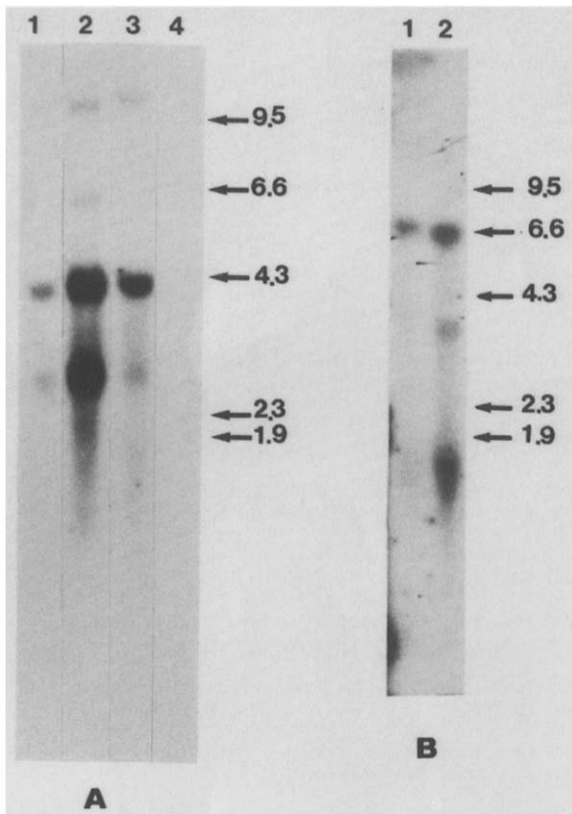


Fig.2. Northern blot analysis of poly($A^+$) mRNA derived from bovine retina or bovine brain and N 115 murine neuroblastoma cell line. Polyadenylated mRNAs were prepared from: bovine retina (A1, 10 $\mu$g; A2 and B1, 20 $\mu$g); bovine brain (A3, 10 $\mu$g; B2, 20 $\mu$g); N 115 neuroblastoma cell line (A4, 10 $\mu$g). They were analyzed for the presence of aFGF (A) and bFGF (B) gene transcripts with cDNA probes. RNAs were hybridized with (A) full-length bovine aFGF cDNA clone or (B) 1.4 kb *Eco*RI fragment from bovine cDNA clone coding for bFGF, as described in section 2. The arrows indicate the $^{32}$P-labelled *Hin*dIII restriction fragments of lambda DNA.

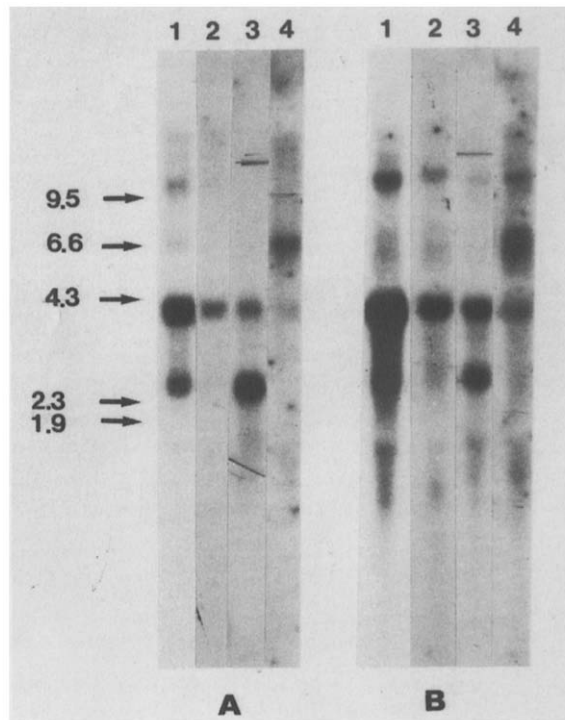


Fig.3. Northern blot analysis of 20 $\mu$g poly($A^+$) mRNA derived from bovine retina (A) and bovine brain (B). Polyadenylated mRNAs were analyzed by hybridization as described in section 2 with full-length bovine retina aFGF cDNA clone (lane 1), aFGF (250 bp) bovine genomic clone (lane 2), *Kpn*I-*Eco*RI restriction fragment from the 3′-end of bovine retina aFGF cDNA clone (lane 3) and *Bam*HI-*Eco*RI restriction fragment from the 5′-end of bovine retina aFGF cDNA clone (lane 4). Arrows indicate the $^{32}$P-labelled *Hin*dIII restriction fragments of lambda DNA.

5′-untranslated region specific probe or by the PCB probe. A similar result was obtained when a *Bam*HI-*Sph*I fragment containing the entire coding sequence or a *Sph*I-*Sph*I fragment were used (not shown). The 5′-untranslated region specific probe hybridized only to the 4.2 kb, the 6.0 kb and the 9.9 kb mRNAs in brain. In the retina, the 6.0 kb was clearly detected while the 9.9 kb mRNA barely hybridized to this probe. This apparent discrepancy between the two hybridization profiles corresponding to brain and retina RNAs probably reflects an underestimation of the amount of the retina poly($A^+$) RNA loaded on the gel as suggested by fig.2A.

## 4. DISCUSSION AND CONCLUSION

Previous studies indicate that there is a single gene on chromosome 5 encoding human aFGF [28] and there is considerable evidence suggesting a role for this mitogen in cell proliferation, wound healing, neovascularization associated with the rapid growth of solid tumor and induction of mesoderm in vertebrate development [29]. The isolation from brain of a 250 bp genomic clone coding for bovine aFGF and a 2.2 kb cDNA clone specific for human aFGF has been reported [17,18]. Acidic FGF has

been purified from a large number of tissues (see review [7]) including bovine retina [1,4]. In the present study we isolated a 4.1 kb cDNA clone from a cDNA library constructed from retina poly($A^+$) RNA. This is the first report of a cDNA clone containing the 465 nucleotides coding for the 155 amino acids of bovine aFGF. The amino acid sequence deduced from the nucleotide sequence agrees well with the reported amino acid sequence of the bovine and human aFGFs. The nucleotide sequence indicates that the amino acid residue of bovine aFGF at position 45 is Gln rather than Phe as previously reported [6,30]. The open reading frame is preceded by 800 bp at the 5'-end and is followed by untranslated region of about 3000 bp. The presence of such a long untranslated region specially at the 3'-end suggests that this region could be implicated in the translational control of aFGF expression as is the case with other growth factors or hormones [31].

The isolation of an aFGF cDNA clone from a retinal library indicates that the aFGF present in the retina is in fact synthesized by this tissue. In situ hybridization experiments are in progress to determine which specific cells of the retina express aFGF mRNA [32]. In all previous reports using either a bovine genomic clone or a human cDNA clone coding for aFGF only one transcript of 4.8 kb was described in human stem cells and in glioma cells [17,18]. More recently two transcripts of 3.3 kb and 2.8 kb were detected in a human smooth muscle cell line [33]. Using our 4.1 kb long cDNA clone we show the presence of four different size mRNAs in two different neural tissues: the brain and the retina. These four transcripts are present in different amounts: the largest ones (the 9.9 and 6.0 kb) are barely detected in 20 $\mu$g poly($A^+$) RNA and possibly represent the nuclear precursors of the aFGF mRNAs. The 4.2 kb transcript gives the strongest hybridization signal when RNAs from retina were probed with the full-length cDNA. This 4.2 kb mRNA probably corresponds to the one previously described. In fact, this transcript is the only one which hybridized to the bovine genomic aFGF clone which encodes the first 56 amino acids of bovine aFGF. Northern analysis of the four messages which hybridized to the aFGF clone indicates that they differ in their 5'- and 3'-untranslated regions. Of special interest is the 2.5 kb transcript, which does not hybridize to the PCB probe. We have also detected no hybridization signal at 2.5 kb when a cDNA fragment that spans only the coding sequence (*Bam*HI-*Sph*I fragment) was used as a probe. These results suggest that the 2.5 kb transcript does not contain any aFGF coding sequences, and may code for a different peptide.

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## NOTE ADDED IN PROOF

Since this paper was submitted the full sequence of the cDNA has been determined and is accessible in the EMBL library under the number x13221.