The EMBO Journal vol.5 no.10 pp.2523-2528, 1986

Human basic fibroblast growth factor: nucleotide sequence and genomic organization

Judith A.Abraham, Jacqueline L.Whang, Annette Tumolo, Ayalew Mergia, Jeff Friedman, Denis Gospodarowicz[1] and John C.Fiddes

California Biotechnology Inc., 2450 Bayshore Parkway, Mountain View, CA 94043, and [1]Cancer Research Institute, School of Medicine, University of California, San Francisco, CA 94143, USA

Communicated by N.J.Proudfoot

Clones encoding the angiogenic endothelial cell mitogen, basic fibroblast growth factor (FGF), have been isolated from human cDNA libraries made from kidney, fetal heart, fetal liver, term placenta, and a breast carcinoma. Basic FGF cDNA clones are present in these libraries at very low levels when compared to the quantity of the growth factor in the tissues. This observation, combined with the fact that several of the clones represent unspliced transcripts, suggests that cytoplasmic basic FGF mRNA is unstable and that the protein is stored in tissues. The amino acid sequence of human basic FGF, deduced from the sequence of these cDNAs and from genomic clones, is 99% homologous to that of bovine basic FGF, implying a strong selection pressure for maintenance of function and structure. As with the bovine factor, human basic FGF does not appear to have a signal peptide sequence. Southern blot analysis of human genomic DNA and mapping of the cloned gene shows that there is only one basic FGF gene. All of the basic, heparin-binding endothelial cell mitogens of similar amino acid composition that have been described must therefore be products of this single gene.

Key words: angiogenesis/endothelial cells/heparin-binding growth factors/nucleotide sequence/secretion

Introduction

The growth of new capillaries (angiogenesis) is a complex process which involves both the migration and proliferation of vascular endothelial cells (reviewed by Folkman, 1985). The signal(s) that naturally trigger angiogenesis *in vivo* are not known but neovascularization can be induced by the addition of certain growth factors. One such growth factor, basic fibroblast growth factor (FGF) (reviewed by Gospodarowicz, 1985, 1986) induces angiogenesis in both the chick chorioallantoic membrane and the rabbit cornea assays (Gospodarowicz *et al.*, 1979, 1984; Esch *et al.*, 1985a). Basic FGF has been shown *in vitro* to be a very potent mitogen and chemoattractant for capillary endothelial cells, and a mitogen for many other cells of mesodermal or neuroectodermal origin (Gospodarowicz, 1985, 1986).

Basic FGF has been purified from a wide range of tissues including pituitary, brain, hypothalamus, retina, adrenal gland, thymus, corpus luteum, kidney, placenta and various tumors (Gospodarowicz, 1986; Lobb *et al.*, 1986; Klagsbrun *et al.*, 1986). Basic FGF is probably identical to several other cationic endothelial cell mitogens that have a high affinity for the glycosaminoglycan, heparin (see, for example, Lobb *et al.*, 1986), and it has also been shown to be 55% homologous to the related mitogen, acidic FGF (Gimenez-Gallego *et al.*, 1985; Esch *et al.*, 1985b). Acidic FGF, in contrast to basic FGF, has only been purified from neural tissues such as brain and retina. The two growth factors have the same intrinsic biological activity but differ in their relative potencies (Gospodarowicz, 1986; Thomas and Gimenez-Gallego, 1986).

We have recently isolated a pituitary cDNA clone that encodes bovine basic FGF (Abraham *et al.*, 1986). Analysis of the nucleotide sequence of this clone showed that basic FGF is probably synthesized initially as a 155 amino acid protein with an amino-terminal extension of nine amino acids not found on the sequenced, 146 amino acid form of the protein (Esch *et al.*, 1985a). Likewise, analysis of a genomic clone encoding the amino-terminus of bovine acidic FGF showed that this factor has a 15 amino acid amino-terminal extension that is homologous to the basic FGF sequence (Abraham *et al.*, 1986). Neither of the two FGFs has a classic signal peptide sequence, raising the question of how these growth factors, which interact with membrane-bound receptors on their target cells (Neufeld and Gospodarowicz, 1985, 1986), are released from the cells in which they are synthesized.

Here, we describe the isolation and characterization of both cDNA and genomic clones that encode human basic FGF. Analysis of these clones shows that the amino acid sequences of human and bovine basic FGF are exceptionally highly conserved, suggesting that there is a very strong selective pressure to maintain biological function. Genomic Southern blot analysis indicates that basic FGF, and probably all other basic, heparin-binding endothelial cell mitogens so far identified, are encoded by the same, single copy gene.

Results

Unspliced basic FGF transcripts

We used the bovine basic FGF cDNA (Abraham *et al.*, 1986) as a hybridization probe to isolate basic FGF clones from human cDNA libraries. Since the nature of the cell types that synthesize basic FGF mRNA was not known, we constructed cDNA libraries from several tissues. Placenta and kidney were chosen because basic FGF has been isolated from these sources (Gospodarowicz, 1986), fetal liver and fetal heart were chosen because angiogenesis occurs during fetal development (Folkman, 1985), and a breast carcinoma was chosen because of the probable identity between basic FGF and tumor angiogenesis factor (Shing *et al.*, 1984; Lobb *et al.*, 1986; Klagsbrun *et al.*, 1986).

The extent of the individual cDNA clones isolated from these libraries is shown in Figure 1. None of the clones represents a complete copy of an FGF transcript but a composite structure for the human basic FGF mRNA (top line, Figure 1) can be obtained from an analysis of these clones and of the genomic isolates described below.

Analysis of the cDNAs reveals several interesting features. In agreement with the observations made on the wide tissue distribu-

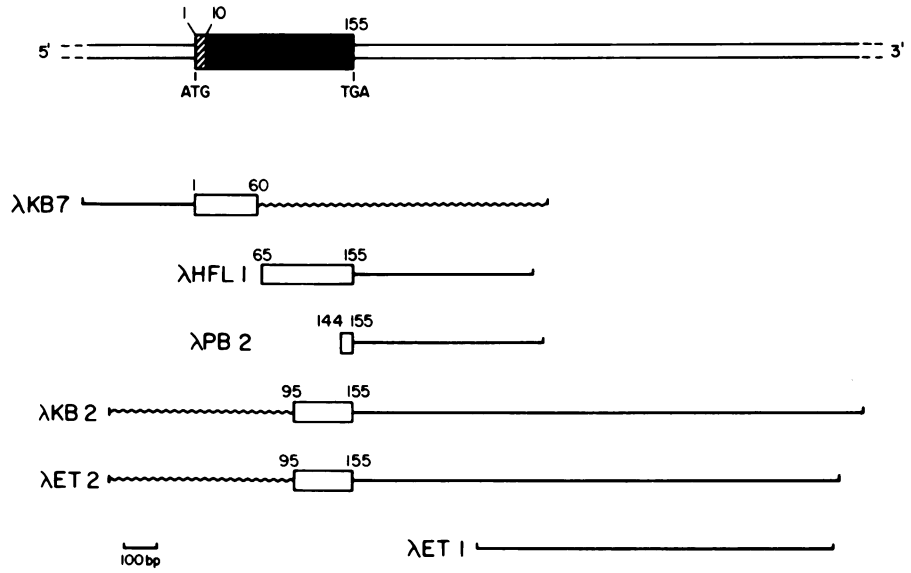

Fig. 1. Human basic FGF cDNA clones. The top line shows schematically the coding sequence for the proposed primary translation product of basic FGF (amino acids 1–155); solid shading indicates the sequenced form of the protein (previously defined as amino acids 1–146) and the cross-hatched region indicates the nine amino acid amino-terminal extension deduced from the cDNA sequence. The full extent of the 5′ and 3′ untranslated regions is not known. In the lower section, the extent of six individual cDNA clones is shown. The names of these clones, the human cDNA libraries from which they were isolated and the number of independent recombinants in the libraries that were screened are as follows: λKB2 and λKB7, adult kidney, 2×10^6; λHFL1, fetal liver, 0.5×10^6; λPB2, term placenta, 0.7×10^6; λET1 and λET2, breast carcinoma, 0.35×10^6. Several of the cDNA clones were isolated repeatedly indicating that the libraries were screened exhaustively. An additional clone (not shown) that contains only 3′ untranslated sequences was isolated from a 0.2×10^6 member fetal heart library. The extent of the coding sequence contained in each cDNA is shown by the open boxed regions; numbers refer to amino acids. The untranslated regions are indicated by straight lines, and wavy lines indicate the intron sequences present in clones λKB2, λKB7 and λET2. The size marker bar at the bottom of the figure indicates 100 bp.

tion of basic FGF (Gospodarowicz, 1986), the mRNA (as represented by cDNA clones) was found in a range of different tissues. However, the frequency of the cDNA clones in the various libraries (averaging 1 in 500 000) is very low. This frequency, which is similar to that observed previously for bovine basic FGF cDNA in a pituitary library (Abraham *et al.*, 1986), is lower than might be expected from the quantity of the protein in these tissues (500 μg/kg pituitary wet weight; Gospodarowicz, 1986).

In the case of the two kidney clones (λKB2 and λKB7) and one of the breast carcinoma clones (λET2), the isolated cDNAs are copies of unspliced transcripts and presumably result from oligo-dT priming on polyadenylate stretches in the introns or untranslated sequences. The identification of the intron–exon boundaries in these cDNA clones was confirmed by comparison with the sequences of the genomic clones described below. Clones that represent unspliced transcripts of a gene have been described recently for both human erythropoietin and colony stimulating factor 1 (Jacobs *et al.*, 1985; Kawasaki *et al.*, 1985). The significance of the unspliced basic FGF transcripts is not known, but a likely explanation, based on the unexpectedly low frequency of basic FGF cDNAs, is that the fully processed transcripts have been degraded in these tissues. Thus, the nuclear precursor basic FGF RNAs represent a significant fraction of the total cellular basic FGF RNA used to make the libraries.

Both the frequency at which the cDNA clones were isolated, and the unspliced nature of several of the clones, is in keeping with our failure to detect basic FGF mRNA by Northern blotting in any of the tissues that were used to generate the libraries (data not shown). The only tissue in which we have so far been able to detect intact basic FGF mRNA is bovine hypothalamus (Abraham *et al.*, 1986). Interestingly, we have found intact mRNA in several actively growing cultured cells, such as the human hepatoma cell line, SK-HEP-1 (Abraham *et al.*, 1986) and cultured bovine capillary endothelial cells (L.Schweigerer, G.Neufeld, J.Friedman, J.A.Abraham, J.C.Fiddes and D.Gospodarowicz, in preparation).

Human and bovine basic FGF proteins are extremely homologous

A composite nucleotide sequence for the coding region of the human basic FGF gene was obtained by sequencing the cDNA clones and parts of the genomic clones described below. Figure 2 shows this nucleotide sequence along with the deduced amino acid sequence, part of the 5′ and 3′ flanking sequences, and the intron–exon junctions. Comparison of the human and bovine sequences suggests that human basic FGF, like its bovine counterpart, is synthesized initially as a 155 amino acid protein; the amino acids of this proposed precursor are numbered 1 to 155 in the figure. The amino acid sequence of the first 41 residues of the 146 amino acid form of human basic FGF has been reported (Böhlen *et al.*, 1985; Gimenez-Gallego *et al.*, 1986) and is in agreement with the amino acid sequence deduced from the nucleotide sequence.

A comparison of the human and bovine nucleotide and amino acid sequences is shown in Figure 3. The most striking feature of this comparison is the exceptionally high degree of sequence conservation. Only two of the 155 amino acids are different between the two species, giving an overall amino acid sequence homology of 98.7%. This is in contrast to the 65.5% homology that has been observed between human and bovine growth hormone, but is similar to the 94.1% homology between human and bovine insulin (Miller and Eberhardt, 1983; Dayhoff, 1976). The striking degree of amino acid homology implies that basic FGF has a highly conserved function and that there has been a strong selection pressure for maintenance of sequence. It is also in keeping with the observation that bovine basic FGF is biologically

```
                    GAATTCATGC CTCTTTCTCT CCTTTTGTTG GTAGACGACT TCAGCCTCTG TCCTTTAATT TTAAAGTTTA TGCCCCACTT GTACCCCTCG
TCTTTTGGTG ATTTAGAGAT TTTCAAAGCC TGCTCTGACA CAGACTCTTC CTTGGATTGC AACTTCTCTA CTTTGGGGTG GAAACGGCTT CTCCGTTTTG AAACGCTAGC
GGGGAAAAAA TGGGGGAGAA AGTTGAGTTT AAACTTTTAA AAGTTGAGTC ACGGCTGGTT GCGCACGAAA AGCCCCGCAG TGTGGAGAAA GCCTAAACGT GGTTTGGGTG
GTGCGGGGGT TGGGCGGGGG TGACTTTTGG GGGATAAGGG GCGGTGGAGC CCAGGGAATG CCAAAGCCCT GCCGCGGCCT CCGACGCGCG CCCCCGCCC CTCGCCTCTC
CCCCGCCCCC GACTGAGGCC GGGCTCCCCG CCGGACTGAT GTCGCGCGCT TGCGTGTTGT GGCCGAAGCC GCCGAACTCA GAGGCCGGCC CCAGAAAACC CGAGCGAGTA
GGGGGCGGCG CGCAGGAGGG AGGAGAACTG GGGGCGCGGG AGGCTGGTGG GTGTGGGGGG TGGAGATGTA GAAGATGTGA CGCCGCGGCC CGGCGGGTGC CAGATTAGCG
GACGGCTGCC CGCGGTTGCA ACGGGATCCC GGGCGCTGCA GCTTGGGAGG CGGCTCTCCC CAGGCGGCGT CCGCGGAGAC ACCCATCTGT GAACCCCAGG TCCCGGGCCG
CCGGCTCGCC GCGCACCAGG GGCCGGCGGA CAGAAGAGCG GCCGAGCGGC TCGAGGCTGG GGGACCGCGG GCGCGGCCGC GCGCTGCCGG GCGGGAGGCT GGGGGGCCGG
GGCCGGGGCC GTGCCCGGAG CGGGTCGGAG GCCGGGGCCG GGGCCGGGGG ACGGCGGCTC CCCGCGCGGC TCCAGCGGCT CGGGGATCCC GGCCGGGCCC CGCAGGGACC
ATG GCA GCC GGG AGC ATC ACC ACG CTG CCC GCC TTG CCC GAG GAT GGC GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys
  1                                 10                                  20                                  30
CGG CTG TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC A gt
Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp Pro His I
                                  40                                  50
gagtgccgacccgctctctccgcctcatttccatttcgtgggttctcg.....................aaggctctttcctctgtggtgctacaaagataatttttttcccgtt

acag TC AAG CTA CAA CTT CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC CGT TAC CTG GCT ATG AAG GAA GAT
     le Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp
                                      70                                  80
GGA AGA TTA CTG GCT TCT gtaagcatactttctgttttcacacgttttttgttagcttttattgctgt.....................taataataataatgataat
Gly Arg Leu Leu Ala Ser
     90
aataacaggtaattcttcctttatttttcag AAA TGT GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC AAT ACT TAC CGG
                                Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Gly Ser Asn Asn Tyr Asn Thr Tyr Arg
                                                100                                 110
TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA GCT ATA
Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala Ile
           120                                 130                                 140
CTT TTT CTT CCA ATG TCT GCT AAG AGC TGA TTTTAATGGC CACATCTAAT CTCATTTCAC ATGAAAGAAG AAGTATATTT TAGAAATTTG TTAATGAGAG TA
Leu Phe Leu Pro Met Ser Ala Lys Ser Ter
           150
AAAGAAAA TAAATGTGTA TAGCTCAGTT TGGATAATTG GTCAAACAAT TTTTTATCCA GTAGTA
```

Fig. 2. Nucleotide and amino acid sequences of human basic FGF. The amino acids are numbered 1−155. In the 5′ flanking region, the extent of the longest cDNA clone, λKB7, is marked by an arrow. The sequence upstream of this was obtained from the genomic clone, λMG4 (see Figure 4). The sequences at the intron−exon junctions are shown along with the first and last 50 nucleotides of the two introns. Only the first 136 nucleotides of the 3′ untranslated region are shown. The potential polyadenylation signal in the 3′ untranslated region and the potential G-C boxes in the 5′ flanking region are underlined.

active in a wide range of species (Gospodarowicz, 1986). The number of silent nucleotide changes (21 out of 465 or 4.5%) is also very low when compared to other bovine and human genes.

Human basic FGF genomic organization

We also used the bovine basic FGF cDNA clone as a hybridization probe to isolate human genomic recombinants. The restriction enzyme maps of three such recombinants, λMG4, λHT1 and λMG10, are shown in Figure 4. Two introns interrupt the human basic FGF coding sequence; DNA sequence analysis established that the first intron splits codon 60 and the second intron separates codons 94 and 95 (see Figure 2). It is not known whether there are any additional introns in the untranslated regions.

Each of the genomic recombinants shown in Figure 4 carries a single coding exon. Since no overlap can be detected between λMG4 and λHT1, or between λHT1 and λMG10, the precise size of the human basic FGF gene cannot be established. The minimum sizes of the two introns are 16 and 17.5 kb, respectively, making the human basic FGF gene at least 34 kb long.

We have previously shown that in a human hepatoma cell line, SK-HEP-1, basic FGF is encoded by two mRNA species of 4.6 and 2.2 kb (Abraham *et al.*, 1986). The position at which basic FGF gene transcription initiates has not yet been localized, but must be at least 323 bp 5′ to the initiating methionine since that is the amount of 5′ untranslated sequence carried on the cDNA clone λKB7. As with the bovine basic FGF cDNA, the 5′ untranslated region of λKB7 is exceedingly G-C rich. Examination of the sequences in the genomic isolate, λMG4, reveals no obvious TATA-like sequence located 5′ to the end of λKB7 that could serve as a promoter. However, in this 5′ region there are several sequences that are homologous with the G-C box sequence that has been characterized in certain promoters and shown to be a binding site for the transcription factor Sp1 (Dynan and Tjian, 1985; see Figure 2). Further experiments are needed to determine whether transcription does in fact initiate in this region.

A potential polyadenylation signal, AATAAA (Proudfoot and Brownlee, 1976), is found 79 bp 3′ to the TGA termination codon (Figure 2). However, four independent cDNA isolates, λHFL1, λPB2, λKB2 and λET2 (Figure 1) are copies of transcripts that did not polyadenylate at this position. If this site is in fact used as a polyadenylation signal to generate the shorter, 2.2-kb mRNA, then transcription must initiate well upstream of the sequence shown in Figure 2. Interestingly, the bovine basic FGF cDNA does not contain an AATAAA sequence in an equivalent position (Abraham *et al.*, 1986), yet bovine capillary endothelial cells

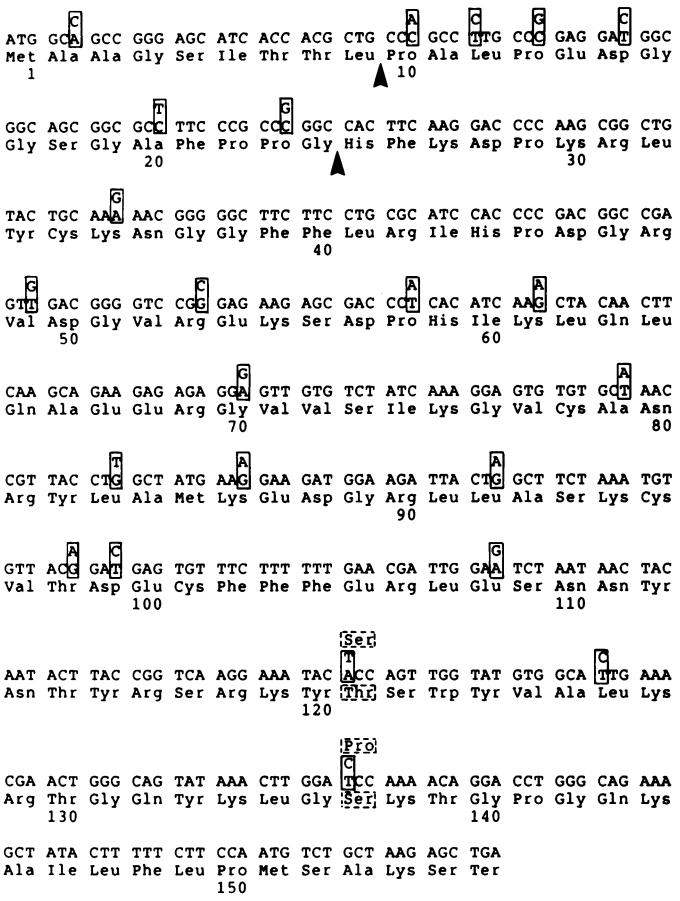

Fig. 3. Comparison of the coding sequences of human and bovine basic FGF. The nucleotide sequence and deduced amino acid sequence of the 155 amino acid form of human basic FGF is shown. The corresponding bovine sequence is shown only where there are differences with respect to the human sequence. The two arrows indicate the amino termini of the two sequenced forms of the basic FGF protein (Esch *et al.*, 1985a; Gospodarowicz, 1986; Gimenez-Gallego *et al.*, 1986).

have recently been shown to contain FGF transcripts that are also 4.6 and 2.2 kb in length (L.Schweigerer, G.Neufeld, J.Friedman, J.A.Abraham, J.C.Fiddes and D.Gospodarowicz, in preparation). No other AATAAA sequences are present in λKB2, the human cDNA clone that has the longest stretch of 3′ untranslated sequence (unpublished results).

Single gene for basic, heparin-binding growth factors

Several endothelial cell mitogens have been described with biochemical properties that are very similar, if not identical, to those of basic FGF. These include chondrosarcoma-derived growth factor (Shing *et al.*, 1984), class 2- and β-heparin-binding growth factors (Lobb and Fett, 1984; Lobb *et al.*, 1985a,b, 1986); eye-derived growth factor I (Courty *et al.*, 1985), β-retina-derived growth factor (Baird *et al.*, 1985a), cartilage-derived growth factor (Sullivan and Klagsbrun, 1985), cationic hypothalamus-derived growth factor (Klagsbrun and Shing, 1985), astroglial growth factor 2 (Pettmann *et al.*, 1985), hepatoma-derived growth factor (Lobb *et al.*, 1986; Klagsbrun *et al.*, 1986), and a component of macrophage-derived growth factor (Baird *et al.*, 1985b). This group of growth factors all elute from heparin–Sepharose at 1.5–2.0 M NaCl, and have an isoelectric point of 9.6; many of them have also been shown to have extremely similar amino acid compositions. The main differences between these factors have been their sites of synthesis and slight molecular

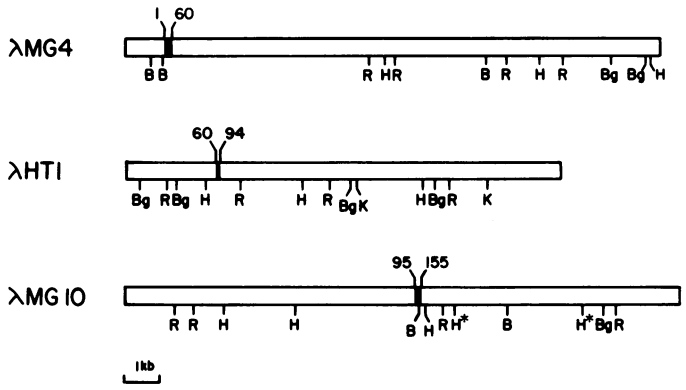

Fig. 4. Restriction enzyme maps of the three genomic recombinants, λMG4, λHT1 and λMG10, containing portions of the human basic FGF gene. Sites are shown for the enzymes *Bam*HI (B); *Eco*RI (R), *Hin*dIII (H); *Bgl*II (Bg) and *Kpn*I (K). *Kpn*I sites were only mapped in λHT1. The region of λMG10 between the two asterisks contains five additional *Hin*dIII sites that have not been mapped. An extra 0.4-kb *Eco*RI fragment is present in λHT1 that has not been mapped. The coding region sequences carried by each of the recombinants are shown by solid shading. Numbers refer to amino acids in the 1–155 amino acid basic FGF. The distances between the ends of λMG4 and λHT1 and between λHT1 and λMG10 are not known.

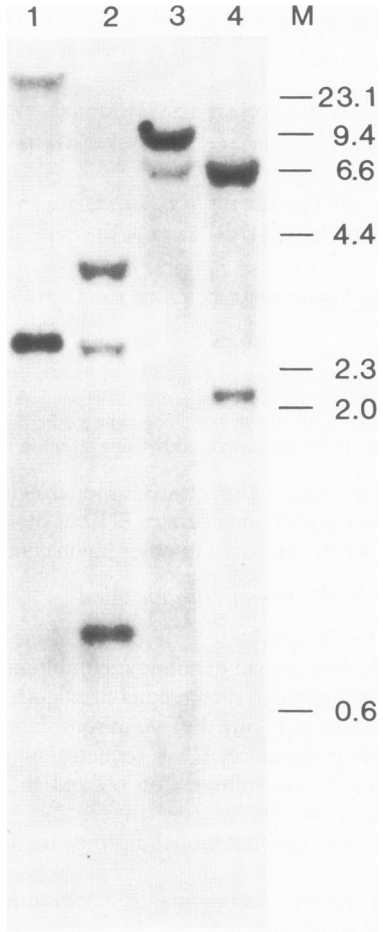

Fig. 5. Human basic FGF genomic Southern analysis. **Lane 1**, *Bam*HI; **lane 2**, *Hin*dIII; **lane 3**, *Pst*I; and **lane 4**, *Eco*RI. Size markers (M) are in kb. In all cases the cDNA probe hybridized very poorly to the exon containing the 5′ end of the gene (e.g. the 9.0-kb *Bam*HI or the 8.0-kb *Hin*dIII fragments). A synthetic oligonucleotide probe for this region (coding exon I) also failed to hybridize. This was presumably due to the very high G-C content of this region.

weight variations (Lobb *et al.*, 1986). These results suggest that either there is a family of related genes, or all of the factors are encoded by a single gene and the variations are due to differential post-translational processing that takes place either *in vivo* or during purification.

To examine these two possibilities, we used the bovine basic FGF cDNA clone to probe Southern blots of human genomic DNA (Figure 5). With *Bam*HI (lane 1), *Hin*dIII (lane 2) and *Eco*RI (lane 4), all enzymes whose sites have been mapped in the genomic clones (Figure 4), the hybridizing genomic fragments all correlate with the restriction enzyme map of the cloned gene. The correlation was confirmed by repeating the hybridization using oligonucleotides specific for coding exons II and III (data not shown). Human basic FGF therefore appears to be encoded by a single-copy gene.

To determine whether any additional basic FGF-like sequences exist in the human genome we repeated the hybridization under low stringency conditions (hybridization at 42°C in 30% formamide, 0.75 M NaCl, 0.075 M sodium citrate; and washes at 50°C in 0.3 M NaCl, 0.03 M sodium citrate). No additional hybridizing fragments were observed (data not shown) indicating that there is no family of basic FGF-like genes.

Discussion

We have isolated cDNA and genomic clones that allowed us to establish the complete amino acid sequence for human basic FGF. This sequence is remarkably homologous to that of bovine basic FGF; only two of the 155 amino acids are different. The basic FGF cDNA clones were isolated from several human tissue sources at very low levels and frequently represented unspliced transcripts of the gene. This observation leads us to propose that basic FGF mRNA synthesis normally takes place in these tissues at a low level and that the fully processed cytoplasmic mRNA is unstable.

The low level and apparent instability of basic FGF mRNA is in contrast to the quantity of basic FGF protein that has been purified from several tissues (Gospodarowicz, 1986). It therefore seems likely that basic FGF is stored in these tissues and that *de novo* basic FGF protein synthesis is not normally taking place. Basic FGF could be stored in an intracellular form or bound to heparan sulfate on the extracellular matrix. Angiogenic stimuli, such as those caused by tissue damage, may, therefore, result in release or activation of stored basic FGF. In support of this hypothesis we readily detected intact basic FGF mRNA in several actively growing cell lines. These include a human hepatoma cell line, SK-HEP-1 (Abraham *et al.*, 1986), and bovine capillary endothelial cells (L.Schweigerer, G.Neufeld, J.Friedman, J.A. Abraham, J.C.Fiddes and D.Gospodarowicz, in preparation). It is possible that cell proliferation activates transcription of the basic FGF gene and that this is related to the production of FGF by tumor cells, in order to promote tumor angiogenesis, and by endothelial cells, in order to stimulate angiogenesis during wound healing.

Since basic FGF has no obvious signal peptide, it may leave the cell in which it was synthesized by some pathway other than transfer across the endoplasmic reticulum. Cell lysis at the site of tissue damage is one possible mechanism whereby basic FGF could be released. An alternative explanation, however, could be the specific transport of basic FGF by some type of specialized mechanism.

No sequence homology has been detected between basic FGF and any known oncogene. However, since basic FGF has been shown to be produced by, as well as stimulate the growth of, capillary endothelial cells (L.Schweigerer, G.Neufeld, J.Friedman, J.A.Abraham, J.C.Fiddes and D.Gospodarowicz, in preparation), it may play a role in stimulating the growth of endothelial cell tumors. Possibly, a change that permits its constitutive export from the cell would lead to basic FGF having oncogenic properties by promoting the uncontrolled growth of endothelial cells.

Materials and methods

Isolation of cDNA clones

Polyadenylated RNA was isolated by the guanidine thiocyanate procedure (Chirgwin *et al.*, 1979) from five different human tissues (adult kidney, fetal liver, fetal heart, term placenta, and a breast carcinoma), Double-stranded cDNA was synthesized from these RNAs using reverse transcriptase; the cDNA was then treated with nuclease SI and cloned via *Eco*RI linkers into λgt10 (Huynh *et al.*, 1985). The resulting libraries were screened (Benton and Davis, 1977) with a 1.4-kb *Eco*RI fragment from the bovine basic FGF cDNA clone, λBB2 (Abraham *et al.*, 1986), which had been labelled by nick translation (Rigby *et al.*, 1977).

Isolation of genomic clones

Two human genomic libraries were screened as described above. Screening of the first library, made from fetal liver DNA cloned in Charon 4A (Lawn *et al.*, 1978), resulted in the isolation of clones λMG4 and λMG10; screening of the second library, made from chemically transformed human fibroblast DNA cloned in Charon 28 (the gift of E.Fritsch) gave clone λHT1. DNA was purified from each of the three recombinant phage and the restriction enzyme maps were derived from a combination of single and double digests.

DNA sequencing

Nucleotide sequences were determined by the dideoxy method (Sanger *et al.*, 1977) following subcloning of cDNA and genomic fragments into M13 vectors (Messing and Vieira, 1982).

Genomic Southern blots

High molecular weight human placental DNA (10 μg) was digested with restriction endonucleases and fractionated on 0.8% agarose gels. After transfer to nitrocellulose (Southern, 1975), the filters were hybridized to the bovine basic FGF cDNA probe as described above.

Acknowledgements

We thank T.Maniatis and E.Fritsch for the human genomic libraries; J.Miller and T.Mikita for help with the DNA sequencing; K.Talmadge for critical reading of the manuscript; E.Stoelting for the artwork and M.Matsumara and E.Pond for preparation of the manuscript. This research was supported by the Biotechnology Research Partners Ltd, and by Small Business Innovation Grant GM36762-01 from the National Institute of General Medical Sciences.

References

Abraham,J.A., Mergia,A., Whang,J.L., Tumolo,A., Friedman,J., Hjerrild,K.A., Gospodarowicz,D. and Fiddes,J.C. (1986) *Science*, **233**, 545–548.

Baird,A., Esch,F., Gospodarowicz,D. and Guillemin,R. (1985a) *Biochemistry*, **24**, 7855–7860.

Baird,A., Mormède,P. and Böhlen,P. (1985b) *Biochem. Biophys. Res. Commun.*, **126**, 358–364.

Benton,W.D. and Davis,R.W. (1977) *Science*, **196**, 180–182.

Böhlen,P., Esch,F., Baird,A., Jones,K.L. and Gospodarowicz,D. (1985) *FEBS Lett.*, **185**, 177–181.

Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J .and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294–5299.

Courty,J., Loret,C., Moenner,M., Chevallier,B., Lagente,O., Courtois,Y. and Barritault,D. (1985) *Biochimie*, **67**, 265-269.

Dayhoff,M.O. (ed.) (1976) *Atlas of Protein Sequence and Structure.* The National Biomedical Research Foundation, Silver Spring, MD, Vol. 5, suppl. 2, p. 127.

Dynan,W.S. and Tjian,R. (1985) *Nature*, **316**, 774–778.

Esch,F., Baird,A., Ling,N., Ueno,N., Hill,F., Denoroy,L., Klepper,R., Gospodarowicz,D., Böhlen,P. and Guillemin,R. (1985a) *Proc. Natl. Acad. Sci. USA*, **82**, 6507–6511.

Esch,F., Ueno,N., Baird,A., Hill,F., Denoroy,L., Ling,N., Gospodarowicz,D. and Guillemin,R. (1985b) *Biochem. Biophys. Res. Commun.*, **133**, 554–562.

Folkman,J. (1985) In Winters,R. (ed.), *Important Advances in Oncology.* J.B.Lippincott Co., Philadelphia, PA, pp. 42–62.

Gimenez-Gallego,G., Rodkey,J., Bennett,C., Rios-Candelore,M., DiSalvo,J. and Thomas,K. (1985) *Science*, **230**, 1385–1388.

Gimenez-Gallego,G., Conn,G., Hatcher,V.B. and Thomas,K.A. (1986) *Biochem. Biophys. Res. Commun.*, **135**, 541–548.

Gospodarowicz,D. (1985) In Ford,R.J. and Maizel,A.L. (eds), *Mediators in Cell Growth and Differentiation.* Raven Press, New York, NY, pp. 109–134.

Gospodarowicz,D. (1986) In *Methods Enzymol.*, in press.

Gospodarowicz,D., Bialecki,H. and Thakral,T.K. (1979) *Exp. Eye Res.*, **28**, 501–514.
Gospodarowicz,D., Cheng,J., Lui,G.-M., Baird,A., Böhlen,P. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 6963–6967.
Huynh,T.V., Young,R.A. and Davis,R.W. (1985) In Glover,D.M. (ed.), *DNA Cloning: A Practical Approach*. IRL Press, Oxford, Vol. **I**, pp. 49–78.
Jacobs,K., Shoemaker,C., Rudersdorf,R., Neill,S.D., Kaufman,R.J., Mufson,A., Seehra,J., Jones,S.S., Hewick,R., Fritsch,E.F., Kawakita,M., Shimizu,T. and Miyake,T. (1985) *Nature*, **313**, 806–810.
Kawasaki,E.S., Ladner,M.B., Wang,A.M., Van Arsdell,J.V., Warren,M.K., Coyne,M.Y., Schweickart,V.L., Lee,M.-T., Wilson,K.J., Boosman,A., Stanley,E.R., Ralph,P. and Mark,D.F. (1985) *Science*, **230**, 291–296.
Klagsbrun,M. and Shing,Y. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 805–809.
Klagsbrun,M., Sasse,J., Sullivan,R. and Smith,J.A. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2448–2452.
Lawn,R.M., Fritsch,E.F., Parker,R.C., Blake,G. and Maniatis,T. (1978) *Cell*, **15**, 1157–1174.
Lobb,R.R. and Fett,J.W. (1984) *Biochemistry*, **23**, 6295–6299.
Lobb,R.R., Alderman,E.M. and Fett,J.W. (1985a) *Biochemistry*, **24**, 4969–4973.
Lobb,R.R., Strydom,D.J. and Fett,J.W. (1985b) *Biochem. Biophys. Res. Commun.*, **131**, 586–592.
Lobb,R., Sasse,J., Sullivan,R., Shing,Y., D'Amore,P., Jacobs,J. and Klagsbrun,M. (1986) *J. Biol. Chem.*, **261**, 1924–1928.
Messing,J. and Vieira,J. (1982) *Gene*, **19**, 269–276.
Miller,W.L. and Eberhardt,N.L. (1983) *Endoc. Rev.*, **4**, 97–130.
Neufeld,G. and Gospodarowicz,D. (1985) *J. Biol. Chem.*, **260**, 13860–13868.
Neufeld,G. and Gospodarowicz,D. (1986) *J. Biol. Chem.*, **261**, 5631–5637.
Pettmann,B., Weibel,M., Sensenbrenner,M. and Labourdette,G. (1985) *FEBS Lett.*, **189**, 102–108.
Proudfoot,N.J. and Brownlee,G.G. (1976) *Nature*, **263**, 211–214.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237–251.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
Shing,Y., Folkman,J., Sullivan,R., Butterfield,C., Murray,J. and Klagsbrun,M. (1984) *Science*, **223**, 1296–1299.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503–517.
Sullivan,R. and Klagsbrun,M. (1985) *J. Biol. Chem.*, **260**. 2399–2403.
Thomas,K.A. and Gimenez-Gallego,G. (1986) *Trends Biochem. Sci.*, **11**, 81–84.

Received on 30 June 1986