Journal of Cellular Biochemistry 47:158–164 (1991)

Transcriptional Regulation of Basic Fibroblast Growth Factor Gene Expression in Capillary Endothelial Cells

Herbert A. Weich, Niggi Iberg, Michael Klagsbrun, and Judah Folkman

Department of Surgery, Children's Hospital (H.A.W., N.I.); Departments of Anatomy and Cellular Biology (J.F.) and Biological Chemistry and Molecular Pharmacology (M.K.), Harvard Medical School, Boston, Massachusetts 02115

Abstract The growth of capillary endothelial cells (BCE) is an important regulatory step in the formation of capillary blood vessels. In vivo, the proliferation of these cells is stringently controlled. In vitro they can be stimulated by polypeptide growth factors, such as acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF). Since bFGF is synthesized and stored by vascular endothelial cells, this mitogen may play an important role in an autocrine growth regulation during angiogenesis. Here, evidence is presented for induction of the mRNA of bFGF by bFGF itself. A similar increase of bFGF mRNA was observed in response to thrombin and after treatment with phorbol ester. These results suggest that an autocrine loop may exist that may serve to modulate the mitogenic response in BCE under various physiological conditions, (e.g., wound healing and new capillary formation).

Key words: bFGF, capillary endothelial cells, transcriptional regulation, autoinduction

bFGF and aFGF are potent growth factors for capillary endothelial cells (BCE) (Moscatelli et al., 1986; Klagsbrun and Shing, 1985; Gospodarowicz, 1984). Furthermore, it has been shown that bFGF is angiogenic in vitro and in vivo (Gospodarowicz et al., 1979; Thomas et al., 1985; Montesano et al., 1986; Sato and Rifkin, 1988). Not only is bFGF a mitogen for endothelial cells, but it is also synthesized by endothelial cells (Hannan et al., 1988; Weich et al., 1990). Recently it has been shown that an antibody specific for bFGF inhibits the growth of capillary endothelial cells in the presence or absence of exogenous bFGF, indicating that this mitogen is necessary for in vitro growth of these cells (Matsuzaki et al., 1989). Taken together, these observations suggest that endothelial cell proliferation might depend on a bFGF mediated autocrine loop.

Growth factors can stimulate the expression of other growth factors. For example, vascular endothelial cells express genes for both the PDGF A-chain and B-chain and PDGF is secreted into the media (DiCorletto and Bowen-Pope, 1983; Collins et al., 1985; Kavanaugh et al., 1988). The transient expression of the A-chain and B-chain is enhanced by TGF-β in vascular endothelial cells (Starksen et al., 1987), whereas thrombin and the phorbol ester 12-*o*-tetradecanoylphorbol-13-acetate (TPA) stimulate B-chain expression, but not A-chain expression (Kavanaugh et al., 1988).

Extensive efforts have been made to determine whether or not paracrine or autocrine growth stimulation are important regulatory mechanisms for cellular transformation and for the formation of naturally occurring tumors (Sporn and Todaro, 1980; Doolittle et al., 1983; Waterfield et al., 1983). It has been shown that cellular transformation can be induced by appropriate growth factors by an autocrine mechanism, whereby one can distinguish between an external and an internal autocrine loop (Keating and Williams, 1988; Bejcek et al., 1989). Unlike PDGF, bFGF and aFGF have no signal peptides, making it difficult for them to participate in autocrine loops. However, it has been shown that cells become transformed if they are transfected with a construct in which bFGF is linked to a signal peptide in order to direct it through the secretory pathway (Rogelj et al., 1988).

Received May 3, 1991; accepted June 17, 1991.

Address reprint requests to Dr. Herbert A. Weich, Molecular Cell Biology, University of Freiburg, Mooswaldallee 1-9, D-7800 Freiburg, Germany.

In this report we demonstrate that stimulation of BCE and BALB 3T3 with exogenous bFGF results in a transient increase of the mRNA for bFGF. TPA and thrombin also increase the level of bFGF mRNA. Stimulation of bFGF mRNA levels by exogenous bFGF or thrombin is maximal after 2 hours, whereas TPA treatment leads to a maximum effect 4 hours after stimulation. The existence of a positive autocrine feedback loop may serve as an amplification mechanism of the pre-replicative phase of BCE proliferation.

MATERIAL AND METHODS

Cell Proliferation

Human recombinant basic fibroblast growth factor was a gift from Takeda Industries, Japan; bovine thrombin, 12-*o*-tetra-decanoylphorbol-13-acetate (TPA), and cycloheximide (CH) were obtained from Sigma.

Bovine capillary endothelial cells (BCE) obtained from primary cultures of calf adrenal glands (Folkman et al., 1976) were cultured on gelatin-coated dishes (1.5% Gibco, Grand Island, NY) in DMEM (Gibco), 10% calf serum (HyClone, Logan, UT), Endothelial Mitogen 25 μg/ml (Biomedical Technologies Inc., Cambridge, MA), penicillin G (100 U/ml), and streptomycin (100 μg/ml) and maintained at 37°C in a 10% CO_2 humidified environment.

To measure BCE proliferation, cells were plated sparsely (1×10^4 cells per gelatinized 2.1 cm^2 well) in DMEM containing 10% calf serum. After overnight attachment, the media were changed and the additions were made according to the experimental design. Fresh mitogens were also added on day 2. The cultures were incubated for 2 and 4 days and the cell number was determined by electronic cell counting.

For the induction experiments, confluent cultures at passages 10–14 were made quiescent by replacing the media with serum-free DMEM containing 2 μg/ml insulin, 5 μg/ml transferrin, and 1 mg/ml bovine serum albumin for 24 h prior to assay. Quiescent, serum-depleted BALB 3T3 cells were used as described (Klagsbrun and Shing, 1985). Confluent, quiescent cultures of capillary endothelial cells were stimulated with basic FGF (2.5–5 ng/ml), thrombin (5 units/ml), TPA (100 ng/ml), or cycloheximide (10 μg/ml) for the indicated times periods, washed once with cold phosphate-buffered saline (PBS), and used for RNA preparation.

Northern Blot Analysis

Total cellular RNA from cultured cells was prepared as described (Chirgwin et al., 1979), except that the protease digestion step was omitted. RNA was quantitated spectrophotometrically by its absorbance at 260 nm. Ten micrograms of total RNA were denatured in 2.2 M formaldehyde/50% of formamide (v/v) in the presence of ethidiumbromide, and RNA gels were prepared by a new surface tension method (Rosen et al., 1990). After elektrophoresis, gels were photographed and briefly rinsed in H_2O. RNA was transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene NH) using 10 × SSC (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate). Following transfer, the position of the ribosomal RNA bands was marked under UV-light and the filter baked in a vacuum oven at 80°C for 2 h. Hybridization with ^{32}P-labeled cDNA probes was performed as previously described (Weich et al., 1986). The blots were washed at 65°C in 60 mM NaCl/0.1% SDS/2 mM EDTA. The bFGF cDNA probe (kindly provided by J. Abraham and J. Fiddes, California Biotechnology, Inc.) was a 1.0 kb Nco I fragment of the bovine bFGF cDNA clone pJJ11-1 (Abraham et al., 1986a,b). The cDNA insert contains the entire protein coding sequence of bovine bFGF, and 580 bp of the 3′-untranslated region. The chicken α-tubulin cDNA was kindly provided by K. Rosen (Department of Neurology Research, Children's Hospital, Boston, MA). The 1.3 kb Hind III fragment was used as a probe to estimate sample loading and integrity of RNA samples after reprobing of each blot. Isolated cDNA fragments were labeled with ^{32}P using a random hexanucleotide priming kit (Boehringer Mannheim). Specific activity of the probes was $0.5–2 \times 10^9$ dpm/μg DNA. Deoxycytidine 5′-[α-^{32}P] triphosphate (~3000 Ci/mmol) was obtained from Amersham Corp.

For quantification of bFGF mRNA induction after Northern blot analysis, blots were exposed to Kodak XAR-5 films for 5–17 days at room temperature without using an intensifying screen. Under these conditions the development of silver grains in the film is proportional to the radioactivity over a wide range if not overexposed. The relative autoradiographic signal from the bFGF probe was then quantified using a Hoefer Scientific GS 300 scanning densitometer. In each lane, the 7 kb band was scanned

three times and the arithmetic mean was used to determine the relative autoradiographic signal.

RESULTS

The effects of adding exogenous bFGF on endogenous bFGF synthesis in BCE were measured. First, BCE were analyzed for their proliferative response to bFGF. Cells were plated at low density in bFGF-free media, and 24 h later the medium was changed to low-serum conditions. Cells were left unstimulated or were stimulated with recombinant human bFGF during a 4-day time period. Under bFGF-free conditions the cell number increased 1.5-fold, but when the bFGF-free media was supplemented with 5 ng/ml bFGF, a concentration which is sufficient for maximal stimulation of BCE growth (Hannan et al., 1988), the cell number increased 8.5-fold (Fig. 1). Optimal BCE growth was obtained in media containing 10% bovine serum supplemented with endothelial mitogen, a crude brain extract containing aFGF and bFGF. Under these conditions cell growth was linear over the indicated time period and cell number increased more than 12-fold (Fig. 1).

In order to analyze the induction of bFGF mRNA (Fig. 2A), confluent BCE were made quiescent by incubation for 24 h in bFGF-free media. These conditions have been previously shown to be sufficient to allow endothelial cells

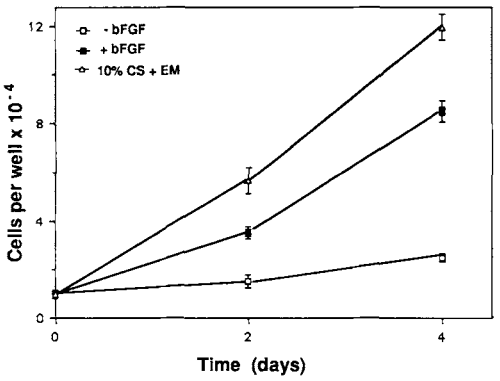

Fig. 1. Analysis of bovine capillary endothelial cell (BCE) proliferation in response to bFGF. 1×10^4 cells were plated in DMEM medium containing 10% calf serum (CS) and incubated for 24 h to allow attachment. Medium was replaced by DMEM/2% CS (day 0) without (−bFGF) or supplemented with 5 ng/ml bFGF (+bFGF) for the time period indicated. Medium was replaced at day 2 and cells were counted at day 2 and 4. To determine optimal growth conditions, cells were also cultured in 10% CS supplemented with endothelial mitogen (10% CS + EM). The data points represent the mean ±SD of an experiment done in triplicate.

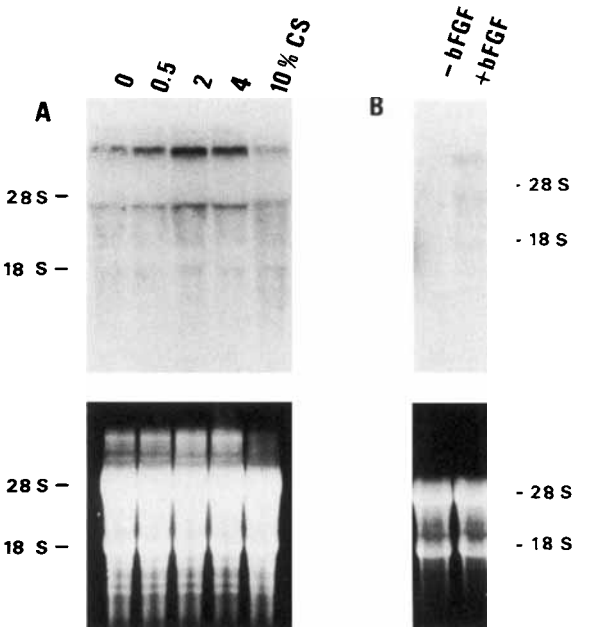

Fig. 2. Northern blot analysis of the effects of bFGF on the levels of bFGF mRNA in bovine capillary endothelial cells (BCE) and BALB 3T3 cells. (A) BCE were grown in serum-containing medium until confluent and then switched to defined media for 24 h as described in "Materials and Methods." RNA was isolated from quiescent BCE after stimulation with 2.5 ng/ml bFGF for 1 to 4 h. Total RNA from cells grown in 10% calf serum (late log phase) was also included. (B) Confluent, serum-depleted BALB-3T3 cells were treated with 2.5 ng/ml bFGF for 2 h and used for RNA preparation. For Northern blot analysis, 10 μg of total RNA per lane was fractionated on a 1.2% agarose-formaldehyde gel and transferred to nitrocellulose membranes. The bFGF transcripts were detected by hybridization to a ^{32}P-labeled 1 kb Nco I bovine cDNA fragment (**top panel**). As a control for sample loading and RNA integrity, the gel used for blotting is shown after ethidium bromide staining (**lower panel**). Migration of 28 S (5 kb) and 18 S (2.1 kb) ribosomal RNA markers are indicated.

to become growth-arrested and to become sensitive to mitogenic stimulation (Starksen et al., 1987). After the addition of bFGF, the levels of bFGF mRNA were analysed by Northern blot hybridization. Addition of the mitogen caused a transient increase in expression of bFGF mRNA (Fig. 2A). Transcripts were approximately 1.9, 4.0, and 7.0 kb in size. All these transcripts were slightly increased after 0.5 h and peaked after 2 h with a maximal increase of 2.1-fold relative to untreated controls (Figs. 2A).

The effect of autoinduction was also analyzed in mouse 3T3 cells (Fig. 2B). In contrast to bovine endothelial cells, these cells constitutively produce only a very low amount of cell-associated bFGF (Iberg et al., 1989). Serum-starved mouse 3T3 cells were treated with bFGF

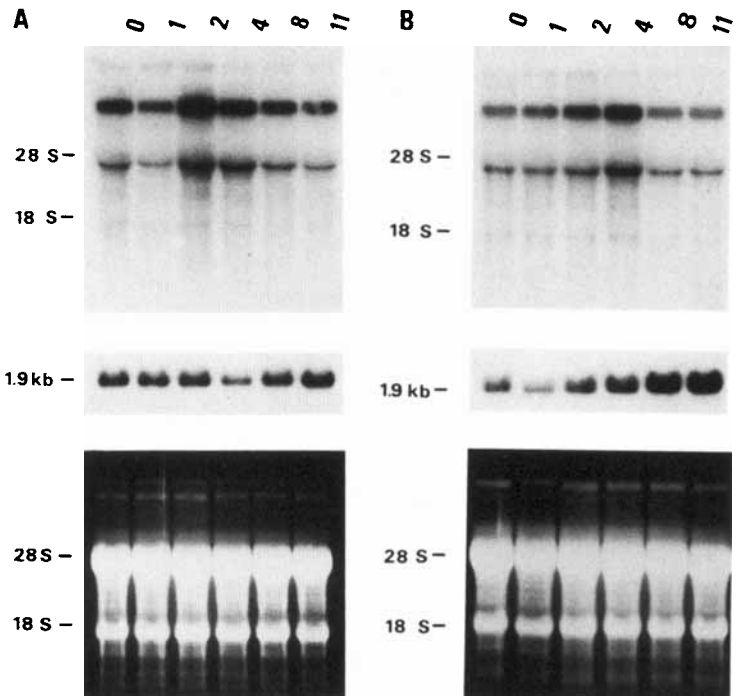

Fig. 3. Effect of thrombin (**A**) and TPA (**B**) on the levels of bFGF mRNA in BCE. Total RNA (10 μg) from quiescent BCE stimulated with thrombin (5 units/ml) or the phorbol ester TPA (100 ng/ml) over 0–11 h was subjected to Northern blot analysis. RNA was size fractionated on a 1.2% agarose-formaldehyde gel and transferred to nitrocellulose membranes (**top panel**). To control for sample transfer, radiolabeled DNA was stripped and the filter reprobed using a chicken α-tubulin cDNA fragment. The level of α-tubulin mRNA, initially used for demonstrating sample loading and RNA integrity, was modulated by TPA treatment (**middle panel**). Equal sample loading was therefore demonstrated by showing ethidium bromide stained gels used for RNA blotting (**lower panel**). Migration of 28 S (5 kb) and 18 S (2.1 kb) ribosomal RNA markers are indicated.

for 2 h and the level of bFGF mRNA was analysed by Northern blot hybridization. Untreated cells exhibited a very low level of bFGF mRNA expression. Following 2 h of treatment, the level increased 4.3-fold (Fig. 2B). Densitometric scanning of the bands indicated an even stronger induction of bFGF mRNA in mouse 3T3 cells than in endothelial cells.

In order to determine whether or not bFGF mRNA levels in BCE are regulated by agents other than bFGF, quiescent cells were stimulated with thrombin or the phorbol ester TPA, and bFGF mRNA levels were determined by Northern blot hybridization. Thrombin is a potent mitogen (Chambard et al., 1987) and TPA activates protein kinase C. Activation of this enzyme by phorbol esters promotes proliferation of cells (Dicker and Rozengurt, 1980, Nishizuka, 1986). Thrombin and TPA increased bFGF mRNA levels transiently (Fig. 3A,B). Stimulation of bFGF mRNA levels by thrombin (Fig. 3A) was maximal after 2 h (2.4-fold).

The time course of induction by thrombin was almost identical to that induced by bFGF (Fig. 4). On the other hand, the time course of TPA induction of bFGF mRNA (Fig. 3B) was different from thrombin (Fig. 4), with the maximal effect occurring 4 h after stimulation (2.6-fold). The different time courses suggest that TPA

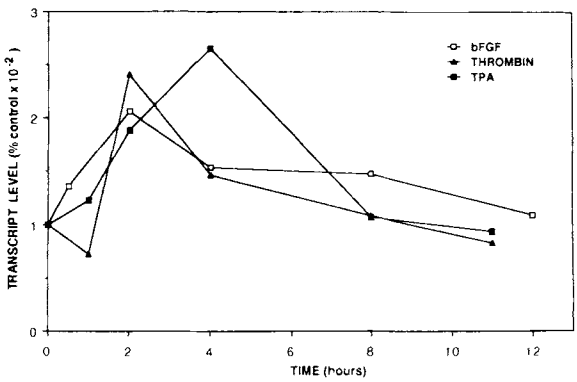

Fig. 4. Densitometric analysis of the time course of bFGF mRNA induction in BCE. mRNA hybridization bands were quantified by scanning densitometry of underexposed unenhanced autoradiographs. The silver grain density of the 7 kb band representing the largest bFGF transcript was compared among treatment groups. Data from the treatment groups are expressed as percentage of control values.

treatment increases bFGF mRNA levels by mechanisms different from those induced by thrombin or bFGF. The maximal stimulation of bFGF mRNA levels was similar for all three agents (2.1- to 2.6-fold). In all cases bFGF mRNA levels were decreased to near control values 8–12 h after treatment.

Studies of bFGF mRNA expression in different cell types have demonstrated the existence of several distinguishable transcripts, of which the 4 kb and 7 kb RNAs are the major forms (Schweigerer et al., 1987; Kurokawa et al., 1987; Sternfeld et al., 1988; Weich et al., 1990). Neither induction with bFGF (Fig. 2) nor treatment with thrombin or TPA (Fig. 3A,B) had a selective effect on the induction of the different transcripts. All of the transcripts were induced equally by bFGF, thrombin and TPA.

To determine whether or not the induction of bFGF expression could be modified by inhibition of de novo protein synthesis, the effect of cycloheximide treatment was analyzed. Quiescent BCE in serum-free media were treated with cycloheximide 30 min before, as well as during, a 2-h bFGF treatment. As shown in Figure 5, the addition of cycloheximide alone had a positive effect, leading to a 3.1-fold increase of bFGF mRNA levels after treatment. bFGF alone increased bFGF mRNA 1.6-fold 2 h after treatment. No additive effect was observed by treatment of quiescent BCE with cycloheximide for 30 min followed by cycloheximide and bFGF together for 2 h (Fig. 5). In fact, the increase of mRNA after bFGF and cycloheximide treatment together was lower than the treatment with cycloheximide alone (2.1-fold v. 3.1 fold).

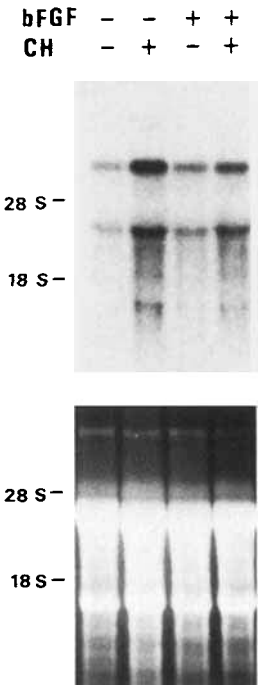

Fig. 5. Northern blot analysis of the effects of cycloheximide on bFGF mRNA levels in BCE. Quiescent BCE were treated for 2 h with cycloheximide (10 μg/ml) and/or bFGF (2.5 ng/ml) as indicated. Northern blot analysis for the detection of the bFGF mRNA **(top panel)** was done as described in Figure 1. To demonstrate sample loading and RNA integrity, the ethidium bromide gel used for RNA blotting is also shown **(lower panel)**. Migration of 28 S (5 kb) and 18 S (2.1 kb) ribosomal RNA markers are indicated.

DISCUSSION

The induction of growth factor mRNA by growth factors and other agents has recently been reported by several groups (Paulsson et al., 1987; Starksen et al., 1987, Weich et al., 1987; Gay and Winkles, 1990). For example, aFGF modulates PDGF gene expression in human endothelial cells (Gay and Winkles, 1990). The addition of aFGF to quiescent cells increased the level of PDGF A-chain mRNA, but not PDGF B-chain mRNA. PDGF A-chain protein was also synthesized and secreted in increased amounts. In experiments with microvessel endothelial cells using TGF-β, thrombin and TPA as stimulatory agents, a transient increase in PDGF A-chain and B-chain mRNA was demonstrated (Starksen et al., 1987; Kavanaugh et al., 1988). Taken together these results indicated a differential regulation of PDGF A-chain und B-chain genes in endothelial cells by growth factors.

Since capillary endothelial cells synthesize bFGF, which remains cell associated (Vlodavsky et al., 1987) and since endogenous bFGF acts as an autocrine mitogen of endothelial cell growth (Schweigerer et al., 1987), we were interested in determining whether or not exogenous bFGF regulates endogenous bFGF expression. To identify possible regulators of bFGF production, capillary endothelial cells were treated with bFGF, TPA, and thrombin, and bFGF mRNA levels were analyzed by Northern blot analysis. It was found, that (i) the bFGF mRNA levels could be induced by bFGF itself, by thrombin, and by TPA; (ii) for each agent the level of the three bFGF transcripts was transiently increased with a maximum at 2–4 h after stimulation; (iii) the degree of bFGF mRNA induction was different for the three agents: TPA had the most stimulatory effect followed by thrombin and bFGF; and (iv) cycloheximide alone was able to induce bFGF mRNA levels, whereas bFGF in combination

with cycloheximide did not superinduce the level of bFGF mRNA in endothelial cells.

Unlike the well-known effects of bFGF on endothelial cell growth, the effects of thrombin and TPA stimulation of protein kinase C on endothelial cell growth are not well characterized. Thrombin is a potent mitogen for several cell types, such as vascular smooth muscle cells (Chambard et al., 1987; Huang and Ives, 1987). The mitogenic effects of thrombin on endothelial cells have not been described. Thrombin causes receptor-mediated activation of a phospholipase C (PLC) leading to the release of IP_3 and diacylglycerol with subsequent activation of PKC (Dicker and Rozengurt, 1980; Nishizuka, 1986). Activation of PKC is important for proliferation of several cell types, and activation of this enzyme by phorbol esters promotes growth in many systems (Nishizuka, 1986). However, TPA is not always mitogenic. In capillary endothelial cells TPA inhibits bFGF mitogenic activity and cell surface binding by down-regulation of bFGF receptors (Doctrow and Folkman, 1988; Doctrow, 1989). This result suggests that the induction of bFGF mRNA by bFGF and thrombin may be due to mitogenic stimulation, but that the induction of mRNA by TPA might be independent of growth stimulation.

To determine whether or not the bFGF effect could be abolished by inhibition of protein synthesis, cells were treated with cycloheximide. Cycloheximide induced an increase of bFGF mRNA expression, but by a different mechanism than bFGF, thrombin, and TPA. Cycloheximide alone may influence bFGF mRNA stability or prevent transcriptional repression (Lau and Nathans, 1987; Almendral et al., 1988). bFGF in combination with cycloheximide did not superinduce the level of bFGF mRNA in endothelial cells, indicating that the effect of bFGF on the induction of bFGF mRNA might depend on de novo protein synthesis. Our studies are consistent with those of Sternfeld (1988), who investigated the differential expression of the bFGF gene in human foreskin fibroblasts. Treatment of fibroblasts with cycloheximide alone resulted in an increase of bFGF mRNA level (4-fold v. 3.1-fold in our studies), but cells treated with cycloheximide before and during serum stimulation did not show any additional induction of bFGF mRNA. On the other hand, if quiescent cells were treated with serum, the level of all bFGF mRNA transcripts increased up to 10-fold (Sternfeld et al., 1988).

Our studies demonstrated that growth factors can induce their own synthesis. Thus, the mitogenic response of cells to a given growth factor might be a complex response involving not only the exogenous growth factors, but the activity of endogenous growth factors as well. Whether or not these endogenous growth factors participate in autocrine loops needs to be determined.

ACKNOWLEDGMENTS

H.A.W. is a recipient of a fellowship from the Deutsche Forschungsgemeinschaft, grant We 1211-1; N.I. is a recipient of a grant from the Swiss National Science Foundation, grant 83.497.0.87, and M.K. was supported by NCI grant CA-37392. This work was also supported by a grant from Takeda Chemical Industries, Ltd. to Harvard University (J.F.).

REFERENCES

Abraham JA, Mergia A, Whang JL, Tumolo, A, Friedman J, Hjerrild KA, Gospodarowicz D, Fiddes JC (1986a): Nucleotide sequences of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. Science 233:545–548.

Abraham JA, Whang JL, Tumolo A, Mergia A, Friedman J, Gospodarowicz D, Fiddes JC (1986b): Human basic fibroblast growth factor: Nucleotide sequence and genomic organization. EMBO J 5:2523–2528.

Almendral JM, Sommer D, Macdonald-Bravo H, Burckhardt J, Perera J, Bravo R (1988): Complexity of the early genetic response to growth factors in mouse fibroblasts. Mol Cell Biol 8:2140–2148.

Bejcek BE, Li DY, Deuel TF (1989): Transformation by *v-sis* occurs by an internal autoactivation mechanism. Science 245:1496–1499.

Chambard JC, Paris S, L'Allemain G, Pouyssegur J (1987): Two growth factor signalling pathways in fibroblasts distinguished by pertussis toxin. Nature 326:800–803.

Chirgwin JM, Pryzyba AE, MacDonald RJ, Ruttner WJ (1979): Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. Biochemistry 18: 5294–5299.

Collins T, Ginsburg D, Boss JM, Orkin SH, Pober JS (1985): Cultured human endothelial cells express platelet-derived growth factor chain 2: cDNA cloning and structural analysis. Nature 316:748–750.

Dicker P, Rozengurt E (1980): Phorbol esters and vasopressin stimulate DNA synthesis by a common mechanism. Nature 287:607–612.

DiCorletto PE, Bowen-Pope DF (1983): Cultured endothelial cells produce a platelet-derived growth factor-like protein. Proc Natl Acad Sci. USA 80:1919–1923.

Doctrow SR (1989): Protein kinase C-mediated modulation of bFGF receptors in capillary endothelial cells. Abstract 1485 Am Soc Cell Biol J Cell Biol 107:Nb6

Doctrow SR, Folkman J (1988): Protein kinase C activators supress stimulation of endothelial cell growth by angiogenic endothelial mitogens. J Cell Biol 104:679–687.

Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Rob-

bins KC, Aaronson SA, Antoniades HN (1983): Simian sarcoma virus *onc* gene, *v-sis*, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221:275–277.

Folkman J, Haudenschild CC, Zetter BR (1976): Long-term culture of capillary endothelial cells. Proc Natl Acad Sci USA 76:5217–5221.

Gay CG, Winkles JA (1990): Heparin-binding growth factor-1 stimulation of human endothelial cells induces platelet-derived growth factor A-chain gene expression. J Biol Chem 265:3284–3292.

Gospodarowicz D (1984): Biological activity in vivo and in vitro of pituitary and brain fibroblast growth factor. Symp Fundam Cancer Res 37:109–134.

Gospodarowicz D, Bialecki H, Thakral TK (1979): The angiogenic activity of the fibroblast and epidermal growth factor. Exp Eye Res 28:501–514.

Hannan RL, Kourembanas S, Flanders KC, Rogelj S, Roberts AB, Faller DV, Klagsbrun M (1988): Endothelial cells synthesize basic fibroblast growth factor and transforming growth factor beta. Growth Factors 1:7–17.

Huang C-L, Ives HE (1987): Growth inhibition by protein kinase C late in mitogenesis. Nature 329:849–850.

Iberg N, Rogelj S, Fanning P, Klagsbrun M (1989): Purification of 18 and 22 kDa forms of basic fibroblast growth factor from rat cells transformed by the *ras oncogene*. J Biol Chem 264:19951–19955.

Kavanaugh WM, Harsh GR, Starksen NF, Rocco CM, Williams LT (1988): Transcriptional regulation of the A and B chain genes of platelet-derived growth factor in microvascular endothelial cells. J Biol Chem 263:8470–8472.

Keating M, Williams LT (1988): Autocrine stimulation of intracellular PDGF receptors in *v-sis*-transformed cells. Science 239:914–916.

Klagsbrun M, Shing Y (1985): Heparin affinity of anionic and cationic capillary endothelial cell growth factors: Analysis of hypothalamus-derived growth factors and fibroblast growth factors. Proc Natl Acad Sci USA 82:805–809.

Kurokawa T, Sasada R, Iwane M, Igasashi K (1987): Cloning and expression of cDNA coding for human basic fibroblast growth factor. FEBS Lett 213:189–194.

Lau LF, Nathans D (1987): Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: Coordinate regulation with c-*fos* or c-*myc*. Proc Natl Acad Sci USA 84:1182–1186.

Matsuzaki K, Yoshitake Y, Matuo Y, Sasaki H, Nishikawa K (1989): Monoclonal antibodies against heparin-binding growth factor II/basic fibroblast growth factor that block its biological activity: Invalidity of the antibodies for tumor angiogenesis. Proc Natl Acad Sci USA 86:9911–9915.

Montesano R, Vassali JD, Baird A, Guillemin, R, Orci L (1986): Basic FGF induces angiogenesis in vitro. Proc Natl Acad Sci USA 83:7297–7301.

Moscatelli D, Presta M, Rifkin DB (1986): Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration. Proc Natl Acad Sci USA 83:2091–2095.

Nishizuka Y (1986): Studies and perspectives of protein kinase C. Science 233:305–312.

Paulsson Y, Hammacher A, Heldin C-H, Westermark B (1987): Possible positive autocrine feedback in the prereplicative phase of human fibroblasts. Nature 328:715–717.

Rogelj S, Weinberg RA, Fanning P, Klagsbrun M (1988): Basic fibroblast growth factor fused to a signal peptide transforms cells. Nature 331:173–175.

Rosen KM, Lamperti ED, Villa-Komaroff L (1990): Optimizing the Northern blot procedure. Biotechniques 8:398–403.

Sato Y, Rifkin DB (1988): Autocrine activities of basic fibroblast growth factor: Regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J Cell Biol 107:1199–1205.

Schweigerer L, Neufeld G, Friedman J, Abraham JA, Fiddes JC, Gospodarowicz D (1987): Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. Nature 325:257–259.

Sporn MB, Todaro GJ (1980): Autocrine secretion and malignant transformation of cells. N Engl J Med 303:878–880.

Starksen NF, Harsch GR, Gibbs VC, Williams LT (1987): Regulated expression of the platelet-derived growth factor A chain in microvascular endothelial cells. J Biol Chem 262:14381–14384.

Sternfeld MD, Hendrickson JE, Keeble WW, Rosenbaum JT, Robertson JE, Pittelkow MR, Shipley D (1988): Differential expression of mRNA coding for heparin-binding growth factor type 2 in human cells. J Cell Physiol 136: 297–304.

Thomas KA, Rios-Candelore M, Gimenez-Gallego G, DiSalvo J, Bennet C, Rodkey J, Fitzpatrick S (1985): Pure brain-derived acidic fibroblast growth factor is a potent angiogenic vascular endothelial cell mitogen with sequence homology to interleukin 1. Proc Natl Acad Sci USA 82:6409–6413.

Vlodavsky I, Fridman R, Sullivan R, Sasse J, Klagsbrun M (1987): Aortic endothelial cells synthesize basic fibroblast growth factor which remains cell associated and platelet-derived growth factor-like protein which is secreted. J Cell Physiol 131:402–408.

Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson A, Westermark B, Heldin C-H, Huang JS, Deuel TF (1983): Platelet-derived growth factor is structurally related to the putative transforming protein $p28^{sis}$ *of simian sarcoma virus*. *Nature* 304:35–39.

Weich HA, Herbst D, Schairer, HU, Hoppe J (1987): Platelet-derived growth factor: Phorbol ester induces the expression of the B-chain, but not of the A-chain, in HEL cells. FEBS Lett 213:89–94.

Weich HA, Iberg N, Klagsbrun M, Folkman J (1990): Expression of acidic and basic fibroblast growth factors in human and bovine vascular smooth muscle cells. Growth Factors 2:313–320.

Weich HA, Sebald W, Schairer HU, Hoppe J (1986): The human osteosarcoma cell line U-2 OS expresses a 3.8 kilobase mRNA which codes for the sequence of the PDGF-B chain. FEBS Lett 198:344–348.