Direct evidence for methylation of arginine residues in high molecular weight forms of basic fibroblast growth factor

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Basic fibroblast growth factor (bFGF) is a heparin-binding angiogenic polypeptide mitogen. Protein sequence analysis of bFGF isolated from tissue sources initially established that it is composed of 146 amino acids (apparent M_r 18 000). More recently larger apparent molecular weight forms have been identified and partially characterized. In addition, these high molecular weight forms (apparent M_r 22 000 and 25 000) have been shown to localize preferentially to nuclear fractions of transfected cells. In this report we demonstrate that the higher molecular weight, amino terminally extended forms of bFGF contain methylated arginine residues. The demonstration is based on 1) amino acid sequence analysis of a protein known to contain methylated arginine (myelin basic protein) and a comparison with amino acid sequence analysis of trypsin-derived fragments of the high molecular weight bFGF purified from guinea pig brain and 2) the ability to label *in vivo* the high molecular weight forms of bFGF with S-adenosyl-L-(methyl- ^3H)-methionine, the substrate of arginine-protein methylase I. These results are suggestive of a role for arginine methylation in directing nuclear localization of certain forms of bFGF.

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Introduction

Basic fibroblast growth factor (bFGF)¹ is a potent mitogen for a variety of cells of mesodermal or neuroectodermal origin. In addition, bFGF has been shown to be angiogenic *in vivo* and to affect other processes such as the induction of mesodermal tissue (for a review see Rifkin and Moscatelli, 1989). Protein sequence analysis of bFGF isolated from tissue sources initially established that it is composed of 146 amino acids (Esch *et al.*, 1985). Analysis of cDNA clones for bFGF revealed the presence of a putative initiator methionine codon from which synthesis of a 155-residue form of bFGF would occur (Abraham *et al.*, 1986). The size of this translation product is identical to that predicted from the cDNA (Jaye *et al.*, 1986) and established for the translation product (Burgess *et al.*, 1986) of the related protein, acidic FGF (aFGF). Thus it seemed likely that bFGF is synthesized as a 155-amino acid protein. In contrast to the cDNA for aFGF, the cDNA encoding bFGF contains an open reading frame 5' to the putative initiator methionine codon. Direct evidence for initiation of translation 5' of this methionine codon was reported by Sommer *et al.* (1987) who demonstrated that bFGF purified from human placenta has a 2-amino acid extension amino terminal to the putative initiating methionine. Subsequently, an apparent M_r 25 000 form of bFGF was identified in guinea pig brain extracts (Moscatelli *et al.*, 1987). Limited amino acid sequence analysis of this form of bFGF indicated the existence of posttranslational modifications in the amino terminal extension. Three positions where the human cDNA predicts an arginine residue (26, 24, and 22 residues amino terminal to the putative initiator methionine) were not cleaved by trypsin. Subsequent phenylthiohydantoin (PTH) amino acid analysis of the residues released in

¹ Abbreviations: bFGF, basic fibroblast growth factor; aFGF, acidic fibroblast growth factor; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high-pressure liquid chromatography; PTH, phenylthiohydantoin; PTC, phenylthiocarbonyl.

the Edman degradation at these positions did not result in the identification of any standard PTH amino acids. It was suggested that these residues may correspond to methylated arginines (Sommer *et al.*, 1989).

A common feature of many of the known methylarginine-containing proteins is nuclear localization and in some cases DNA-binding activity. For example, it is believed that arginine methylation may play an important role in the transport of histones from the cytosol to the nucleus, whereas subsequent lysine methylation may be involved in the chromatin condensation before mitosis (reviewed in Paik and Kim, 1985). Baldin *et al.* (1990) have demonstrated nuclear and nucleolar localization of exogenously added bFGF in adult bovine aortic endothelial cells. Nuclear uptake was observed only in cells that were growing or undergoing synchronous G₁ → S transition. Renko *et al.* (1990) demonstrated nuclear localization of bFGF in NIH 3T3 cells transfected with a full-length bFGF cDNA and in SK-Hep-1 cells, which produce relatively high levels of the protein (Moscatelli *et al.*, 1986). In these studies only the forms of bFGF that would contain the sites of potential arginine methylation (apparent *M_r* 22 000 and 24 000) were observed in the nuclear fraction. In this report we provide direct evidence for the methylation of arginine residues in the "high" molecular weight forms of bFGF.

Results

Figure 1 shows a Coomassie blue stained gel of an aliquot of the apparent *M_r* 25 000 form of bFGF isolated from guinea pig brains that was used as the starting material for the studies described here. Further purification of the high molecular weight bFGF before protein sequence analysis was achieved by transfer of the preparation from the sodium dodecyl sulfate (SDS) gel to nitrocellulose membranes. The high molecular weight bFGF was detected by reversible staining with Ponceau S, the band cut from the membrane and subjected to *in situ* protease digestion (Abersold *et al.*, 1987) using trypsin. After separation of the trypsin-derived peptides by reversed-phase microbore high-pressure liquid chromatography (HPLC), a total of nine peptides were subjected to amino acid sequence analysis. The sequences of all of these peptides were in agreement with the guinea pig peptide sequences or guinea pig cDNA-derived sequences reported by Sommer *et al.* (1989). The sequence of one of these peptides was re-

Figure 1. SDS-PAGE analysis of apparent *M_r* 25 000 bFGF isolated from guinea pig brains. (a) Molecular weight standards of 94 000, 67 000, 43 000, 30 000, 20 100, and 14 000. (b) An aliquot of the guinea pig bFGF preparation that was used for *in situ* proteolytic degradation.

ported by Sommer *et al.* (1989) to be V-G-G-X-G-X-G-T-A-A-A-R where X refers to the presence of a PTH-amino acid that did not align with any standard PTH-amino acid. The sequence of this peptide begins 29 residues amino terminal to the putative initiator methionine identified in the cDNA sequence of human bFGF (Abraham *et al.*, 1986). It was suggested that these residues may be methylated arginine residues based on the presence of arginine codons in the corresponding positions of the human bFGF cDNA, the failure of trypsin to cleave the protein at these positions and the repetitive nature of the G-X-G sequence surrounding these residues (Sommer *et al.*, 1989). Our amino acid sequence analysis of the corresponding peptide also resulted in the presence of unusual PTH-derivatives at these positions (Figure 2 and below).

To determine whether the unusual PTH-derivatives corresponded to PTH-methylarginine, we isolated the tryptic peptide of myelin basic protein containing the predominantly N^G-monomethylated arginine at residue 107 (Brostoff and Eylar, 1971) and subjected this peptide to amino acid sequence analysis to establish the elution position of PTH-N^G-monomethylarginine. Figure 2 shows the elution profiles of standard PTH amino acids (Figure 2a), the PTH derivatives obtained from amino acid sequence analysis of the myelin basic protein-derived peptide corresponding to residue 107 (Figure 2b) and the PTH derivatives obtained from

Figure 2. Selected chromatograms of the PTH-amino acid analysis of selected cycles of automated Edman degradation of tryptic peptides derived from myelin basic protein and *M*, 25 000 bFGF. (a) Chromatogram of standard PTH amino acids labeled with standard single letter code. (b) PTH analysis of residue 107 of myelin basic protein which consists predominantly of N^ε-monomethylarginine (arrow). (c–h) Successive cycles of Edman degradation (cycles 3–8) of a trypsin-derived fragment of apparent *M*, 25 000 bFGF. The assigned PTH derivatives are noted with a G (glycine) with a single arrow (N^ε-monomethylarginine) or a pair of arrows (probable combination of N^ε-monomethyl and dimethylated arginine).

amino acid sequence analysis of residues 3 through 8 of the tryptic peptide derived from high molecular weight bFGF described above (Figure 2, c–i). The PTH-derivatives identified with one or two arrows in cycles 4, 6, and 8 of bFGF (Figure 2, d, f, and h, respectively) elute at positions similar to that observed for the PTH-derivative corresponding to residue 107 of myelin basic protein (Figure 2b), which is known to be predominantly N^G-monomethylarginine. The single broad peak seen in Figure 2d when compared with the broad doublet seen in Figure 2, f and h, indicates the presence of N^G-monomethylarginine in cycle 4 and a mixture of monomethylated and dimethylated arginines in cycles 6 and 8 of this bFGF peptide. This conclusion is supported by the observation that we are unable to resolve the symmetric and asymmetric PTH-dimethylarginines from one another, whereas both can be resolved (later elution position) from PTH-N^G-monomethylarginine (data not shown). Further evidence that these PTH-derivatives represent methylated arginine residues in bFGF is provided by the fact that their elution positions can be altered by the ionic strength of buffer A used in the PTH analysis, a feature shared by PTH-histidine, PTH-arginine, and the PTH derivative of myelin basic protein residue 107 (data not shown). Amino acid composition analysis of the tryptic peptide containing residue 107 of myelin basic protein and the bFGF-derived tryptic peptide described above revealed the presence of a co-migrating PTC-derivatized amino acid for the two peptides that did not correspond to any standard phenylthiocarbonyl (PTC) amino acid (data not shown). Together these data provide strong evidence for the methylation of at least three arginine residues in guinea pig-derived high molecular weight bFGF.

Current data indicates that a single enzyme, S-adenosyl-L-methionine protein-arginine N-methyltransferase or protein methylase I (EC 2.1.1.23) is responsible for the post-translational formation of all three identified forms of methylated arginine, i.e., N^G-mono-, N^G, N^G-di, and N^G,N^G-dimethylarginine (Paik and Kim, 1985). To establish further the methylation of arginine residues of high molecular weight forms of bFGF, we examined the ability of NIH 3T3 cells transfected with a human bFGF cDNA expression vector to incorporate label provided by S-adenosyl-L-(methyl-³H)-methionine into bFGF. Florkiewicz and Sommer (1989) demonstrated that the human cell line SK-HEP-1 coexpresses four forms of bFGF (apparent M_r , 17 800, 22 500, 23 100, and 24 200) and that

transfection of a human bFGF cDNA expression vector into COS-1 cells results in the translation of these same forms. The results shown in Figure 3 demonstrate that the three forms of bFGF $> \sim 18\,000\ M_r$ incorporate label from the methyltransferase I substrate whereas the $M_r\ 18\,000$ form does not. Each of the three forms of bFGF that incorporate label from S-adenosyl-L-(methyl-³H)-methionine include the corresponding peptide sequence of guinea pig bFGF shown to contain methylarginine residues.

Discussion

Quantitative subcellular fractionation experiments established that high molecular weight forms (apparent M_r 22 000, 22 500, and 24 000) of human bFGF were concentrated in the nuclear and ribosomal fractions whereas the 18 000 form was found almost exclusively in the cytosolic fraction (Renko *et al.*, 1990). The amino terminal extensions of these three high molec-

Figure 3. Immunoprecipitation of biosynthetically labeled bFGF from NIH 3T3 cells transfected with human bFGF cDNA expression vector. Semi-confluent cultures of transfected cells were labeled overnight with L-(³⁵S)-methionine (a) or S-adenosyl-L-(methyl-³H)-methionine (b). The cell lysates were concentrated with a heparin-Sepharose column as described in Materials and methods. The 2 M NaCl eluates of the heparin-Sepharose were immunoprecipitated with polyclonal antibodies raised against recombinant human bFGF. The immunoprecipitates were subjected to SDS-PAGE under reducing conditions and the gel was processed for fluorography and exposed to X-ray film at -70°C .

ular weight bFGFs result from translation initiation at in frame CUG codons (Florkiewicz and Sommer, 1989) are rich in arginine residues and contain several repeats of the sequence gly-arg-gly according to the cDNA sequence of human bFGF. These repeats have been shown to be a site of arginine methylation in other proteins (Boffa *et al.*, 1977; Lapeyre *et al.*, 1986). In addition, it has been suggested that methylation of arginine residues plays a role in directing translocation of proteins to the nucleus (reviewed in Paik and Kim, 1985). This suggestion is based on the observation that methylarginines are found in many proteins associated with nucleic acids (Paik and Kim, 1967; Chang *et al.*, 1976; Boffa *et al.*, 1977, 1979; Christensen *et al.*, 1977; Marvil *et al.*, 1980; Plank and Wilson, 1980; Lischive *et al.*, 1985; Lapeyre *et al.*, 1986; Cruz-Alvarez and Pellicer, 1987). Sommer *et al.* (1989) reported the presence of unusual PTH-amino acids at three positions in the protein sequence of a high molecular weight form of guinea pig bFGF. They suggested these residues may correspond to methylated arginines, based on the fact that arginine residues are predicted at these positions according to the human cDNA sequence. The studies reported here provide direct evidence that 1) these three positions indeed contain methylated arginines, 2) that the methylation pattern of the residues is heterogeneous, i.e., exclusively monomethyl-arginine or a mixture of mono- and dimethyl-arginine, and 3) that methylation of arginines in human bFGF also occurs *in vivo*. These data indicate that arginine methylation may be an important posttranslational modification responsible for directing the nuclear translocation of these forms of bFGF. It has been demonstrated that the high molecular weight forms of bFGF that contain these methylated arginines and the 18 000 form that does not have the same apparent affinity for immobilized heparin are capable of binding the same population of receptors and are capable of stimulating plasminogen-activator production by endothelial cells at equivalent concentrations (Moscatelli *et al.*, 1987). However, some cells transfected with synthetic DNAs that code only for the high molecular weight forms of bFGF show impaired growth (N. Quarto *et al.*, 1991, manuscript in preparation). These observations are particularly interesting in light of the recent finding that a high molecular weight form of the FGF-related protein int-2 also occurs as a result of translation initiation at an in frame CUG codon (Acland *et al.*, 1990). The amino terminal extension of the int-2 protein is rich in arginine residues and,

in contrast to the shorter AUG-initiated protein that is secreted, localizes to the nucleus (Acland *et al.*, 1990). The methylation states of the arginines in this amino terminal extension have not been determined.

Lapeyre *et al.* (1986) reported that the majority of the arginines in the glycine-rich carboxy terminus of the nucleolar specific protein, nucleolin, are dimethylated. The results reported here provide a rational approach for determining the role of methylation, or specifically dimethylation, of arginine residues of bFGF in directing its localization to the nucleus by site-directed mutagenesis of the arginines, which have been shown to be mono- or mono- and dimethylated.

Methods

Polyacrylamide gel electrophoresis (PAGE) and transfer to nitrocellulose

Proteins were subjected to PAGE in the presence of SDS using a 15% acrylamide resolving gel with a 4.5% acrylamide stacking gel and the buffer system of Laemmli (1970). Electrophoresis was conducted using the Mighty Small apparatus (Hoeffer, San Francisco, CA). Proteins were visualized with Coomassie blue R-250. For proteolytic digestion, the proteins were transferred from the gels to a nitrocellulose membrane (0.45 μ m, Schleicher and Schuell, Keene, NH) using the buffer system of Towbin *et al.* (1979). Transfers were for 2 h at 100 V using a Hoeffer Mighty Small blotting chamber.

Purification, proteolytic digestion, and amino acid sequence analysis of bFGF and myelin basic protein

The apparent *M*_r 25 000 form of bFGF was purified to near homogeneity from guinea pig brains as described (Moscatelli *et al.*, 1987). The preparation was concentrated using a Centricon-10 (Amicon, Danvers, MA) and subjected to PAGE in the presence of SDS. The proteins were transferred from the gel to nitrocellulose for *in situ* protease digestion using the procedure described by Abersold *et al.* (1987). The apparent *M*_r 25 000 form of bFGF was visualized on the nitrocellulose membrane after staining with Ponceau S (Sigma, St. Louis, MO). The band was excised from the membrane and digested with trypsin (Worthington, Freehold, NJ) at 37° for 20 h at an approximate ratio of 1:20 (enzyme:substrate). The digest was fractionated using an Applied Biosystems (Foster City, CA) Model 130 HPLC and RP-300 reversed-phase cartridge. Peptides were eluted using 0.1% trifluoroacetic acid (buffer A) and 0.08% trifluoroacetic acid in acetonitrile/H₂O (70:30) with a linear gradient of 5% to 95% buffer B. Peptides were subjected to automated Edman degradation using an Applied Biosystems Model 477A protein sequencer equipped with an on line Model 120A PTH analyzer.

Myelin basic protein was purchased from Sigma and purified further using the Model 130A HPLC as described above for peptide generation. The protein was digested with trypsin in a solution of 0.1 M NH₄HCO₃ at 37° for 20 h at a 1:20 ratio of enzyme to substrate. Peptides were isolated and sequenced as described above for bFGF.

Amino acid analysis

Amino acid analysis was performed on samples that were hydrolyzed *in vacuo* in 6 M HCl/0.1% phenol for 24 h at

115°C. Amino acid compositions were determined based on reversed-phase separation of the phenylthiocarbamoyl derivatives, using the general procedures outlined by Waters Associates (Milford, MA). Data collection and reduction was performed using a Waters Associates 840 system.

Radiolabeling of cultured cells and immunoprecipitation

Mouse NIH 3T3 cells transfected with an expression vector containing a 1.1 kb *EcoRI* fragment of human bFGF cDNA (Quatro *et al.*, 1989) were used in the labeling experiments. Semiconfluent cultures in 100-mm dishes were labeled overnight with either L-(³⁵S)-methionine (1066 Ci/mmol, DuPont/New England Nuclear) at a final activity of 25 µCi/ml or S-adenosyl-L-(methyl-³H) methionine (78.9 Ci/mmol, DuPont/New England Nuclear, Boston, MA) at a final activity of 100 µCi/ml. Labeled cells were scraped into cold phosphate-buffered saline and pelleted by centrifugation at 4000 × *g* for 10 min. The pellet was suspended in 10 volumes of 20 mM tris(hydroxymethyl)aminomethane-HCl pH 7.5, 3 mM EDTA, 0.5M NaCl and sonicated for 3 min at 50 W with a Branson sonicator (Plainview, NY). The extract was centrifuged (10 000 × *g* for 1 h) and the supernatant was passed through a column (10 × 30 mm) of heparin-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with the same buffer. The column was washed with equilibration buffer and eluted with 2 M NaCl in the same buffer. Fractions were measured for radioactivity and aliquots of the most active fractions were immunoprecipitated with anti-bFGF antibodies as described by Presta *et al.* (1986). The samples were subjected to SDS-PAGE with a gradient (10–20%) of polyacrylamide resolving gel and 3% polyacrylamide stacking gel. The gel was processed for fluorography and then exposed to Kodak (Rochester, NY) XAR-5 film at –70°C.

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References

Abersold, R.H., Leavitt, J., Saavedra, R.A., Hood, L.E., and Kent, B.H. (1987). Internal amino acid sequence of proteins separated by one- or two-dimensional gel electrophoresis after in situ protease digestion on nitrocellulose. *Proc. Natl. Acad. Sci. USA* **84**, 6970–6974.

Abraham, J.A., Mergia, A., Whang, J.L., Trluno, A., Friedman, J., Hjerrild, K.A., Gospodarowicz, D., and Fiddes, J.C. (1986). Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. *Science* **233**, 545–548.

Acland, P., Dixon, M., Peters, G., and Dickson, C. (1990). Subcellular fate of the int-2 oncoprotein is determined by choice of initiation codon. *Nature* **343**, 662–665.

Baldin, V., Roman, A.-M., Bosc-Bierne, I., Amalric, F., and Bouche, G. (1990). Translocation of bFGF to the nucleus is G₁ phase cell cycle specific in bovine aortic endothelial cells. *EMBO J.* **9**, 1511–1517.

Boffa, L.C., Karn, J., Vidali, G., and Allfrey, V.G. (1977). Distribution of N^G N^G-dimethylarginine in nuclear protein fractions. *Biochem. Biophys. Res. Commun.* **74**, 969–976.

Boffa, L.C., Sterner, R.R., Vidali, G., and Allfrey, V.G. (1979). Post-synthetic modifications of nuclear proteins: high mobility group proteins are methylated. *Biochem. Biophys. Res. Commun.* **89**, 1322–1327.

Brostoff, S., and Eylar, E.H. (1971). Localization of methylated arginine in the A1 protein from myelin. *Proc. Natl. Acad. Sci. USA* **68**, 765–769.

Burgess, W.H., Mehlman, T., Marshak, D.R., Fraser, B.A., and Maciag, T. (1986). Structural evidence that endothelial cell growth factor β is the precursor of both endothelial cell growth factor α and acidic fibroblast growth factor. *Proc. Natl. Acad. Sci. USA* **83**, 7216–7220.

Chang, F.N., Navickas, I.J., Chang, C.N., and Dancis, B.M. (1976). Methylation of ribosomal proteins in HeLa cells. *Arch. Biochem. Biophys.* **172**, 627–633.

Christensen, M.E., Beyer, A.L., Walker, B., and LeStourgeon, W.M. (1977). Identification of N^G, N^G-dimethylarginine in nuclear protein from the lower eukaryote polycephalum homologous to the major proteins of mammalian 40S ribonucleoprotein particles. *Biochem. Biophys. Res. Commun.* **74**, 621–629.

Cruz-Alvarez, M., and Pellicer, A. (1987). Cloning of a full-length complementary DNA for an artemia salina glycine-rich protein. *J. Biol. Chem.* **262**, 13377–13380.

Esch, F., Baird, A., Ring, N., Veno, N., Hill, F., Denoroy, L., Klepper, R., Gospodarowicz, D., Bohlen, P., and Guillemin, R. (1985). Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. *Proc. Natl. Acad. Sci. USA* **82**, 6507–6511.

Florievicz, R.Z., and Sommer, A. (1989). Human basic fibroblast growth factor gene encodes four polypeptides: three initiate translation from non-AUG codons. *Proc. Natl. Acad. Sci. USA* **86**, 3978–3981.

Jaye, M., Howk, R., Burgess, W.H., Ricca, G.A., Chiu, I.-M., Ravera, M.W., O'Brien, S.J., Modi, W.S., Maciag, T., and Drohan, W.N. (1986). Human endothelial cell growth factor: cloning, nucleotide sequence, and chromosome localization. *Science* **233**, 541–545.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lapeyre, B., Amalric, F., Ghaffari, S.H., Venkatarama Rao, S.V., Dumbar, T.S., and Olson, M.O.J. (1986). Protein and cDNA sequence of a glycine-rich, dimethylarginine containing region located near the carboxyl-terminal end of nucleolin (C23 and 100 kDa). *J. Biol. Chem.* **261**, 9167–9173.

Lischive, M.A., Ochs, R.L., Reddy, R., Cook, R.G., Yeoman, L.C., Tano, E.M., Reichlin, M., and Busch, H. (1985). Purification and partial characterization of a nucleolar scleroderma antigen (M_A = 34,000; pI, 8.5) Rich in N^G, N^G-dimethylarginine. *J. Biol. Chem.* **260**, 14304–14310.

Marvil, D.K., Nowak, L., and Szer, W. (1980). A single-stranded nucleic acid-binding protein from artemia salina. *J. Biol. Chem.* **255**, 6466–6472.

Moscatelli, D., Joseph-Silverstein, J., Manejias, R., and Rifkin, D.B. (1987). M_{25,000} heparin-binding protein from guinea pig brain is a high molecular weight form of basic fibroblast growth factor. *Proc. Natl. Acad. Sci. USA* **84**, 5778–5782.

- Moscatelli, D., Presta, M., Joseph-Silverstein, J., and Rifkin, D.B. (1986). Both normal and tumor cells produce basic fibroblast growth factor. *J. Cell. Physiol.* **129**, 273–276.
- Paik, W.K., and Kim, S. (1967). Enzymatic methylation of protein fractions from calf thymus nuclei. *Biochem. Biophys. Res. Commun.* **29**, 14–20.
- Paik, W.K., and Kim, S. (1985). Protein methylation. In: *The Enzymology of Post-Translational Modification of Proteins*, Vol. 2, eds. R.B. Freedman and H.G. Hawkins, London: Academic Press, 187–228.
- Plank, S.R., and Wilson, S.H. (1980). Studies on the structure of mouse helix-destabilizing protein-1. *J. Biol. Chem.* **255**, 11547–11556.
- Presta, M., Moscatelli, D., Joseph-Silverstein, J., and Rifkin, D.B. (1986). Purification from a human hepatoma cell line of a basic fibroblast growth factor-like molecule that stimulates capillary endothelial cell plasminogen activator production, DNA synthesis, and migration. *Mol. Cell Biol.* **6**, 4060–4066.
- Quatro, N., Talarico, D., Sommer, A., Florkiewicz, R., Basilico, C., and Rifkin, D.B. (1989). Transformation by basic fibroblast growth factor requires high levels of expression: a comparison with transformation by hst/Kfgr. *Oncogene Res.* **5**, 101–110.
- Renko, M., Quarto, N., Morimoto, T., and Rifkin, D.B. (1990). Nuclear and cytoplasmic localization of different basic fibroblast growth factor species. *J. Cell. Physiol.* **144**, 108–114.
- Rifkin, D.B., and Moscatelli, D. (1989). Recent developments in the cell biology of basic fibroblast growth factor. *J. Cell. Biol.* **109**, 1–6.
- Sommer, A., Brewer, M.T., Thompson, R.C., Moscatelli, D., Presta, M., and Rifkin, D.B. (1987). A form of human basic fibroblast growth factor with an extended amino terminus. *Biochem. Biophys. Res. Commun.* **144**, 543–550.
- Sommer, A., Moscatelli, D., and Rifkin, D.B. (1989). An amino-terminally extended and post-translationally modified form of a 25kD basic fibroblast growth factor. *Biochem. Biophys. Res. Commun.* **160**, 1267–1274.
- Towbin, H., Jibson, M., Muller, D., and Arnon, R. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **81**, 4350–4354.