Vol. 200, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1994 Pages 598-605

EFFECTS OF CYTOKINES AND GROWTH FACTORS ON PHOSPHORYLATED FETUIN BIOSYNTHESIS BY ADULT RAT HEPATOCYTES IN PRIMARY CULTURE

Tomokazu Ohnishi[1], Osamu Nakamura[1], Naokatu Arakaki[1],
Hiroomi Miyazaki[2] and Yasushi Daikuhara[1§]

[1]Department of Biochemistry, Kagoshima University Dental School,
35-1 Sakuragaoka-8, Kagoshima 890, Japan

[2]Second Department of Internal Medicine, Faculty of Medicine, Kagoshima University,
35-1 Sakuragaoka-8, Kagoshima 890, Japan

Received March 3, 1994

SUMMARY. Recently, we showed that a 59 kDa non-phosphorylated sialoprotein purified from rat bone matrix is the rat counterpart of bovine fetuin and human α_2-HS glycoprotein and that fetuin synthesized and secreted by adult rat hepatocytes in primary culture is mostly phosphorylated (phosphofetuin), though fetuin is known to contain no phosphorus. Here we report that the rate of synthesis of phosphofetuin by hepatocytes in culture was reduced by inflammatory cytokines such as human interleukin (hIL)-6, human tumor necrosis factor-α and hIL-1α, but dose-dependently stimulated by growth factors of hepatocytes, such as hepatocyte growth factor (HGF)/scatter factor (SF), epidermal growth factor and insulin, as determined by metabolic labeling and Northern blot analysis using cDNA for rat fetuin as a probe. We also showed that administration of HGF/SF stimulated gene expression of rat fetuin *in vivo*.

Fetuin, the first fetal protein found in bovine serum, which was reported by Pedersen [1] in 1944, has been found to be the bovine counterpart of human α_2-HS glycoprotein (α_2-HSG) [2], a major non-phosphorylated sialoprotein in plasma [3, 4]. Human α_2-HSG is a negative acute-phase reactant [5] and its synthesis in HepG2 cells, a human hepatoma cell line, is reported to be down-regulated by cytokines [6]. Human α_2-HSG is also known to be a member of the cystatin superfamily [7], and to be synthesized by the liver [8] and incorporated into mineralized tissues, where it is concentrated 30- to 100-fold [8 - 10], although biological functions of fetuin/α_2-HSG is not clear at present. We have purified a 59 kDa non-phosphorylated sialoprotein from rat bone matrix and shown that this protein is synthesized and secreted from both hepatocytes

§To whom reprint requests should be addressed.

Abbreviations used in this paper: α_2-HSG, α_2-HS glycoprotein; C/EBP, CCAAT/enhancer-binding protein; hEGF, human epidermal growth factor; hHGF, human hepatocyte growth factor; hIL, human interleukin; hTNF, human tumor necrosis factor; NF-IL6, nuclear factor for IL-6 expression; PITC, phenyl isothiocyanate; pp63, phosphorylated 63 kD N-glycoprotein; SF, scatter factor.

0006-291X/94 $5.00

and osteoblasts in primary culture [11]. We have also cloned a cDNA for this sialoprotein from a rat liver cDNA library by immunoscreening [12]. A computer search of protein and nucleic acid data bases revealed that this sialoprotein obtained from bone matrix is the rat counterpart of bovine fetuin and human α_2-HSG, and is a dephosphorylated form of pp63, a rat 63 kDa phosphorylated N-glycoprotein [12]. pp63 (phosphorylated fetuin; phosphofetuin) has been purified from the conditioned medium of rat hepatocytes in primary culture and was shown to inhibit insulin receptor tyrosine kinase and receptor auto-phosphorylation, and to antagonize the growth-promoting action of insulin on FaO hepatoma cells [13]. Since immunoreactive fetuin in the conditioned medium of hepatocytes in culture has been shown to be mainly phosphorylated [12] but fetuin isolated from rat serum has been identified as the dephosphorylated form of pp63 [14], phosphofetuin synthesized and secreted from the liver is likely to be dephosphorylated during the circulation then accumulated into mineralized tissues.

In this paper, we report that production of phosphofetuin by rat hepatocytes in culture was reduced by some inflammatory cytokines and stimulated by hepatotrophic growth factors such as human hepatocyte growth factor (hHGF), human epidermal growth factor (hEGF) and insulin. We also show that hHGF increased in mRNA level of rat fetuin in the liver *in vivo*.

MATERIALS AND METHODS

Materials. [α-^{32}P]dCTP (1,111 TBq/mmol) was purchased from Du Pont-New England, Boston, MA; carrier-free (^{32}P)orthophosphate was from Japan Atomic Energy Research Institute, Ibaragi, Japan; recombinant human interleukin-1α (hIL-1α) (1 x 10^8 U/mg), recombinant hIL-6 (4 x 10^6 U/mg), and recombinant human tumor necrosis factor-α (hTNF-α) (2 x 10^7 U/mg) were from Genzyme Corp., Boston, MA; hIL-3 (1 x 10^8 U/mg) was from Amersham Japan, Tokyo; phenyl isothiocyanate (PITC) and a Wakosil-PTC column for HPLC were from Wako Pure Chemical Industries, Osaka; bovine insulin, *O*-phospho-L-serine, *O*-phospho-L-threonine and *O*-phospho-L-tyrosine were from Sigma Chemical Co., St. Louis, MO; phosphate-free Williams medium E was from Kyokuto Pharmaceutical Industrial Co., Tokyo, and X-ray film for autoradiography was from Konica Corp., Tokyo. Recombinant hEGF was generously supplied from Wakunaga Pharmaceutical Co., Osaka. Recombinant hHGF was prepared as reported previously [15, 16]. Rat fetuin (unphosphorylated form) was purified from bone matrix as described before [11]. Anti-rat fetuin monoclonal antibody was prepared as described previously [11]. Other materials used for immunoprecipitation, electrophoresis and preparation of adult rat hepatocytes in primary culture were as described elsewhere [11, 12, 17].

Preparation of Primary Cultures of Parenchymal Cells from Adult Rat Liver. Adult rat hepatocytes in primary culture were prepared as described previously [17]. The basal culture medium was Williams medium E containing 5 nM dexamethasone, 100 U/ml of penicillin and 100 μg/ml of streptomycin which was supplemented with FCS at the indicated concentrations. Unless otherwise mentioned, cells were plated into 24-well plastic plates (A/S Nunc, Roskilde, Denmark) at a density of 3 x 10^5 cells/0.2 ml/cm^2 in the basal medium containing 10 % FCS. Four hours after seeding, the medium was changed to basal medium containing 5 % FCS, and the cells were incubated for 24 h before use. Because FCS contains bovine fetuin, the cells were washed twice with FCS-free basal medium before experiments. Then the medium was changed to FCS-free basal medium in the presence or absence of growth factors and cytokines, and the cells were incubated as described in figure legends.

Determination of Phosphofetuin Synthesis by the Hepatocytes in Culture. For determination of biosynthesis of phosphofetuin, phosphate-free Williams medium E was used instead of standard

Williams medium E. Cells were labeled with [^{32}P]phosphate (7.4 MBq/ml) in the basal medium with or without growth factors and cytokines for 20 h, and the conditioned medium was immunoprecipitated with anti-rat fetuin monoclonal antibody, sheep anti-mouse IgG antibody and Protein A-Sepharose CL-4B. The immunoprecipitates were subjected to autoradiography after SDS-PAGE under reducing conditions as described previously [12]. The radioactivity of immunoprecipitates was determined by scanning the exposed film with a densitometer (model CS-910, Shimazu Corp., Kyoto, Japan) and expressed in arbitrary units per μg of cellular protein.

Analysis of Phosphorylated Amino Acids of Phosphofetuin. [^{32}P]-Labeled phosphofetuin synthesized and secreted by hepatocytes in primary culture was immunoprecipitated from the conditioned medium as described above. After removing inorganic [^{32}P]phosphate by ultrafiltration with Centricon-10 (Amicon Division, Grace Japan, Tokyo), immunoprecipitated materials were hydrolyzed by treatment with 6 N HCl for 24 h at 108 °C under vacuum in a vacuum reaction tube (Pierce Chemical Co., Rockford, IL). Then amino acid residues derived from the immunocomplex were labeled with PITC and separated in an HPLC apparatus (model CCPM, Tosoh Corp., Tokyo) with a Wakosil-PTC column (4 x 200 mm) as described previously [12]. Fractions of PITC-phosphoserine, PITC-phosphothreonine, and PITC-phosphotyrosine were collected and their radioactivities were measured in a liquid scintillation counter (model LSC-901S, Aloka Corp., Tokyo).

Northern Blotting. Total cellular RNA was extracted by the acid guanidium thiocyanate-phenol-chloroform method [18] from 1.2 x 10^7 cells and analyzed as described previously [12]. Equal loading of the lanes was checked by ethidium bromide staining [19].

In Vivo Experiments. Male rats of Wistar strain (150 - 200 g) received either 30% partial hepatectomy or carbon tetrachloride, and the livers were removed 20 h after the injury. Carbon tetrachloride was given by a stomach tube with 33% (v/v) solution in olive oil at a dose of 1 ml CCl_4/kg body weight. Recombinant hHGF was continuously administered at a dose of 0.02 μg/h using a mini-osmotic pump (Alzet 2001D, Alza Corp., Palo Alto, CA) which was set in the peritoneal cavity for 1 week. Total cellular RNA was then extracted from the livers and Northern blot analysis was performed as described above with the exception that radioactivity on hybridized membranes was determined by a Bioimaging analyzer (model BAS-1000, Fujifilm Inc., Tokyo).

Other Methods. Measurement of protein and SDS-PAGE were carried out as described previously [11].

RESULTS AND DISCUSSION

Analysis of Phosphorylated Amino Acid Residue of Rat Phosphofetuin Secreted from Hepatocytes in Primary Culture.

Le Cam *et al.* [20] showed that the phosphorylated amino acid residue of pp63 is serine by thin-layer chromatography. We analyzed the phosphorylated amino acid residue in [^{32}P]-labeled rat phosphofetuin secreted from hepatocytes in primary culture by HPLC and confirmed that it was only serine (data not shown). The production of phosphofetuin by hepatocytes was induced by hepatotrophic factors, as described below, but the specific radioactivity of phosphoserine in [^{32}P]-labeled phosphofetuin secreted from hepatocytes was not altered by stimulation with hHGF, hEGF or insulin (data not shown).

Although rat fetuin contains 13 serine residues [13, 14], we do not know which residue(s) is phosphorylated, at present. However, 9 of 13 serine residues of rat fetuin are conserved in both

bovine fetuin and human α_2-HSG at the amino acid positions of 83, 116, 120, 168, 178, 265, 295, 298 and 300, starting from the N-terminal of mature rat fetuin [2, 7, 13]. Therefore, these serine residues may be responsible for phosphorylation.

Effects of Inflammatory Cytokines and Growth Factors on Phosphofetuin Synthesis by Rat Hepatocytes in Primary Culture.

We first examined the effects of inflammatory cytokines on the synthesis and secretion of [^{32}P]-labeled phosphofetuin by hepatocytes because rat fetuin is the counterpart of human α_2-HSG, which is known to be a negative acute phase reactant [5, 21] and the mRNA level of α_2-HSG in adult rat liver is reported to decrease in response to *in vivo* acute inflammation or injection of TNF [22]. As shown in Fig. 1*a*, 10 ng/ml of hIL-6 and 10 ng/ml of hTNF-α inhibited the synthesis and secretion of [^{32}P]-labeled phosphofetuin by rat hepatocytes in primary culture, as judged by immunoprecipitation of the conditioned medium with anti-rat fetuin monoclonal antibody. hIL-1α (10 ng/ml) also slightly inhibited its synthesis and secretion, but hIL-3 had no effect (Fig. 1*a*). Fig. 1*b* shows levels of rat fetuin mRNA of 1.5 kb determined by Northern blot hybridization: all these cytokines, except hIL-3, suppressed *de novo* synthesis of phosphofetuin by hepatocytes. These results are consistent with a report that IL-6, IL-1β and conditioned medium from lipopolysaccharide-activated monocytes depress secretion of α_2-HSG and expression of the mRNA of α_2-HSG in hepatoma HepG2 cells [6].

We next examined the effects of growth factors for hepatocytes, such as hHGF, hEGF and insulin, on their synthesis and secretion of phosphofetuin in primary cultures. As shown in Figs. 2*a* and 2*b*, hHGF, hEGF and insulin dose-dependently increased the synthesis and secretion of [^{32}P]-labeled phosphofetuin by the cells. Of these growth factors, insulin was the most potent stimulator of the synthesis of phosphofetuin by hepatocytes, causing significant increase at 10^{-9} M and a maximum of about 3.5-fold increase over the control at 10^{-7} M. Changes in the levels of rat fetuin mRNA induced by these growth factors showed almost the same pattern as that of phosphofetuin secretion from the cells (Fig. 2*c*).

The mechanism regulating the suppression and induction of phosphofetuin mRNA expression by cytokines and growth factors is still unknown, but in this regard, it is noteworthy that a pentanucleotide sequence, 5'-GCAAT, which matches the CCAAT/enhancer-binding protein (C/EBPα) binding sequence [23], is present in 5'-flanking region of the rat fetuin (pp63) gene [24]. Other genes for negative acute proteins such as albumin and transthyretin are also known to contain the C/EBPα binding sequence in their promoter regions [25], and IL-6 is shown to suppress the C/EBPα expression and increase NF-IL6, a nuclear factor for IL-6 expression in mouse liver [25, 26]. NF-IL6, which was shown to be highly homologous with C/EBPα, has been shown to be involved in regulation of the gene expressions of various proteins including positive and negative acute phase reactants [25, 27]. NF-IL6 mRNA is not normally expressed in any tissues, but it can be induced by stimulation with inflammatory cytokines such as IL-1, IL-6 and TNF [27]. Thus, as Isshiki *et al.* [26] postulated, C/EBPα may stimulate expression of a broad spectrum of liver specific genes of negative acute phase proteins under normal conditions, but once acute inflammation occurs, NF-IL6 may be rapidly induced and bind to the C/EBPα binding sites thereby interfering with the binding of C/EBPα to the fetuin gene.

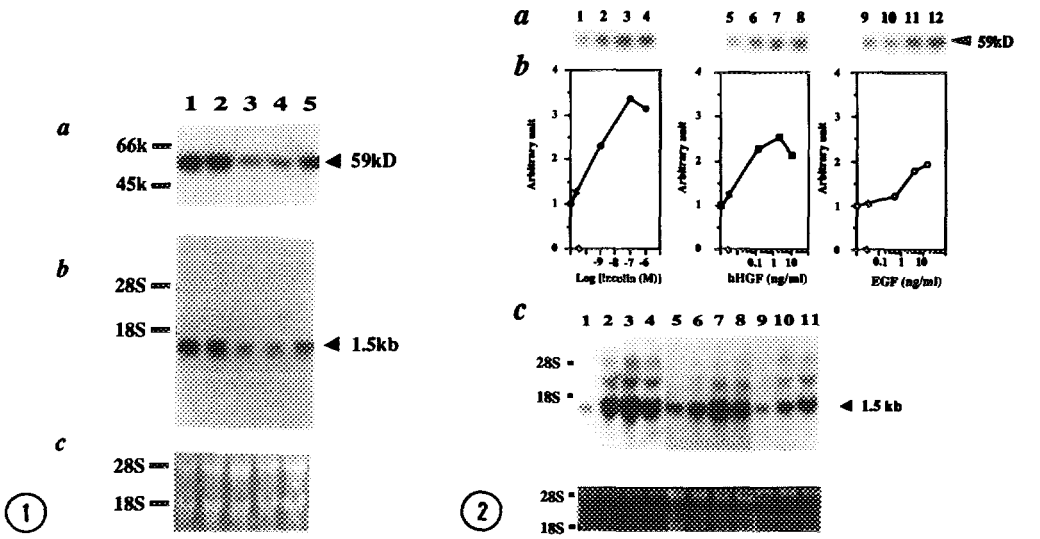

Figure 1. **Effects of inflammatory cytokines on phosphofetuin synthesis and fetuin mRNA expression in rat hepatocytes in primary culture.** (*a*) Cells (3 x 10^5/0.2 ml/cm^2) were labeled with [^{32}P]phosphate in phosphate-free Williams E medium without FCS for 20 h in the absence (lane 1) or presence of 10 ng/ml of hIL-3 (lane 2), 10 ng/ml of hIL-6 (lane 3), 10 ng/ml of hTNF-α (lane 4), or 10 ng/ml of hIL-1α (lane 5). The conditioned media were immunoprecipitated with anti-rat fetuin monoclonal antibody, and the immunoprecipitates were solubilized and subjected to SDS-PAGE under reducing conditions. Then radioactive materials were located by autoradiography. (*b*) Total cellular RNA (7.5 μg per lane) extracted from the cells cultured in the same conditions as Fig. 2*a* except [^{32}P]phosphate which was omitted was electrophoresed and blotted onto a nylon membrane and then fetuin mRNA was determined by Northern blotting with ^{32}P-labeled cDNA for rat fetuin as a probe. (*c*) Ethidium bromide staining of the agarose gel used for Northern blotting shown in *b*, indicating equal loadings of total RNA. The positions of rat fetuin (59 kDa) and rat fetuin mRNA (1.5 kb) are indicated at the right, and the positions of molecular mass marker proteins in daltons (Fig. 1*a*) and those of ribosomal RNAs (Figs. 1*b* and *c*) are indicated at the left.

Figure 2. **Effects of growth factors on phosphofetuin synthesis and fetuin mRNA expression in rat hepatocytes in primary culture.** Experimental conditions were as described in the legend for Fig. 1, except for additions during the incubation period. (*a*) Autoradiogram of immuoprecipitates of the conditioned media with anti-rat fetuin monoclonal antibody after SDS-PAGE. Additions were: 0, 10^{-9}, 10^{-7}, and 10^{-6} M insulin, for lanes 1-4, respectively; 0, 0.2, 2.0, and 10 ng/ml of hHGF for lanes 5-8, respectively; and 0, 0.5, 5, and 20 ng/ml of hEGF for lanes 9-12, respectively. (*b*) Relative amounts of phosphofetuin synthesized and secreted by the cells determined by densitometric quantitation of the autoradiograms shown in *a* are plotted. Values are means in arbitrary optical density units per μg of cellular protein and are represented relative to the control value (without a growth factor). (*c*) Total cellular RNA was extracted and Northern blot analysis (5.0 μg/lane) with ^{32}P-labeled cDNA for rat fetuin was performed as described in the legend for Fig. 1. Lanes 1-4, insulin at 0, 10^{-9}, 10^{-7}, and 10^{-6} M, respectively; lanes 5-8, hHGF at 0, 0.2, 2.0, and 10 ng/ml, respectively, and lanes 9-11, hEGF at 0, 1.0, and 10 ng/ml, respectively. Ethidium bromide staining of the agarose gel used for Northern blotting is shown at the bottom.

Effects of Administration of hHGF and Liver Injuries on Fetuin mRNA Level in the Rat Liver.

We confirmed the effect of hHGF on stimulation of rat fetuin biosynthesis in the liver by administration of recombinant hHGF to rats. As shown in Fig. 3, administration of hHGF in-

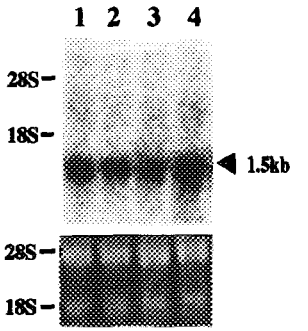

Figure 3. **Effect of hepatic injuries and administration of recombinant hHGF (rhHGF) on fetuin mRNA level in the rat liver.** Rats were treated as described in 'Materials and Methods' and total cellular RNA (10 μg/lane) extracted from the liver was analyzed by Northern blotting with ^{32}P-labeled cDNA for rat fetuin as described in the legend for Fig. 1. Lane 1, normal control; lane 2, 20 h after 30% partial hepatectomy; lane 3, 20 h after CCl_4 administration; lane 4, rhHGF administration for 1 week. Ethidium bromide staining of the agarose gel used for Northern blotting is shown at the bottom. The positions of ribosomal RNAs are indicated at the left and that of rat fetuin mRNA (1.5 kb) is indicated at the right.

creased the level of mRNA for fetuin in the liver to about 180% of the control. On the other hand, hepatic injuries such as partial hepatectomy and administration of carbon tetrachloride decreased the levels to about 75% of the control. These results are consistent with *in vitro* observations described above. In this connection, it is noteworthy that the phosphofetuin (pp63) mRNA level in the rat liver remnant is reported to be decreased after partial hepatectomy [22, 28], but the level of TNF-α mRNA in the liver is reported to be increased by administration of carbon tetrachloride [29].

hHGF was first identified in the plasma of patients with fulminant hepatitis [30] and purified from plasma of these patients [17]. hHGF is a strong mitogen for both human and rat hepatocytes in primary culture [16], and for intrahepatic human biliary epithelial cells in culture [31], and thought to be involved in liver regeneration [for review, see Ref. 32]. Although hHGF was originally thought to be a liver-specific growth factor, recent studies have identified it with the scatter factor (SF) [33] and the tumor cytotoxic factor [34]. Thus, hHGF/SF is now known to be a broad-spectrum and multi-functional cytokine with effects on various types of cells including epithelial and endothelial cells as well as hepatocytes [for reviews, see a book Ref. 35]. It is also shown that HGF stimulates albumin synthesis by rat hepatocytes in primary culture [36]. We recently showed that the production of hHGF/SF by fibroblasts, which have been shown to be a cellular source(s) of hHGF/SF, was stimulated by some inflammatory cytokines such as IL-1(α and β) and TNF-α, possibly via induction of NF-IL6 [37]. Thus, if the hypothesis on regulation of phosphofetuin expression described above is true, hHGF/SF and other growth factors are likely to induce C/EBPα or suppress NF-IL6 in hepatocytes, and stimulate expression of phenotype of mature hepatocytes.

Since fetuin/α_2-HSG was found in bovine and human serum about 50 years ago, many possible biologic functions for this protein, including bone remodeling, have been proposed [22,

38 - 42; for references before 1986, see Ref. 21], but its actual biological roles are still obscure. Our results reported in this paper indicate that biosynthesis of phosphofetuin in hepatocytes is regulated by inflammatory cytokines and growth factors for hepatocytes and that rat phosphofetuin is an negative acute reactants and a phenotype of mature hepatocytes.

Acknowledgments. We thank Dr. Takehisa Ishii, Research Center, Mitsubishi Kasei Corporation, for providing recombinant hHGF. We also thank Mrs. Elizabeth Ichihara for assistance in preparing this manuscript and Miss Michi Uesugi for secretarial assistance. This work was supported in part by Grants-in-Aid for Cancer Research and for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Pedersen, K. O. (1944) Nature 154, 575.
2. Dziegielewska, K. M., Brown, W. M., Casey, S.-J., Christie, D. L., Foreman, R. C., Hill, R. M., and Saunders, N. R. (1990) J. Biol. Chem. 265, 4354-4357.
3. Spiro, R. G. (1960) J. Biol. Chem. 235, 2860-2869.
4. Schmid, K., and Bürgi, W. (1961) Biochim. Biophys. Acta 47, 440-453.
5. Lebreton, J. P., Joisel, F., Raoult, J. P., Lannuzel, B., Rogez, J. P., and Humbert, G. (1979) J. Clin. Invest. 64. 1118-1129.
6. Daveau, M., Davrinche, C., Julen, N., Hiron, M., Arnaud, P., and Lebreton, J. P. (1988) FEBS Lett. 241, 191-194.
7. Kellermann, J., Haupt, H., Auerswald, E.-A., and Müller-Esterl, W. (1989) J. Biol. Chem. 264, 14121-14128.
8. Triffitt, J. T., Gebauer, U., Ashton, B. A., Owen, M. E., and Reynolds, J. J. (1976) Nature 262, 226-227.
9. Ashton, B. A., Höhling, H.-J., and Triffitt, J. T. (1976) Calcif. Tissue Res. 22, 27-33.
10. Mbuyi, J.-M., Dequeker, J., Bloemmen, F., and Stevens, E. (1982) Calcif. Tissue Int. 34, 229-231.
11. Ohnishi, T., Arakaki, N., Nakamura, O., Hirono, S., and Daikuhara, Y. (1991) J. Biol. Chem. 266, 14636-14645.
12. Ohnishi, T., Nakamura, O., Ozawa, M., Arakaki, N., Muramatsu, T., and Daikuhara, Y. (1993) J. Bone Miner. Res. 8, 367-377.
13. Auberger, P., Falquerho, L., Contreres, J. O., Pages, G., Le Cam, G., Rossi, B., and Le Cam, A. (1989) Cell 58, 631-640.
14. Rauth, G., Pöschke, O., Fink, E., Eulitz, M., Tippmer, S., Kellerer, M., Häring, H.-U., Nawratil, P., Haasemann, M., Jahnen-Dechent, W., and Müller-Esterl, W. (1992) Eur. J. Biochem. 204, 523-529.
15. Yoshiyama, Y., Arakaki, N., Naka, D., Takahashi, K., Hirono, S., Kondo, J., Nakayama, H., Gohda, E., Kitamura, N., Tsubouchi, H., Ishii, T., Hishida, T., and Daikuhara, Y. (1991) Biochem. Biophys. Res. Commun. 175, 660-667.
16. Strain, A. J., Ismail, T., Tsubouchi, H., Arakaki, N., Hishida, T., Kitamura, N., Daikuhara, Y., and McMaster, P. (1991) J. Clin. Invest. 87, 1853-1857.
17. Gohda, E., Tsubouchi, H., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Miyazaki, H., Hashimoto, S., and Daikuhara, Y. (1988) J. Clin. Invest. 81, 414-419.
18. Chomezynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156-159.
19. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloninig. A Laboratory Manual. Second Edition, Book 1. p. 7.43. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

20 Le Cam, A., Magnaldo, I., Le Cam, G. and Auberger, P. (1985) J. Biol. Chem. 260, 15965–15971.
21. Arnaud, P., Miribel, L., and Emerson, D. L. (1988) Methods Enzymol. 163, 431–441.
22. Daveau, M., Davrinche, C., Djelassi, N., Lemetayer, J., Julen, N., Hiron, M., Arnaud, P., and Lebreton, J. P. (1990) FEBS Lett. 273, 79–81.
23. Landschulz, W. H., Johnson, P. F., Adashi, E. Y., Graves, B. J., and McKnight, S. L. (1988) Genes Dev. 2, 786–800.
24. Falquerho, L., Patey, G., Paquereau, L., Rossi, V., Lahuna, O., Szpirer, J., Szpirer, C., Levan, G., and Le Cam, A. (1991) Gene 98, 209–216.
25. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T. Hirano, T., and Kishimoto, T. (1990) EMBO J. 9, 1897–1906.
26. Isshiki, H., Akira, S., Sugita, T., Nishio, Y., Hashimoto, S., Pawlowski, T., Suematsu, S., and Kishimoto. T. (1991) The New Biologist 3, 63–70.
27. Hirano, T., Akira, S., Taga, T., and Kishimoto, T. (1990) Immunol. Today 11, 443–449.
28. Colombo, B. M., Falquerha, L., Manenti, G., Dragani, T. A., and Le Cam, A. (1991) Biochem. Biophys. Res. Commun. 180, 967–971.
29 Czaja, M. J., Flanders, K. C., Biempica, L., Klein, C., Zern, M. A., and Weiner, F. R. (1989) Growth Factors 1, 219–226.
30. Nakayama, H., Tsubouchi, H., Gohda, E., Koura, M., Nagahama, J., Yoshida, H., Daikuhara, Y., and Hashimoto, S. (1985) Biomed. Res. 6, 231–237.
31. Joplin, R., Hishida, T., Tsubouchi, H., Daikuhara, Y., Ayres, R., Neuberger, J. M., and Strain, A. J. (1992) J. Clin. Invest. 90, 1284–1289.
32. Tsubouchi, H., Gohda, E., Strain, A. J., and Daikuhara, Y. (1993) In Hepatocyte Growth Factor–Scatter Factor (HGF–SF) and the C–Met Receptor (I. D. Goldberg, and E. M. Rosen, Eds.), pp. 251–274, Birkhäuser Verlag, Basel.
33. Weidner, K. M., Arakaki, N., Hartmann, G., Vandekerckhove, J., Weingart, S., Rieder, H., Fonatsch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y. and Birchmeier, W. (1991) Proc. Natl. Acad. Sci. USA 88, 7001–7005.
34. Shima, N., Nagao, M., Ogaki, F., Tsuda, E., Murakami, A., and Higashio, K. (1991) Biochem. Biophys. Res. Commun. 180, 1151–1158.
35. Goldberg, I. D., and Rosen E. M. (Eds.) (1993) Hepatocyte Growth Factor–Scatter Factor (HGF–SF) and the C–Met Receptor. pp. 1–397, Birkhäuser Verlag, Basel.
36. Takehara, T., Matsumoto, K. and Nakamura, T. (1992) J. Biochem. 112, 330–334.
37. Tamura, M., Arakaki, N., Tsubouchi, H. Takada, H., and Daikuhara, Y. (1993) J. Biol. Chem. 268, 8140–8145.
38. Abdullah, M., Crowell, J. A., Tres, L. L., and Kierszenbaum, A. L. (1986) J. Cell Physiol. 127, 463–472.
39. Colclasure, G. C., Lloyd, W. S., Lamkin, M., Connerman, W., Troxler, R. F., Offner, G. D., Bürgi, W., Schmid, K., and Nimberg, R. B. (1988) J. Clin. Endocrin. Metab. 66, 187–192.
40. Kumbla, L., Cayatte, A. J., and Subbiah, M. T. R. (1989) FASEB J. 3, 2075–2080.
41. Cayatte, A. J., Kumbla, L., and Subbiah, M. T. R. (1990) J. Biol. Chem. 265, 5883–5888.
42. Yang, F., Schwartz, Z., Swain, L. D., Lee, C.–C., Bowman, B. H., and Boyan, B. D. (1991) Bone 12, 7–15.