Insulin-like growth factor (IGF) action is mediated by high affinity cell surface IGF receptors and modulated by a family of secreted IGF binding proteins (IGFBPs). IGFBP-5, the most conserved of six IGFBPs characterized to date, uniquely potentiates the anabolic actions of IGF-I for skeletal cells. In osteoblasts, IGFBP-5 production is stimulated by prostaglandin E$_2$ (PGE$_2$), a local factor that mediates certain effects induced by parathyroid hormone, cytokines such as interleukin-1 and transforming growth factor-$
abla$, and mechanical strain. In this study, we show that transcriptional and posttranscriptional events initiated by PGE$_2$ collaborate to enhance IGFBP-5 gene expression in primary fetal rat osteoblast cultures. PGE$_2$ treatment enhanced promoter activity by $\sim$2-fold. Similar stimulatory effects were seen with forskolin. A DNA fragment with only 51 base pairs of the 5-flanking sequence retained hormonal responsiveness, which may be mediated by a binding site for transcription factor AP-2 located at positions $\sim$44 to $\sim$36 in the proximal IGFBP-5 promoter. Incubation of osteoblasts with the mRNA transcriptional inhibitor 5,6-dichloro-1-$\beta$-D-ribofuranosylbenzimidazole demonstrated that PGE$_2$ enhanced IGFBP-5 mRNA stability by 2-fold, increasing the $t_1/2$ from 9 to 18 h. The effects of PGE$_2$ on steady-state IGFBP-5 transcripts were abrogated by preincubating cells with cycloheximide, indicating that the effects of PGE$_2$ on both gene transcription and mRNA stability required ongoing protein synthesis. Therefore, both promoter-dependent and -independent pathways converge to enhance IGFBP-5 gene expression in response to PGE$_2$ in osteoblasts.

Insulin-like growth factors I (IGF-I) and -II are abundant locally produced growth regulators in skeletal tissue (1). While the synthesis of IGFs is hormonally controlled, their actions are ultimately determined by way of signal-transducing receptors. IGF binding proteins (IGFBPs) are a family of secreted proteins that also avidly bind IGFs and modify their actions by altering their access to cell surface receptors (2, 3). Of the six known IGFBPs, fetal rat osteoblasts synthesize five, IGFBP-2, -3, -4, -5, and -6 (4). However, IGFBP-5 is the only IGFBP with a demonstrated ability to potentiate IGF actions in bone cells (5). Therefore, agents that stimulate IGFBP-5 synthesis may have an important anabolic role in skeletal growth and the maintenance of skeletal integrity.

The initial identification of IGFBP-5 was based upon its ability to augment IGF-I activity in bone cell cultures, as well as its structural and sequence similarities to other IGFBPs (6–9). The mechanism of potentiation of IGF action by IGFBP-5 has been attributed to its association within pericellular compartments (cell membrane and matrix components), resulting in a high local concentration of IGFs in close proximity to cell surface IGF receptors (5, 10, 11). Recent studies suggest the ability of IGFBP-5 to bind ligand decreases after its association with matrix or select polysaccharides and the actions of IGFBP-5-selective proteases (12–15). The subsequent release of highly concentrated IGF in the pericellular environment thus enhances IGF receptor binding and its biological effects. IGFBP-5 has the additional attribute of high affinity binding to the calcium phosphate component of bone, which may serve to concentrate IGFs in inorganic bone matrix for storage and subsequent activation during periods of localized bone resorption (8).

In bone cell cultures, both IGFBP-5 and IGF-I synthesis are regulated by prostaglandin E$_2$ (PGE$_2$), parathyroid hormone (PTH), other agents that stimulate cAMP synthesis, or by cAMP itself (4, 16). PGE$_2$ is produced by osteoblasts in response to PTH, to cytokines such as interleukin-1 and transforming growth factor-$
abla$, and to mechanical strain, and PGE$_2$ has been shown to mediate various biological actions on osteoblasts initiated by these stimuli (17–22). PGE$_2$ may thus serve as a local analog of PTH. Unlike PTH, however, its influence may be more highly focused due to its synthesis within the skeleton. Furthermore, PGE$_2$ has demonstrated anabolic actions in bone that depend on the cellular state of differentiation and on dose and duration of treatment (23–25).

While the molecular mechanisms by which PGE$_2$ regulates IGF-I synthesis in osteoblasts are only currently being eluci-
Mechanisms Regulating IGFBP-5 by PGE₂

EXPERIMENTAL PROCEDURES

Cell Cultures—Primary osteoblast-enriched cell cultures were prepared from parietal bones obtained from 22-day-old Sprague-Dawley rat fetuses (Charles River Laboratories, Raleigh, NC). Rats were housed and euthanized by methods approved by the Yale University Animal Care and Use Committee. Cranial sutures were removed by dissection, and the bones were digested with collagenase for five sequential 20-min intervals. The cells released during the last three digestions exhibit biochemical characteristics associated with differentiated osteoblasts, including high levels of alkaline phosphatase, PTH receptors, type I collagen synthesis, and a rise in osteocalcin expression. The cells released during the last three digestions were then plated at 9,400/cm² in Dulbecco's modified Eagle's medium containing 20 mM HEPES (pH 7.2), 0.1 mg/ml ascorbic acid, penicillin, and streptomycin (from Life Technologies, Inc.) and 10% fetal bovine serum (Sigma). To examine IGFBP-5 transcription stability, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB, Sigma) was added to the cultures at a final concentration of 75 μM. Cycloheximide (Sigma) was used at a final concentration of 2 μM, and its use preceded other treatments by 15 min to assure its effectiveness prior to vehicle or PGE₂ treatments.

Plasmids—Rat IGFBP-5 cDNA was kindly provided by Drs. S. Shimaski and N. Ling. Murine IGFBP-5 promoter constructs have been described previously (32). Plasmids were propagated in Escherichia coli strain DH5α with ampicillin selection and were prepared by a modification of the alkaline extraction method (33, 34).

RNA Analysis— Cultures of 9.6 cm² were solubilized in buffer consisting of 5 mM guanidine thiocyanate, 25 mM trisodium citrate, 0.5% Sarkosyl, and 0.1% 2-mercaptoethanol, followed by extraction with phenol/chloroform/isoamyl alcohol (75:25:1) in the presence of 0.2 M sodium acetate (35). Total RNA was precipitated, ethanol washed, dried, and resuspended in diethylpyrocarbonate-treated water, and concentration and purity were determined by absorbance at 260 and 280 nm. Fifteen micrograms of RNA was denatured with 2.2 M formaldehyde, 12.5 mM formamide at 65°C for 15 min and fractionated on a 1.5% agarose, 2.2 M formaldehyde gel. Co-electrophoresed RNA standards were excised and ethidium bromide stained, and the remaining gel was blotted onto charged modified nylon (GeneScreen Plus, DuPont NEN). A restriction fragment containing the rat IGFBP-5 cDNA clone was blotted onto charged modified nylon (GeneScreen Plus, DuPont NEN). A restriction fragment containing the rat IGFBP-5 cDNA clone was hybridized with [32P]-labeled rat IGFBP-5 cDNA and [32P]-labeled 18S rRNA, washed, and visualized by autoradiography. On the left are pooled data for four independent Northern blots. On the right is a representative blot with IGFBP-5 transcripts shown in the upper panel and the 18S rRNA pattern shown below. RNA standards (Life Technologies, Inc.) were used to determine the length (in kilobases [kb]) of IGFBP-5 transcripts, shown in the right panel.

Transfection Studies—IGFBP-5 promoter-luciferase reporter plasmids (1.0–1.5 μg 96-cm² culture well) were co-transfected with a vector carrying the β-galactosidase gene under SV40 promoter control (1.0 μg culture well; pSV-β-galactosidase Control Vector, Promega Corp.) to normalize for transfection efficiency. Cultures at 75% confluence density were rinsed in serum-free medium and exposed to plasmids in the presence of Lipofectin (Life Technologies, Inc.) for 3 h. The solution was then replaced with growth medium containing 5% fetal bovine serum, and the cultures were grown to confluence (48 h). Confluent cultures were rinsed with serum-free medium and treated for 6 or 24 h with vehicle (ethanol diluted 1/1000 or greater), PGE₂ or forskolin (both from Sigma). At the end of the treatment interval, the medium was aspirated, and the cultures were rinsed with phosphate-buffered saline and then lysed in 100 μl of 25 mM Tris phosphate (pH 7.8), 2 mM dithiothreitol, 2 mM 1,2-diaminoxydohexane-N,N,N’,N’-tetraacetic acid, 1% glycerol, 1% Triton X-100 (cell lysis buffer, Promega Corp.). Nuclei were pelleted at 12,000 × g for 5 min, and the supernatants were stored at 75°C until assay. Commercial kits were used to measure luciferase (Promega Corp.) and β-galactosidase (Tropix, Bedford, MA). Protein was determined by the Bradford assay (38).

Statistical Analysis—When statistical analysis was conducted, data were assessed by one-way analysis of variance, using Kruskal-Wallis or Bonferroni methods for post hoc analysis.

RESULTS

PGE₂ increases the level of IGFBP-5 mRNA and protein in primary fetal rat osteoblast-enriched cultures (4). The magnitude of the rise in transcript abundance is time-dependent. In agreement with earlier studies (4), IGFBP-5 mRNA levels increased by 1.4–7-fold following 4–32 h of PGE₂ (1 μM) treatment (Fig. 1). A subsequent analysis by RNase protection assay confirmed these observations and additionally demonstrated a time-dependent rise in abundance of nascent transcripts, first observed at 3 h after PGE₂ treatment, with a 3-fold increase seen at 8 h (data not shown). These results suggest that PGE₂ may stimulate IGFBP-5 transcription, although this effect appears delayed when compared with another PGE₂-stimulated gene, IGF-I, for which nascent transcripts increase within 30 min of PGE₂ treatment (26).

To investigate potential transcriptional mechanisms influenced by PGE₂ treatment, gene transfer experiments were conducted. In initial studies, segments of the murine IGFBP-5 promoter containing various lengths of 5′-flanking DNA and 120 base pairs (bp) of exon 1 were fused to the luciferase reporter gene, transiently transfected into osteoblast-enriched...
Mechanisms Regulating IGFBP-5 by PGE₂

Various IGFBP-5 promoter-luciferase reporter plasmids (depicted in the left panel) were co-transfected with pSV-β-galactosidase control vector into osteoblast-enriched cultures (9.6 cm²) using Lipofectin. Cultures were grown to confluence (48 h), the growth medium was aspirated, and the cultures were rinsed with serum-free Dulbecco’s modified Eagle’s medium. Cultures were exposed to control medium (containing ethanol vehicle) for 6 h. Cytoplasmic extracts were prepared, and luciferase activity was determined as described under “Experimental Procedures.” Data were corrected for transfection efficiency (β-galactosidase expression) and for protein content of cytoplasmic extracts. Transfections were performed in duplicate or triplicate, and results are pooled data for three or more separate experiments for a total of eight or more replicate cultures. The mean ± S.E. for pooled experiments is shown. Luciferase activity was determined by single channel photon counting, and background levels were ~930 cpm/µg of protein. Control transfections included an SV40 promoter/enhancer-luciferase reporter plasmid (pGL2-Control) and promoterless pGL-2 Basic (both from Promega Corp.). Numbers on the far right in parentheses indicate the percent maximal luciferase expression determined from the mean value for each construct, as compared with the mean value for IGFBP5-Luc3, which has been set at 100%.

cultures, and analyzed for reporter gene expression 48 h later. As shown in Fig. 2, IGFBP5 promoter constructs with 1406, 1004, and 156 bp (IGFBP5-Luc3, -Luc4, and -Luc5) of 5′-flanking DNA directed comparably high luciferase activity (100, 95.6, and 84.3%, relative to IGFBP5-Luc3). In contrast, fusion plasmids with 75 bp or less of 5′-flanking DNA (IGFBP5-Luc6, -Luc7, and -Luc8) had progressively diminished activity (22.9 to 5.9%, relative to IGFBP5-Luc3). Luciferase expression for a positive control viral promoter-driven construct, pGL2-Control, was included for comparison. These data indicate that regions between −156 and −51 bp contain cis-acting regulatory element(s) needed for high level basal promoter activity in osteoblast cultures, analogous to the areas that we defined in Hep G2 (hepatocyte) and C2I (myoblast) cell lines (32, 37). Two recombinant plasmids with longer 5′-flanking segments of 4100 and 3000 bp (IGFBP5-Luc1 and -Luc2) directed lower luciferase activity, suggesting the presence of inhibitory elements in the 5′-flanking region of the promoter. Promoter activity was orientation-specific. When the 1004-bp promoter fragment was inserted into the luciferase vector in the reversed orientation (IGFBP5-Luc4 rev, Fig. 2), reporter expression was minimal, being comparable with the promoterless pGL2-Basic parental plasmid.

Recombinant IGFBP-5 promoter-luciferase fusion constructs were next used to identify promoter elements that participated in PGE₂-stimulated IGFBP5 expression. While basal luciferase activity for IGFBP5-Luc2 through -Luc7 varied up to 14-fold (Fig. 2), 6 h of PGE₂ treatment enhanced their ability to drive luciferase expression to a similar extent, ranging from 2.3- to 1.6-fold (Fig. 3). The shortest construct responsive to PGE₂, IGFBP5-Luc7, contained only 51 bp of 5′-flanking DNA. However, deletion of the next 20 bp (IGFBP5-Luc8) eliminated the effect of PGE₂ on reporter gene expression. Using IGFBP5-Luc 4, a comparable increase was seen after 24 h of PGE₂ treatment, and these effects were duplicated by treatment with forskolin (10 µM), a strong inducer of adenylate cyclase activity (Fig. 4). Plasmids with internal deletions spanning −69 to −51 bp and −52 to −32 bp in the background of the very active IGFBP5-Luc4 construct had diminished basal activity as shown previously (32). Importantly, however, each of these two deletion constructs clearly responded to PGE₂ treatment, although at modestly reduced levels (1.4- and 1.7-fold, respectively; Fig. 5).

In aggregate, these results show that the IGFBP-5 promoter is very active in primary osteoblast cultures but demonstrate that PGE₂ stimulated only a 2-fold increase in IGFBP5 gene transcription, even after a 24-h incubation. This contrasts sharply with Northern blot data showing up to a 6-fold rise in steady-state IGFBP-5 mRNA levels at 24 h (Fig. 1). Thus, the discrepancy between maximal promoter activity and steady-state transcripts encoding IGFBP5 in response to PGE₂ indicates the participation of transcriptional and post-transcriptional mechanisms in regulating IGFBP-5 gene expression. Therefore, the RNA polymerase II selective inhibitor DRB was used to examine the influence of PGE₂ on IGFBP-5 mRNA stability. Cultures were first treated with vehicle alone or PGE₂ (1 µM) for 24 h, followed by DRB (75 µM) to arrest gene transcription. PGE₂ treatment caused a 2-fold rise in the half-life of IGFBP5 mRNA; vehicle-treated control cultures had a τ₀/2 = 9 h, while cultures pretreated with PGE₂ had τ₀/2 = 18 h (Fig. 6). Consequently, PGE₂ enhanced the stability of IGFBP5 transcripts. To explore further the mechanisms involved in induction of IGFBP5 mRNA after PGE₂ treatment, we examined the effect of the protein synthesis inhibitor, cycloheximide. At a dose of 2 µM, cycloheximide blocked >90% of ongoing protein synthesis, as measured by incorporation of [3H]proline into trichloroacetic acid-precipitable material in PGE₂-treated cultures (−cycloheximide, 12.6 ± 0.9 × 10³ cpm versus +cyclo-
Mechanisms Regulating IGFBP-5 by PGE₂

DISCUSSION

IGFBP-5 expression is activated through cAMP-dependent pathways in osteoblasts and in other cell culture models (4, 16, 39). While IGFBP-5 transcripts accumulate in response to PGE₂ and other agents that elevate cAMP, little is known about the mechanisms of hormone-induced IGFBP-5 synthesis in osteoblasts. We now present data demonstrating stimulation of IGFBP-5 promoter activity by PGE₂ and show that PGE₂ also enhances IGFBP-5 mRNA stability. These results indicate that promoter-dependent and -independent mechanisms function together to regulate IGFBP-5 gene expression.

Unstimulated fetal rat osteoblasts synthesize IGFBP-5 mRNA and protein (4, 40, 41). Results from transient transfection experiments confirm our earlier studies that near-maximal basal promoter activity resides within the first 156 bp of 5′-flanking DNA and that over 20% of basal activity is controlled by the proximal 75 bp of the promoter (32). Similar to our earlier evidence, promoter function is attenuated by internal deletions that eliminate nucleotides −69 to −51 or −52 to −32, encoding segments that span a DNase I-footprinted re-
region identified with Hep G2 nuclear protein extracts (32).

In transient transfections of osteoblasts, PGE2 treatment for 6 or 24 h increased luciferase activity driven by the IGFBP-5 promoter by 1.6–2.3-fold in constructs containing as little as 51 bp or as much as 3000 bp of 5′-flanking DNA. The shortest promoter fragments mediating PGE2-induced gene transcription do not contain a consensus cAMP response element. However, a potential binding site for transcription factor AP-2 is present between nucleotides −44 and −36, and at least six AP-2 sites are dispersed throughout the 3000 bp of the IGFBP-5 promoter. The apparent decline in response to PGE2 treatment seen with recombinant plasmids having progressively shorter promoter segments may reflect loss of individual AP-2 binding sites or other potential cAMP-responsive cis-elements.

While this paper was in preparation, Duan and Clemmons (42) reported the involvement of AP-2 in basal and cAMP-mediated IGFBP-5 transcription in human dental fibroblast cell lines. In their study, forskolin stimulated IGFBP-5 promoter activity ∼2.8-fold, a result similar in magnitude to our observation in osteoblasts. They identified an AP-2 site within nucleotides −55 to −36 in the human IGFBP-5 promoter as the key hormone response element (42). An identical AP-2 site is present in a comparable location in the murine promoter, as noted above. While deletion of this site in construct IGFBP5-LucAb reduced basal promoter activity, it caused only a modest decline in the effect of PGE2. Therefore, additional AP-2 sites or alternative cAMP response elements may be functional in osteoblasts.

The modest ∼2-fold effect of PGE2 on IGFBP-5 transcription does not account for the 6-fold increase in steady-state IGFBP mRNA seen following a 24-h incubation. As demonstrated here, PGE2 also caused a doubling of IGFBP-5 transcript half-life, from ∼9 to ∼18 h. Of note, the t1/2 for IGFBP-5 mRNA in osteoblast-enriched cultures under basal conditions, ∼9 h, is similar to the 11-12-h transcript half-life measured by us in C2l myoblasts (37) but differs somewhat from reported values of ∼14 and ∼20 h obtained under basal conditions in a similar osteoblast culture model (40, 41), which may be accounted for by small differences in experimental design. Thus, both transcriptional and post-transcriptional effects of PGE2 contribute to the induction of IGFBP-5 mRNA following hormone treatment. These dual actions on IGFBP-5 gene expression can be distinguished from transcriptional effects of PGE2 on the IGF-I gene, which appear to be mediated through an element found in the proximal part of promoter 1, the major IGF-I gene promoter (27). In addition, the actions of PGE2 to enhance IGFBP-5 gene expression require ongoing protein synthesis, since they were obliterated by preincubation with cycloheximide, while PGE2-stimulated IGF-I gene transcription occurs even in the absence of new protein synthesis.

Levels of IGFBP-5 in extracellular compartments are modulated not only by rates of gene expression and protein biosynthesis but also by post-translational mechanisms. The existence of IGFBP-5-selective proteases has been documented in a variety of cultured cells, including normal human osteoblasts (12–14, 43), and IGF-mediated stabilization of IGFBP-5 abundance has been described in culture models derived from bone and other cell types (44). Since IGFBP-5 enhances the anabolic actions of IGF-I in bone cells (5), analysis of the multiple mechanisms involved in modifying IGFBP-5 availability within the skeleton should have direct impact in understanding how growth factors regulate skeletal cell metabolism.

4T. L. McCarthy, M. J. Thomas, Y. Umayahara, H. Shu, M. Centrella, and P. Rotwein, unpublished observation.
REFERENCES

1. McCarthy, T. L., and Centrella, M. (1993) in Current Directions in Insulin-like Growth Factor Research (LeRoith, D., and Raizada, M. K., eds), pp. 407-414, Plenum Press, New York

2. Clemmons, D. R. (1991) in Insulin-like Growth Factors: Molecular and Cellular Aspects (LeRoith, D., ed) Vol. 1, CRC Press, Inc., Boca Raton, FL

3. Kiefer, M. C., Schmid, C., Waldvogel, M., Schlaffer, I., Futa, E., Masarz, F. R., Green, K., Barr, P. J., and Zapf, J. (1992) J. Biol. Chem. 267, 12692-12699

4. McCarthy, T. L., Casighino, S., Centrella, M., and Canalis, E. (1994) J. Cell. Physiol. 160, 163-175

5. Andress, D. L., Loop, S. M., Zapf, J., and Kiefer, M. C. (1993) J. Biol. Chem. 268, 22467-22472

6. Andress, D. L., and Birnbaum, R. S. (1991) J. Biol. Chem. 266, 10646–10653

7. Shimasaki, S., Shimonaka, M., Zang, H.-P., and Ling, N. (1991) J. Biol. Chem. 266, 10646–10653

8. Bautista, C. M., Baylink, D. J., and Mohan, S. (1991) J. Biol. Chem. 266, 10646–10653

9. Kiefer, M. C., Masiarz, F. R., Bauer, D. M., and Zapf, J. (1991) J. Biol. Chem. 266, 10646–10653

10. Andress, D. L., and Birnbaum, R. S. (1991) J. Biol. Chem. 266, 10646–10653

11. Jones, J. I., Gockerman, A., Busby, W. H., Jr., Camacho-Hubner, C., and Clemmons, D. R. (1993) J. Biol. Chem. 268, 22467-22472

12. Kanzaki, S., Hilliker, S., Baylink, D. J., and Mohan, S. (1991) Biochem. Biophys. Res. Commun. 176, 213-218

13. Nam, T. J., Busby, W. H., Jr., and Clemmons, D. R. (1994) Endocrinology 135, 1385–1391

14. Claussen, M., Zapf, J., and Braulke, T. (1994) Endocrinology 134, 1364–1366

15. Aral, T., Parker, A., Busby, W. H., Jr., and Clemmons, D. R. (1994) J. Biol. Chem. 269, 20388-20393

16. Conover, C. A., Bale, L. K., Clarkson, J. T., and Tarriss, R. (1993) Endocrinology 132, 2025-2030

17. Klein-Nulend, J., Bowers, P. N., and Raisz, L. G. (1990) Endocrinology 126, 1070–1075

18. Peck, M. J., and Lanyon, L. E. (1989) Cold. Tissue Int. 45, 34–40

19. Ngan, P., Saito, S., Saito, M., Lanese, R., Shanfield, J., and Davidovich, A. (1990) Arch. Oral Biol. 35, 717–725

20. Rawlinson, S. P., El-Haj, A. J., Minter, S. L., Tavares, I. A., Bennett, A., and Lanyon, L. E. (1991) J. Bone Miner. Res. 6, 1345–1351

21. Chow, J. W. M., and Chambers, T. J. (1994) Am. J. Physiol. 267, E287-E292

22. Marusici, A., Kalinowski, J. F., Harrison, J. R., Centrella, M., Raisz, L. G., and Lorenzo, T. J. (1991) J. Immunol. 146, 2633–2638

23. Chyon, Y. S., and Raisz, L. G. (1984) Prostaglandins 27, 97–103

24. Centrella, M., Casighino, S., and McCarthy, T. L. (1994) Endocrinology 135, 1611–1620

25. Jee, W. S. S., Mori, S., Li, X., and Chan, S. (1990) Bone Miner. 15, 175–192

26. Bidwell, D. P., Rotwein, P., and McCarthy, T. L. (1993) Endocrinology 133, 1020–1028

27. McCarthy, T. L., Thomas, M. J., Centrella, M., and Rotwein, P. (1995) Endocrinology 136, 3901–3908

28. McCarthy, T. L., Centrella, M., and Canalis, E. (1988) J. Bone Miner. Res. 3, 401–408

29. Centrella, M., Canalis, E., McCarthy, T. L., Orloff, J. J., Stewart, A. F., and Insogna, K. L. (1989) Endocrinology 125, 199–208

30. Centrella, M., Kim, J., Pham, T., Casighino, S., Rosen, V., Wozney, J., and McCarthy, T. L. (1995) Mol. Cell. Biol. 15, 3273–3281

31. Centrella, M., Casighino, S., Gundberg, C., McCarthy, T. L., Wozney, J., and Rosen, V. (1996) Ann. N.Y. Acad. Sci., in press

32. Kou, K., Mittanck, D. W., Fu, C., and Rotwein, P. (1995) DNA Cell Biol. 14, 241–249

33. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

34. Nicoletti, V. G., and Condorelli, D. F. (1993) BioTechniques 14, 532–536

35. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159

36. Feinberg, A. P., and Vogelstein, B. (1984) Anal. Biochem. 153, 6–15

37. Rotwein, P., James, P. L., and Kou, K. (1995) Mol. Endocrinol. 9, 913–923

38. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254

39. Canirnu-Hubner, C., Busby, W. H., Jr., McCusker, R. H., Wright, G., and Clemmons, D. R. (1992) J. Biol. Chem. 267, 11949–11956

40. Canlis, E., and Gabbitas, B. (1995) J. Biol. Chem. 270, 10771–10776

41. Gabbitas, B., and Canalis, E. (1995) Endocrinology 136, 2397–2403

42. Duan, C., and Clemmons, D. R. (1995) J. Biol. Chem. 270, 24844–24851

43. Thrailkill, K. M., Quarles, L. D., Nagase, H., Suzuki, K., Serra, D. M., and Canalis, E. (1995) J. Biol. Chem. 270, 24844–24851

44. Conover, C. A., and Kiefer, M. C. (1993) J. Clin. Endocrinol. Metab. 76, 1153–1159
Promoter-dependent and -independent Activation of Insulin-like Growth Factor Binding Protein-5 Gene Expression by Prostaglandin E in Primary Rat Osteoblasts
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