Progesterone Stimulation of Human Insulin-like Growth Factor-binding Protein-5 Gene Transcription in Human Osteoblasts Is Mediated by a CACCC Sequence in the Proximal Promoter

(Received for publication, December 31, 1998, and in revised form, June 22, 1999)

Viroj Boonyaratankornkit§§, Donna D. Strong¶¶, Suburraman Mohan***, David J. Baylink‡§, Candice A. Beck‡‡, and Thomas A. Linkhart§§§

From the J. L. Pettis Veterans Affairs Medical Center and the Departments of ¶Biochemistry, ¶¶Microbiology and Molecular Genetics, §§Pediatrics, **Physiology, and Medicine, Loma Linda University, Loma Linda, California 92357 and the ¶¶University of Colorado Health Sciences Center, Department of Pathology, Denver, Colorado 80262

Insulin-like growth factor-binding protein-5 (IGFBP-5) is produced by osteoblasts and potentiates insulin-like growth factor mitogenic stimulation in osteoblast cell cultures. Progesterone (PG) increased IGFBP-5 expression in normal human osteoblasts and increased IGFBP-5 transcription in U2 human osteosarcoma cells. We developed a chloramphenicol acetyltransferase reporter construct containing the human IGFBP-5 proximal promoter sequence, which includes TATA and CAAT boxes, and five putative PG response element half-sites. 10^{-8} M PG increased promoter activity of this construct in U2 cells co-transfected with a PG receptor isoform A (PR_A) expression vector. Analysis of 5' deletion constructs indicates that PG transactivation of IGFBP-5 promoter activity does not require the PG response element half-sites but does require the region −162 to −124 containing two tandem CACCC box sequences. Mutation of the proximal CACCC box at −139 eliminated PG transactivation. Gel shift assays using a −162 to −124 DNA fragment, U2 cell nuclear extracts, and purified PR_A protein indicate that nuclear factors bind to a CACCC sequence at −139 and that PR_A alters the pattern of transcription factor interaction with the CACCC sequence. Using a luciferase reporter construct containing base pairs −252 to +24 of the IGFBP-5 promoter, we found that both PR_A and PR_B isoforms mediated PG stimulation of promoter activity. These results suggest that PG may stimulate IGFBP-5 gene transcription via a novel mechanism involving PR and CACCC-binding factors.

Insulin-like growth factor-binding protein-5 (IGFBP-5) belongs to a family of six structurally related proteins that are unrelated to the IGF receptors and that bind IGF-I and IGF-II with high affinity (1–3). IGFBPs modulate the mitogenic and metabolic activities of the IGFs in vitro, suggesting an important role in IGF physiology in vivo. Of the various IGFBPs, IGFBP-5 is unique in that it stimulates IGF-induced osteoblastic cell proliferation when added simultaneously with IGF I or II to serum-free osteoblast-like cell cultures (4–7). Recent evidence suggests that IGFBP-5 may stimulate cell proliferation by an IGF-independent mechanism involving IGFBP-5 specific cell surface binding sites (7, 8). In addition, IGFBP-5 binds with high affinity to hydroxyapatite and to various extracellular matrix proteins, properties that are consistent with a role for this binding protein in fixing IGFs to extracellular matrices including mineralized bone (9, 10). IGFBP-5 may be a more potent enhancer of IGF activities when bound to extracellular matrix than in solution (10). Coding sequences and 5'-flanking regions of IGFBP-5 genes are highly conserved in human, rat, and mouse (4, 5, 11–13), and the mouse and human 5'-flanking regions have been found to impart basal promoter activity to reporter gene constructs in several cell types, including osteoblasts (12–16).

IGFBP-5 is expressed by a number of cell types including fibroblasts, myoblasts, and osteoblasts, and production of IGFBP-5 is regulated by growth factors, cytokines, and systemic hormones (15–21). Among the nuclear receptor activating hormones, glucocorticoids and retinoic acid have been shown to alter IGFBP-5 mRNA and protein levels in human and rat osteoblast-like cells (16, 22–24). However, effects of progesterone on IGFBP-5 expression have not been previously reported.

Pertinent to this study, we found that progesterone (PG) stimulated human osteoblast cell proliferation (25, 26) and increased IGFBP-5 mRNA levels (27). However, the molecular mechanism by which PG increased IGFBP-5 mRNA levels is not known. As a first step toward understanding how bone cell production of IGFBP-5 is regulated by PG, we isolated the 5'-flanking region of the human IGFBP-5 gene, linked the IGFBP-5 proximal promoter to a chloramphenicol acetyltransferase (CAT) reporter, and measured basal and progesterone-inducible promoter activity in transient transfection assays with U2 human osteosarcoma cells. We also examined the roles of progesterone receptor isoforms PR_A and PR_B in mediating PG induction of IGFBP-5 transcription. PRs are expressed in various cells as two different isoforms that are transcribed from separate promoters in the PR gene (28, 29). The B-receptor isoform (PR_B) is 933 amino acids in length, and the A-receptor isoform (PR_A) lacks 164 amino acids at the N terminus.

electrophoretic mobility shift assay(s); RCE, retinoblastoma control element; MNF, myocyte nuclear factor; GRE, glucocorticoid response element.

This paper is available on line at http://www.jbc.org 26431
**Experimental Procedures**

**Materials—** Progesterone acetate was from Sigma, and R5020 (progesterone) was from NEN Life Science Products. U-2 OS (U2) human osteosarcoma cells (HTB96) were from the American Type Culture Collection (Manassas, VA). Dulbecco's modified Eagle's medium (DMEM) was from Mediatech, Inc. (Herndon, VA). Iron-supplemented bovine calf serum (CS) and charcoal-dextran-treated fetal bovine serum (CD-FBS) were from Hyclone (Logan, UT). Penicillin-streptomycin, and trypsin were from Life Technologies, Inc. Bovine serum albumin, crystallized, and guanidine isothiocyanate were from Fluka (Ronkonkoma, NY). The RNA polymerase inhibitor 5,6-dichloro-benzimidazole-1-β-D-rifosuranoside was from Calbiochem (San Diego, CA). Recombinant human IGFBP-5 cDNA was the generous gift of Drs. C. Dony and K. Lang of Roche Molecular Biochemicals Therapeutics (Penzberg, Germany). PR mammalian expression vectors pPR-A and pPR-B (30) were provided by Dr. B. O'Malley (Baylor College of Medicine, Houston, TX). Purified PRα and a specific monoclonal antibody (ABS2) that recognizes PRα (31) were provided by Dr. D. P. Edwards (University of Colorado Health Science Center, Denver, CO). Mouse mammary tumor virus (MMTV) long terminal repeat promoter constructs pMSG-CAT and pMVT-Luc were from Amersham Pharmacia Biotech and Dr. Ron Evans (Salk Institute, San Diego, CA), respectively. 5'-threo-1,2-[32P]Chloramphenicol, [α-32P]dCTP, and [γ-32P]ATP were from ICN Biochemical, Inc. (Irvine, CA). [α-32P]UTP was from NEN Life Science Products. All other molecular biology grade chemicals were obtained from U. S. Biochemical Corp., Sigma, and Calbiochem.

Oligodeoxynucleotides were synthesized by Integrated DNA Technologies (CorvalIa, IA). For gel shift assays complimentary molecules were used to prepare double-stranded probes. The numbered and upper strand sequences of these oligodeoxynucleotides are: BP5-PDREm1, 5'-CTCTTCCATACCAACCACTATGAG-3'; BP5-PDREm2, 5'-CTCTTCCAATACAACCCACTGT-3'; BP5-PDREm5, 5'-CTCTTCCATACCAACCACTATGAG-3'; pCAT reporter construct, cells were plated in 6-well plates at 120,000/well. All experiments were performed in quadruplicate. For the CAT reporter construct studies except that amounts of pLUC-252 and pCMV-β-gal were kept constant at 1.0 μg and CAT activity was expressed per unit of protein. Northern Blot Analysis—Total RNA was extracted from cell cultures by a single-step acid guanidinium thiocyanate-phenol-chloroform method (38). 20 μg of total RNA was subjected to Northern analysis as described previously (23), using Magnagraph hybridization membranes (MSI, Westboro, MA). The human IGFBP-5 cDNA was a 0.25-kilobase fragment containing the coding region of exon 1 and was labeled with [32P] and hybridized as described (23). Relative abundance of each mRNA species was quantitated by laser densitometry (Biomed Instruments, Fullerton, CA) and corrected as described (39) for the amounts of RNA transferred to the hybridization membrane by dividing the densities of 28S and 18S RNA bands stained with ethidium blue by densitometric scanning. Relative mRNA levels are presented as the ratio of treated to control.

**Nuclear Run-on Analysis—**The effect of PG on the rate of IGFBP-5 gene transcription was determined with a modified nuclear run-on analysis as described (40). Briefly, U2 cells were treated with 10 μM PG or with vehicle for 1 and 4 h. The cells were rinsed and scraped into ice-cold phosphate-buffered saline, pH 7.0, and nuclear extracts were sonicated. DNA fragments were incubated in transcription buffer consisting of 0.6 μM KCl, 12.5 μM MgCl2, 2.5 mM of ATP, CTP, and GTP, and 100 μCi of [32P]UTP (800 Ci/Mmol). RNA was isolated and equal amounts (2 x 106 dpm) were hybridized to 10 μg of alkali-denatured plasmid DNA fragments (IGFBP-5 cDNA, β-actin cDNA, and pBluescript DNA) that had been immobilized to Hybond-N filters. The filters were hybridized in a solution containing 10 mM TE, pH 7.4, 10 mM EDTA, 0.2% SDS, and 0.6 M NaCl at 68 °C.
Effect of PG and RU486 on IGFBP-5 Levels in Normal Human Osteoblasts—Previously we found that PG increased IGFBP-5 mRNA levels in normal human osteoblasts and in MG63 and U2-OS osteosarcoma cells (27). In the present study we replicated this earlier finding with normal human vertebral osteoblasts and determined whether the progestin antagonist RU486 would affect PG induction of IGFBP-5 expression. Treatment with 10^{-8} M PG for 4 h increased IGFBP-5 mRNA levels to 190% of control (Fig. 1). 10^{-9} M RU486 by itself did not affect IGFBP-5 mRNA levels and prevented the increase in IGFBP-5 mRNA levels induced by PG. These results support the conclusion that PG increases IGFBP-5 expression by a PR-dependent mechanism.

Effect of PG on IGFBP-5 Transcription—U2 cells were selected to investigate the mechanism of PG action in the present study because both IGFBP-5 expression and transfection efficiency were higher in this cell type than in other human osteoblast-like cell lines. PG treatment for 1 h increased IGFBP-5 gene transcription determined by nuclear run-on analysis to 334% of control (p < 0.002), whereas the rate of β-actin gene transcription was not significantly altered (Fig. 2). After 4 h of PG treatment, the IGFBP-5 transcription rates in both the treated and control groups were elevated such that the difference between PG-treated and control groups was less compared with that found after 1 h of PG treatment (data not shown). To determine whether PG affected IGFBP-5 mRNA stability, U2 cells were treated with PG for 4 h, and thereafter RNA polymerase II-mediated transcription was inhibited with 4 × 10^{-5} M 5,6-dichloro-2-benzamidine-1-β-D-ribofuranoside. IGFBP-5 mRNA levels in control and PG-treated cultures were determined at 5–24 h by Northern analysis. In these experiments, IGFBP-5 mRNA half-life was estimated to be approximately 24 h and was not affected by PG (data not shown). The long IGFBP-5 mRNA half-life and absence of a PG effect on half-life are consistent with the results of nuclear run-on analysis, suggesting that PG rapidly increased IGFBP-5 mRNA levels in human osteoblasts primarily by a transcriptional mechanism.

**Identification of IGFBP-5 Promoter Sequences That Confer PG Responsiveness**—In experiments for assessing effects of PG treatment, concentrations of steroids and other factors in serum were minimized by maintaining U2 cells for 72 h in phenol red-free DMEM plus 5% CD-FBS and then plating the cells in 5% CD-FBS at two-thirds of the cell density used in the basal promoter expression experiments. Cells were co-transfected with pCAG753 and pCMV-β-gal, plus PR expression vector pHPR-A (44), or a PR control vector from which the PR coding region was removed. Cells were incubated for 48 h in medium with 10% CS. CAT activity in cells that were transfected with pCAG753 or pCAG461 sense constructs was 620 and 730%, respectively, of CAT activity in cells transfected with pHFCAT1. In contrast, CAT activity did not increase in U2 cells transfected with antisense promoter constructs.

**Functional Analysis of the IGFBP-5 Gene**—We isolated a pWE15 human genomic cosmID clone containing the IGFBP-5 gene and then isolated a 4.6-kilobase EcoRI fragment containing the 5′-flanking region. The nucleotide sequence from position –461 through the first exon was identical to that reported previously (12). The proximal 5′-flanking region contains a TATA box, a CAAT box, and several putative response elements. Primer extension analysis in U2 cells confirmed a single transcription start site 33 nucleotides from the TATA box (data not shown) that was reported previously in a study of human breast cancer cells (12).

To determine whether the putative IGFBP-5 promoter functioned in human osteoblasts, CAT reporter constructs were made containing a PstI fragment from –753 to +23 (pCAT753) and a 484-bp HindIII/PstI fragment from –461 to +23 (pCAT461). U2 cells were transiently transfected with sense reporter constructs, antisense constructs, or the pHFCAT1 promoterless control vector and then incubated for 48 h in medium with 10% CS. CAT activity in cells that were transfected with pCAG753 or pCAG461 sense constructs was 620 and 730%, respectively, of CAT activity in cells transfected with pHFCAT1. In contrast, CAT activity did not increase in U2 cells transfected with antisense promoter constructs.

**RESULTS**

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**Identification of IGFBP-5 Promoter Sequences That Confer PG Responsiveness**—In experiments for assessing effects of PG treatment, concentrations of steroids and other factors in serum were minimized by maintaining U2 cells for 72 h in phenol red-free DMEM plus 5% CD-FBS and then plating the cells in 5% CD-FBS at two-thirds of the cell density used in the basal promoter expression experiments. Cells were co-transfected with pCAT753 and pCMV-β-gal, plus PR expression vector pHPR-A (44), or a PR control vector from which the PR coding region was removed. Cells were incubated for 48 h in phenol red-free DMEM supplemented with 2% CD-FBS in the absence or presence of 10 nM PG. In these conditions basal promoter activity from pCAT753 was 285% of activity from the promoterless control pHFCAT1. The reduction of basal promoter activity in lower density U2 cells maintained in 2% CD-FBS compared with 10% CS suggests that serum factors and cell density-dependent signals may increase promoter activity.

When U2 cells were co-transfected with the expression vector pHPR-A, along with pCMV-β-gal and pCAT753, PG reproduc-


**Progesterone and IGFBP-5 Promoter Activity**

**FIG. 3.** Analysis of IGFBP-5 promoter deletion constructs. A schematic representation of the IGFBP-5 proximal promoter-reporter construct indicates relative positions of putative PRE half-sites (solid bars), CACC box sequences (hatched box), CAAT box (oval), TATA box (open box), and the start of transcription (+1). Constructs shown below contain 753, 461, 345, 325, 252, 162, and 124 bp of IGFBP-5 5′-flanking region +23 bp of untranslated Exon 1 inserted into pJCAT1. Basal promoter activities in cultures co-transfected with each construct and pCMV-β-gal and then maintained in 10% CS are shown in the first column, expressed as percentages of promoterless pJCAT1 activity. Progesterone inductions of promoter activity in cultures co-transfected with each IGFBP-5 promoter construct, pPR-A and pCMV-β-gal, and then changed to CD-FBS and treated with solvent or 10−6 M PG are shown in the second column, expressed as percentages of the respective solvent control group. All data are based on normalizing CAT to β-galactosidase activities, and are means ± S.E. of at least three independent experiments for each construct. There was no significant difference in fold induction of CAT expression by PG among groups pCAT753pCAT162 as determined by analysis of variance. PG induction of CAT expression in the pCAT124 group was not significant and was significantly lower than in all other groups (p < .001). Basal promoter activities (minus PG) in pCAT162 and pCAT124 were the same, approximately 3-fold of pJCAT1 control.

**FIG. 4.** Mutation of CACC box eliminates PG induction of IGFBP-5 promoter activity. U2 cells were transfected as described under “Experimental Procedures” with pPR-A, with pCMV-β-gal, and with pCAT124, wild type pCAT162, or mutant pCAT162 promoter-reporter constructs. In pCAT162mut both CACC boxes at positions −147 to −134 were mutated from CCCACCCCCCCCC to aaaaACCCCAACC. Only the distal CACC box was mutated to aaaaACCCCAACC in pCAT162mDi, and only the proximal CACC box was mutated to CCCACaaaaACCC in pCAT162mPr. Transfected cells were incubated in 2% CD-FBS in the presence or absence of 10−6 M PG. CAT activity was normalized to β-galactosidase activity and expressed as a percentage of respective control group (transfected cells incubated without PG), mean ± S.E. of three independent experiments. PG significantly increased CAT activity in pCAT162- and pCAT162mDi-transfected cells (p < 0.001) but did not significantly affect CAT activities in pCAT162mut, pCAT162mPr, or pCAT124-transfected cells.

**FIG. 5.** EMSA with U2 cell nuclear extract proteins. 32P-Labeled double-stranded synthetic oligodeoxynucleotides corresponding to the human IGFBP-5 promoter, EMSA were performed. 

**Table 1**

| Construct | Basal CAT Activity (% of Control) | Induced CAT Activity (% of Control) |
|-----------|----------------------------------|-----------------------------------|
| pCAT753   | 630 ± 80                         | 210 ± 15                          |
| pCAT461   | 730 ± 120                        | 220 ± 15                          |
| pCAT345   | 1800 ± 120                       | 247 ± 52                          |
| pCAT325   | 1970 ± 110                       | 230 ± 13                          |
| pCAT162   | 790 ± 90                         | 205 ± 15                          |
| pCAT162   | 350 ± 26                         | 240 ± 27                          |
| pCAT124   | 300 ± 20                         | 110 ± 11                          |

**Table 2**

| Construct | Basal CAT Activity (% of Control) | Induced CAT Activity (% of Control) |
|-----------|----------------------------------|-----------------------------------|
| pCAT753   | 630 ± 80                         | 210 ± 15                          |
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**Materials and Methods**

**Introduction of IGFBP-5 Promoter CACC Box Sequences with Nuclear Extract Proteins—**To determine whether U2 cell nuclear proteins interact with the CACC box motif of the IGFBP-5 promoter, EMSA were performed. 

**1P-Labeled double-stranded synthetic oligodeoxynucleotides corresponding to the wild type IGFBP-5 promoter sequence from −155 to −128, designated the IGFBP-5 progesterone-dependent responsive element (BP5-PDRE), were incubated with U2 cell nuclear extract proteins. Analysis by EMSA resulted in a reproducible set of shifted bands (Fig. 5A). Unlabeled BP5-PDRE oligodeoxynucleotide or different oligodeoxynucleotides containing CACC box core motif sequences from enhancers of other promoters were used to compete for binding to labeled BP5-PDRE. These enhancers were RCE sequences from the TGF-β and c-fos promoters (32, 33) and the MNF-binding site sequence from the myoglobin promoter (34). Other competitors tested were the consensus AP-2-binding site sequence from the metallothionein-IIA promoter (35) and the consensusSpl-binding site sequence from Simian virus 40 (36) because Sp1 and AP2 have been shown to bind to nonconsensus sequences containing CACC boxes (52, 57, 59). As shown in Fig. 5A, 50-fold excess

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unlabeled BP5-PDRE specifically competed with labeled BP5-PDRE for binding to U2 cell nuclear proteins and eliminated bands 1–4. At a 50-fold excess molar ratio of unlabeled DNA to labeled BP5-PDRE DNA, consensus AP-2, Sp-1, MNF, and the RCE sequences from the TGF-β promoter competed as effectively as unlabeled BP5-PDRE for binding in complexes 1, 3, and 4 (Fig. 5A). AP-2, Sp-1, and c-fos RCE sequences did not compete as effectively as BP5-PDRE, MNF, or TGF-β RCE sequences for protein binding in complex 2. Competition with 25-, 50-, and 100-fold molar excess of unlabeled BP5-PDRE and MNF-binding sequences are compared in Fig. 5B. Competition for binding to band 5 was variable and may be nonspecific. These results suggest that nuclear protein(s) in complexes 1, 3, and 4 may be more specific for the CCCCACCCC sequence, which is identical to the consensus CBP/CBP binding sequence (Fig. 5E). The ability of binding to the CCCCACCCC sequence was dependent on the presence of nuclear extract proteins, as observed previously (31), and produced a strong mobility shift band, which was supershifted by incubating the protein-DNA complexes with the PR antibody (Fig. 7A). Purified PR_A protein alone did not bind the BP5-PDRE oligodeoxynucleotide, which is similar to the result with the MMTV-PRE. Incubation of labeled BP5-PDRE with 5 μg of U2 cell nuclear protein alone produced multiple shifted bands (Fig. 7B), as in Fig. 4. When increasing amounts of PR_A were preincubated with nuclear extract proteins and then mixed with labeled BP5-PDRE, the
Progesterone and IGFBP-5 Promoter Activity

The human IGFBP-5 promoter does not contain classic consensus palindromic progesterone or glucocorticoid response elements (GRE/PRE), defined as two hexameric half-sites with a 3-bp spacer (60). However, the IGFBP-5 promoter contains five putative GRE/PRE half-sites that have strong sequence similarity to the hexanucleotide motifs 5′-TGTTC-3′ of the MMTV promoter and 5′-TGTTC-3′ of the uteroglobin gene promoter (50, 60). Although GR and PR do not bind to GRE/PRE half-sites as effectively as to the palindromic GRE/PRE consensus sequence, clusters of GRE/PRE half-site sequences in MMTV, uteroglobin, and other promoters have been shown to bind the receptors and effectively mediate ligand-dependent transcription through synergistic interactions between the multiple half-sites (50, 60). Furthermore, PR as well as other steroid hormones receptors affect transcription of many genes without directly binding to consensus hormone response elements (61–66). Many of these actions of nuclear receptors involve interactions with co-activators and co-repressors (67, 68). Although the presence of GRE half-sites in the IGFBP-5 promoter suggested the possibility that they might mediate PG transactivation, our results demonstrate that this is not the case. Rather, results of the series of reporter construct IGFBP-5 promoter deletions suggest that a repeated CACCC box motif (BP5-PDRE) proximal to the GRE half-sites mediates PG transactivation.

The CACCC box is an important promoter element required for efficient and accurate gene expression in the TGF-β and IGF I genes (32, 33, 52, 59). In addition, a number of transcription factors including the RCE-binding protein, MNF, and AP2 bind to CACCC box sequences and effect transcription (35, 54). CACCC-binding proteins can either bind directly to the CACCC box sequence or interact with other transcription factors and bind as a complex (58). Duan and Clemmons (14) reported that human IGFBP-5 promoter activity is increased by AP-2. They found that the two overlapping AP-2-binding sites in the CACCC box motif at positions −162 to −124 were not required for AP-2 stimulation of promoter activity in human hepatoma cells and skin fibroblasts. Rather, an alternate consensus AP-2-binding sequence 5′-GCCNNNGGC-3′ at positions −52 to −35, adjacent to the TATA box, mediated AP-2 transactivation. Both of the sequences bound purified AP-2 protein in EMSA experiments even though only the sequence adjacent to the TATA box was involved in AP-2 transactivation of promoter activity. In contrast, we found that PR, transactivation of the IGFBP-5 promoter in osteoblastic cells required the AP-2-binding sequence at position −139. This suggests that AP-2 and PR transactivate the IGFBP-5 promoter by different mechanisms.

Although EMSA analysis suggested that purified PR did not bind to BP5-PDRE directly, addition of PRA altered the gel shift band pattern produced by binding of nuclear extract pro-
teins to the BP5-PDRE DNA sequence. This result suggests that PRα interacted with BP5-PDRE-binding proteins, although the identities of the binding proteins are not known. Of the CACCC box sequences tested in competition EMSA experiments, the RCE and MNF-binding sites of the TGF-β and myoglobin promoters (33, 34) are most similar to the BP5-PDRE sequence, and the RCE and MNF sequences most effectively competed with the BP5-PDRE in binding nuclear extract proteins. These results, although not definitive, are consistent with the possibility that RCE, MNF, or related proteins may be involved in binding BP5-PDRE.

Our results indicate that both PRα and PRβ can mediate PG induction of hIGFBP-5 promoter activity. For many genes that are induced by progestins, human PRβ is more active than human PRα (44, 69, 70), although PRα has been reported to be a stronger transactivator than PRβ for chicken ovalbumin and human tyrosine aminotransferase genes (44, 48). In many PG-inducible genes that have been examined, activated PRβ increases transcription by binding to a PRE within the gene promoter, and PRβ functions as a negative regulator without directly binding to a PRE (44, 69). It remains to be established whether PRβ mediates transactivation of the IGFBP-5 promoter by a mechanism that is similar to or different from that of PRα (70).

PRα and PRβ are differentially expressed in a cell type- and species-specific manner (71, 72). Depending on the cell type, either both isoforms are induced by estrogen or PRα is differentially induced (73–75). In human osteoblasts both isoforms of PR are expressed, and estrogen induced both A and B promoters (74). Progestins have been found to stimulate osteoblast proliferation, differentiation, and growth factor expression (25, 26, 76–80) and to increase bone formation (81, 82). In ovariectomized dogs, PG increased bone formation (83) and in postmenopausal women progestins combined with estrogen increased bone density to a greater extent than estrogen alone (84, 85). Our results indicate that both PRA and PRB can mediate PG induction of IGFBP-5 promoter activity. For many genes that are inducible genes that have been examined, activated PRB in-
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