Journal of Cellular Biochemistry 57:488–494 (1995)

Cortisol Downregulates Osteoblast α1(I) Procollagen mRNA by Transcriptional and Posttranscriptional Mechanisms

Anne M. Delany, Bari Y. Gabbitas, and Ernesto Canalis

Departments of Research (A.M.D., B.Y.G., E.C.), Medicine (E.C.), and Orthopedic Surgery (E.C.), Saint Francis Hospital and Medical Center, Hartford, Connecticut 06105 and The University of Connecticut School of Medicine (A.M.D., E.C.), Farmington, Connecticut 06030

Abstract Glucocorticoids decrease osteoblast proliferation and type I collagen production, and this may play a role in the development of glucocorticoid-induced osteoporosis. Osteoblast-enriched cultures derived from fetal rat calvaria were used to determine the mechanisms by which cortisol decreases α1(I) procollagen expression in bone cells. A 24 h treatment with cortisol decreased collagen synthesis in these cultures in a dose-dependent manner. Cortisol decreased α1(I) procollagen transcripts in a dose- and time-dependent manner as well. Repression of α1(I) procollagen transcripts was evident as early as 2 h of treatment and was maximal after 48 h of treatment. Nuclear run-off assays showed that cortisol downregulated transcription of the α1(I) procollagen gene. In addition, pretreatment with cortisol decreased the stability of α1(I) procollagen mRNA in transcription-arrested osteoblast cultures. The ability of cortisol to downregulate α1(I) procollagen transcripts was sensitive to cycloheximide treatment, suggesting that the gene is under "secondary control" by glucocorticoids. Since cortisol decreases α1(I) procollagen gene transcription in osteoblasts but does not affect α1(I) procollagen gene transcription in fibroblasts, we suggest that the mechanisms controlling glucocorticoid repression of collagen expression are cell-type specific.

Key words: glucocorticoid, bone, osteoporosis, fibroblast, RNA stability

Collagen is the most abundant protein in the body, and type I collagen is the major structural protein in bone. The development of osteoporosis in response to glucocorticoid excess is well documented, and this loss of bone mass is associated with both decreased bone formation and increased bone resorption [Lukert and Raisz, 1990]. In vitro, glucocorticoids stimulate bone resorption in organ cultures and in bone chips implanted subcutaneously into rats, suggesting a direct effect on osteoclast activity [Defranko et al., 1992; Reid et al., 1986; Gronowicz et al., 1990]. Glucocorticoids have two major inhibitory effects on bone formation. They inhibit cell replication, which decreases the population of cells able to synthesize type I collagen, and they downregulate osteoblast collagen gene expression [Canalis, 1983].

In bone, glucocorticoids decrease collagen synthesis and type I collagen mRNA levels, but the mechanisms mediating these effects have not been explored [Kim and Chen, 1989]. In human and rat skin fibroblasts, glucocorticoids decrease steady-state procollagen mRNA by decreasing the stability of the mRNA, without changing gene transcription [Hämäläinen et al., 1985; Raghow et al., 1986]. However, recent studies have suggested that the level of regulation of some genes may be different in osseous compared to fibroblastic cells [Pavlin et al., 1992]. Because decreased collagen synthesis plays an important role in the bone loss associated with glucocorticoid-induced osteoporosis and because glucocorticoids may regulate collagen expression by tissue-specific mechanisms, we examined the regulation of the type I collagen gene by cortisol in cultures of osteoblast-enriched cells derived from fetal rat calvaria (Ob cells).

Received July 1, 1994; accepted August 12, 1994.

Address reprint requests to Anne Delany, Department of Research, Saint Francis Hospital and Medical Center, 114 Woodland Street, Hartford, CT 06105.

MATERIALS AND METHODS

Cell Culture

Parietal bones from 22-day-old fetal rats, obtained from timed-pregnant Sprague-Dawley rats (Charles River Breeding Laboratories, Wilm-

ington, MA), were dissected free of sutures and subjected to five sequential digestions with bacterial collagenase (Worthington Biochemical, Freehold, NJ). The cell population obtained from digestions three to five were pooled and plated at 6,500–9,800 cells/cm^2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with nonessential amino acids, penicillin, streptomycin, 20 mM HEPES, 100 μg/mL ascorbic acid, and 10% fetal bovine serum (Gibco, Grand Island, NY). These cultures have been shown previously to be enriched in osteoblasts and to have osteoblastic characteristics [McCarthy et al., 1988; Wong et al., 1975]. The cells were grown to confluence, washed, and cultured in serum-free DMEM for 24 h prior to testing. Then cells were cultured in serum-free medium in the presence or absence of test reagents for 2–48 h. The test reagents used in this study were cortisol, cycloheximide, and 5,6-dichlorobenzimidazole riboside (DRB) (Sigma Chemical Co., St. Louis, MO). These reagents were dissolved in ethanol, and the appropriate vehicle controls were included in the experiments.

Total Collagen Synthesis

Ob cells were cultured for 24 h and pulse-labeled with 25 μCi/ml (2,3-^{3}H)proline (25–40 Ci/μM; New England Nuclear, Boston, MA) for the last 2 h of culture. Cellular proteins were extracted with 0.3% Triton, sonicated, precipitated with TCA, washed with acetone, and resolubilized in 0.5 M acetic acid. An aliquot of the extract was incubated with repurified bacterial collagenase (Worthington Biochemical, Freehold, NJ), and labeled proline incorporated into collagenase digestible protein (CDP) was measured by the method of Peterkofsky and Diegelmann [1971]. Data are expressed as disintegration per minute (dpm)/culture well.

RNA Extraction and Northern Blot Analysis

Cells were washed with phosphate-buffered saline and lysed in a solution of 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sarkosyl, 100 mM 2-mercaptoethanol (Sigma). The lysate was extracted with acid phenol and chloroform, precipitated once with isopropanol and once with ethanol, and quantitated by spectrophotometry [Chomczynski et al., 1987]. RNA samples (10–20 μg/lane) were denatured in 50% formamide/1.8 M formaldehyde and subjected to electrophoresis through 1% agarose gels containing 0.66 M formaldehyde, as described [Sambrook et al., 1989]. The fractionated RNA was transferred to GeneScreen Plus (New England Nuclear) according to the manufacturer's directions. cDNA probes were labeled with α^{32}P-dCTP (3,000 Ci/mM; New England Nuclear) using random primers (Prime-a-Gene; Promega, Madison, WI) [Feinberg and Vogelstein, 1983]. Blots were hybridized with labeled cDNA in 50% formamide, 5× SSPE, 5× Denhardts solution, and 0.4% SDS (Sigma). The blots were washed with high stringency (0.5× SSC/0.5% SDS) and exposed to X-ray film for autoradiography. Appropriate exposures of the resulting autoradiograms were subjected to scanning densitometry (EDC; Helena Laboratories, Beaumont, TX). The blots were probed with a 1.6 kb PstI fragment of the rat α1(I) procollagen cDNA (pα$_1$R1) [Genovese et al., 1984] and a full-length human glyceraldehyde-3-phosphate dehydrogenase (GAPD) cDNA [Yun Tso et al., 1985] (American Type Culture Collection, Rockville, MD).

Nuclear Run-Off

Subconfluent cultures of Ob cells were treated briefly with trypsin (0.25%; Gibco) and subcultured in DMEM containing 10% fetal bovine serum [Rao et al., 1977]. When confluent, cultures were serum-deprived for 24 h and treated in serum-free DMEM in the presence or absence of 1 μM cortisol for 2, 6, or 24 h. Nuclei were isolated by Dounce homogenization in the presence of Nonidet P-40. Nascent RNA transcripts were labeled in vitro with α^{32}P-UTP (3,000 Ci/mM; New England Nuclear), and the labeled RNA was purified by treatment with DNase I and proteinase K, followed by phenol/chloroform extraction and ethanol precipitation [Greenberg and Ziff, 1984]. In vitro labeled RNA (2.6×10^6 cpm) from each treatment was hybridized to immobilized cDNA insert (0.5 μg) for α1(I) procollagen and GAPD. Linearized pGL2Basic (Promega) was used as a control for nonspecific hybridization. Membranes were washed and treated with RNase A (Sigma) and subjected to autoradiography.

RESULTS

The ability of cortisol to decrease collagen synthesis in Ob cell cultures was documented by pulse-labeling the cells with ^{3}H proline and quantitating its incorporation into collagenase digestible protein [Canalis and Avoli, 1992]. Treatment with cortisol for 24 h decreased collagen synthe-

sis in a dose-dependent manner, with 1 μM cortisol causing a 30% decrease compared to the untreated control cultures (data not shown). Northern blot analysis showed that the cortisol-mediated decrease in collagen synthesis corresponds to a decrease in steady-state α1(I) procollagen mRNA levels (Fig. 1). A 24 h treatment with cortisol decreased α1(I) procollagen transcripts in a dose-dependent manner, similar to that observed with the collagen protein levels.

The time course by which cortisol decreases α1(I) procollagen mRNA was determined using Northern blot analysis (Fig. 2). Downregulation of α1(I) procollagen transcripts was apparent as early as 2 h of treatment with 1 μM cortisol. α1(I) procollagen mRNA was decreased by ~45% by 6 h, and maximal repression was seen after 48 h of treatment with cortisol. To determine the transcriptional component of this response, nuclear run-off assays were performed. Cortisol decreased transcription of the α1(I) procollagen gene by ~50% after 2 h of treatment, and this level of repression was also observed at 6 h of treatment (Fig. 3). Inhibition of α1(I) procollagen gene transcription by cortisol was less obvious at 24 h, possibly due to downregulation

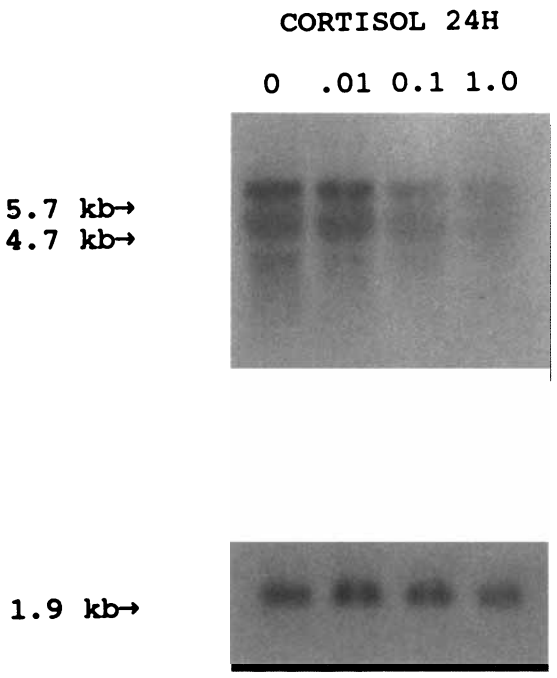

Fig. 1. Cortisol decreases α1(I) procollagen transcripts in a dose-dependent manner. Northern blot analysis of RNA from Ob cells cultured for 24 h in the presence of cortisol at the indicated doses (μM). The blot was probed with cDNA for α1(I) procollagen (**top panel**) and with cDNA for GAPD (**bottom panel**). These results are representative of three cultures.

of the glucocorticoid receptor [Oakley and Cidlowski, 1993]. Cortisol treatment did not affect transcription of the GAPD gene.

The effect of cortisol on α1(I) procollagen mRNA stability was determined in transcription-arrested Ob cells. Cultures were pretreated with or without cortisol for 12 h, followed by addition of the RNA polymerase II inhibitor DRB [Zandomeni et al., 1983]. RNA was harvested at selected times after DRB addition and subjected to Northern blot analysis. Figure 4 shows the results of one such experiment; the α1(I) procollagen mRNA levels are represented as a percentage of the mRNA present at the time of DRB addition. The slopes of the curves indicate that α1(I) procollagen mRNA decayed faster in Ob cells pretreated with cortisol than in control cells. Pretreatment with cortisol did not alter the stability of GAPD mRNA in transcription-arrested Ob cells (data not shown).

To determine if protein synthesis is necessary for the downregulation of α1(I) procollagen transcripts by cortisol, Ob cells were cultured with the protein synthesis inhibitor cycloheximide at 3.6 μM in the presence or absence of 1 μM cortisol for 24 h. Northern blot analysis of the RNA isolated from these cells showed that treatment with cycloheximide alone decreased the abundance of α1(I) procollagen mRNA, and cycloheximide abolished the ability of cortisol to further decrease collagen transcripts (Fig. 5). This indicates that protein synthesis is necessary for the downregulation of α1(I) procollagen transcripts by cortisol, suggesting that this gene is under "secondary control" by glucocorticoids [Landers and Spelsberg, 1992].

DISCUSSION

In fibroblasts, glucocorticoids decrease α1(I) procollagen mRNA levels by decreasing transcript stability but not by affecting gene transcription (Hämäläinen et al., 1985; Raghow et al., 1986). We have shown that in osteoblasts cortisol decreases transcription of the rat α1(I) procollagen gene, suggesting that the mechanisms by which cortisol affects gene expression are cell-type specific. This may be related to differences in the mechanisms by which fibroblasts and osteoblasts regulate basal level collagen gene expression. Recently, Pavlin et al. [1992] found that the promoter of the rat α1(I) procollagen gene contains a 2.3 kb sequence which is necessary for high level expression of a reporter gene when the construct is transfected

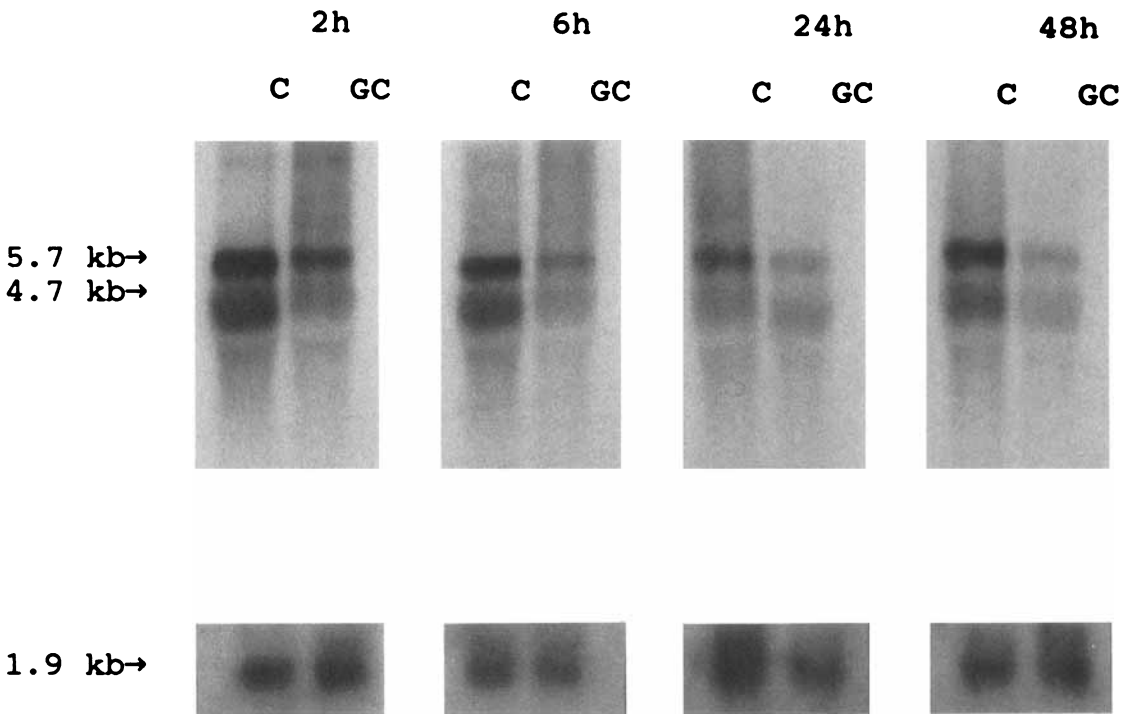

Fig. 2. Time course by which cortisol decreases α1(I) procollagen transcripts. Northern blot analysis of RNA isolated from Ob cells cultured in the absence (C) or presence of the glucocorticoid cortisol (GC) at 1 μM for 2–48 h. The blot was probed with cDNA for α1(I) procollagen **(top panel)** and with cDNA for GAPD **(bottom panel).** These results are representative of three cultures.

into osseous cell lines but is not necessary when the construct is transfected into fibroblastic cell lines. Other evidence for cell-type-specific transcriptional regulation of the α1(I) procollagen gene comes from studies of the Mov13 mouse strain, which carries a retroviral insert in the first intron of the α1(I) collagen gene [Breindl et al., 1984]. Transcription of the mutant allele is blocked in almost all mesodermal cell types, except odontoblasts and osteoblasts [Kratochwil et al., 1993]. These results support the hypothesis that osteoblasts and fibroblasts may express a different quantity or complement of transcription factors associated with collagen gene transcription [Ravazzolo et al., 1991].

The sequences necessary for transcriptional regulation of the α1(I) procollagen gene by glucocorticoids have not yet been characterized experi-

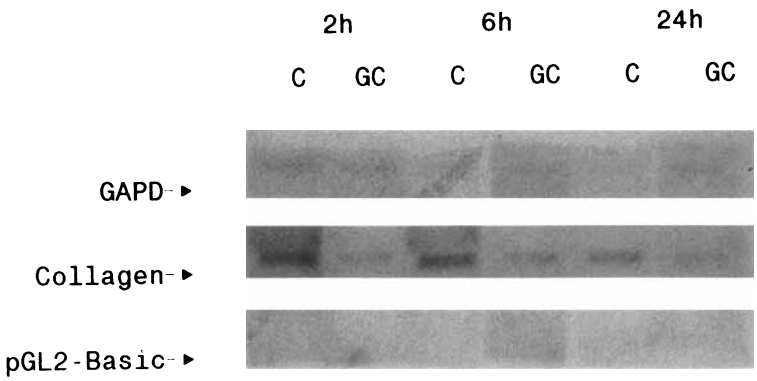

Fig. 3. Cortisol decreases α1(I) procollagen gene transcription. Nuclear run-off assays were performed on nuclei isolated from Ob cells cultured in the absence (C) or presence of the glucocorticoid cortisol (GC) at 1 μM for 2, 6, or 24 h. The in vitro labeled RNA was hybridized to immobilized pGL2-Basic and cDNA for GAPD and α1(I) procollagen.

mentally. However, the 5′ flanking DNA of the gene contains sequences which are similar to the binding sites for the transcriptional activators AP-1, Spi-1/PU.1, and NF-kB [Lichtler et al., 1989]. Interaction of these factors with the glucocorticoid receptor represses their ability to activate gene transcription [Mukaida et al., 1994; Ray and Prefontaine, 1994; Gauthier et al., 1993; König et al., 1992; Kerppola et al., 1993; Jonat et al., 1990], and this may be a mechanism by which cortisol decreases α1(I) procollagen transcription. Intragenic sequences may also be involved in the repression of collagen transcription by glucocorticoids. The first intron of the human α1(I) procollagen gene contains an AP-1–like sequence which is part of an orientation-specific *cis*-acting element [Katai et al., 1992; Liska et al., 1990]. If the rat α1(I) procollagen gene contains a similar intronic element, interaction of the glucocorticoid receptor with AP-1 may play a role in transcriptional downregulation.

In cultures of human osteoblast-like cells, glucocorticoids increase the steady-state level of the mRNAs for Fos and Jun by tenfold and twofold, respectively [Subramaniam et al., 1992]. The relative ratio of Fos to Jun has been shown to determine whether glucocorticoids increase or decrease transcription from the composite GRE in the proliferin promoter: Jun, in the presence of high levels of Fos, decreases transcription

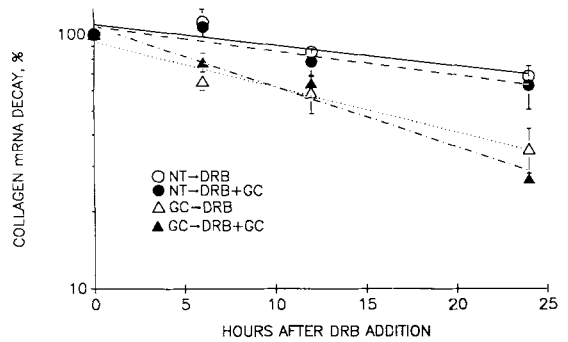

Fig. 4. Cortisol decreases α1(I) procollagen transcript stability in transcription arrested Ob cells. Ob cells were cultured for 12 h with (▲, △) or without (○, ●) 1 μM cortisol (GC). The cultures were washed and treated with 75 μM DRB in the presence (●, △) or absence (○, ▲) of 1 μM cortisol for up to 24 h. Northern blots of RNA from these cells were probed with cDNA for α1(I) procollagen, and appropriate exposures of the resulting autoradiograms were analyzed by scanning densitometry. The results are presented as a percentage of the collagen mRNA present at the time of DRB addition and are the mean ± SEM of triplicate cultures. ○, slope = −0.019; ▲, slope = −0.041; ●, slope = −0.022; △, slope = −0.054.

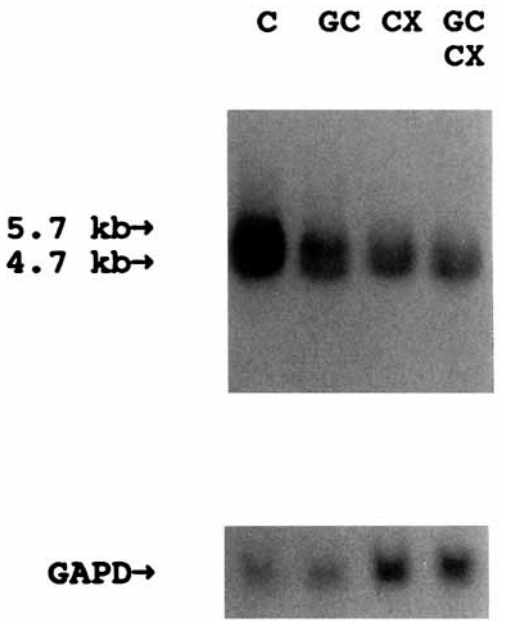

Fig. 5. Downregulation of α1(I) procollagen transcripts by cortisol is protein-synthesis-dependent. Northern blot analysis of RNA isolated from Ob cells cultured for 24 h in the absence (C) or presence of the glucocorticoid cortisol (GC) at 1 μM, and with or without cycloheximide (CX) at 3.6 μM. The blot was probed with cDNA for α1(I) procollagen **(top panel)** and with cDNA for GAPD **(bottom panel).** These results are representative of three cultures.

from the element in response to glucocorticoids, while in the presence of low levels of Fos, transcription is activated in the presence of glucocorticoids [Diamond et al., 1990]. If a composite GRE, similar to the one present in the proliferin promoter, is active in the α1(I) procollagen gene, changing ratios of Fos to Jun could be another mechanism by which glucocorticoids exert their control. Such a mechanism agrees with the finding that protein synthesis is necessary for the action of glucocorticoids on collagen gene expression in osteoblasts and may also explain, in part, the tissue-specific nature of the regulation of α1(I) procollagen gene expression by glucocorticoids. Fibroblasts and osteoblasts may have different basal levels of Fos, Jun, and other members of this family of transcription factors [Distel and Speigelman, 1990; Vogt and Bos, 1990; Curran and Franza, 1988], and the regulation of these genes by glucocorticoids may be tissue-specific.

Cortisol decreases the stability of α1(I) procollagen mRNA in transcription arrested Ob cells. This effect of glucocorticoids on procollagen transcripts agrees with the results obtained in fibroblasts using an in vivo RNA pulse-chase protocol

[Hämäläinen et al., 1985; Raghow et al., 1986]. The 3′ untranslated region of the α1(I) procollagen RNA contains highly conserved regions [Määttä et al., 1991] which may play a role in posttranscriptional regulation of gene expression. Recently, Määttä and Penttinen [1993] characterized a 67 kD cytoplasmic RNA binding activity, α1-RBF_{67}, isolated from NIH3T3 cells and from human skin fibroblasts. This factor specifically bound a conserved region in the 3′ untranslated region of the human α1(I) procollagen RNA, and the binding activity of this factor was decreased in extracts from cells cultured in the presence of dexamethasone [Määttä and Penttinen, 1993]. Since glucocorticoids decrease the stability of α1(I) procollagen transcripts in both fibroblasts and osteoblasts, these cells may share a common regulatory mechanism, possibly involving α1-RBF_{67}.

In conclusion, our investigations reveal that in osteoblasts glucocorticoids decrease α1(I) procollagen mRNA levels by transcriptional and posttranscriptional mechanisms. Further study of the regulation of collagen gene expression by glucocorticoids is of interest because glucocorticoid-induced osteoporosis is an important clinical problem and because dissecting the differential and tissue-specific regulation of gene expression by glucocorticoids will contribute to our understanding of steroid and bone biology.

ACKNOWLEDGMENTS

This work was supported by a grant (DK45227) from the National Institute of Diabetes, Digestive and Kidney Disorders. We thank Dr. David Rowe and Dr. Barbara Kream for providing the α1(I) procollagen cDNA. We gratefully acknowledge the expert technical assistance of Ms. Sheila Rydziel, Ms. Deena Kjeldsen, and Ms. Kathy Boucher. We also thank Ms. Beverly Faulds for excellent secretarial help.

REFERENCES

Breindl M, Harbers K, Jaenisch R (1984): Retrovirus-induced lethal mutation in collagen I gene of mice is associated with an altered chromatin structure. Cell 38:9–16.

Canalis E (1983): Effect of glucocorticoids on type I collagen synthesis, alkaline phosphatase activity, and deoxyribonucleic acid content in cultured rat calvariae. Endocrinology 112:931–939.

Canalis E, Avoli L (1992): Effects of deflazacort on aspects of bone formation in cultures of intact calvaviae and osteoblast-enriched cells. J Bone Miner Res 7:1085–1092.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Curran T, Franza BR Jr (1988): Fos and Jun: The AP-1 connection. Cell 55:395–397.

Defranco DJ, Lian JB, Glowacki J (1992): Differential effects of glucocorticoid on recruitment and activity of osteoclasts induced by normal and osteocalcin-deficient bone implanted rats. Endocrinology 131:114–121.

Diamond MI, Miner JN, Yoshinaga SK, Yamamoto KR (1990): Transcription factor interactions: Selectors of positive or negative regulation from a single DNA element. Science 249:1266–1272.

Distel RJ, Spiegelman BM (1990): Protooncogene c-fos as a transcription factor. Adv Cancer Res 55:37–55.

Feinberg AP, Vogelstein B (1983): A technique for radiolabeling restriction fragments to high specific activity. Anal Biochem 132:6–13.

Gauthier J-M, Bourachot B, Doucas V, Yaniv M, Moreau-Gachelin F (1993): Functional interference between the Spi-1/PU.1 oncoprotein and steroid hormone or vitamin receptors. EMBO J 12:5089–5096.

Genovese C, Rowe D, Kream B (1984): Construction of DNA sequences complementary to rat α_1 and α_2 collagen mRNA and their use in studying the regulation of type I collagen synthesis by 1,25-dihydroxyvitaminD. Biochemistry 23: 6210–6216.

Greenberg ME, Ziff EB (1984): Stimulation of 3T3 cells induced transcription of the c-fos proto-oncogene. Nature 311:433–438.

Gronowicz G, McCarthy MB, Raisz LG (1990): Glucocorticoids stimulate resorption in fetal rat parietal bones in vitro. J Bone Miner Res 5:1223–1230.

Hämäläinen L, Oikarinen J, Kivirikko KI (1985): Synthesis and degradation of type I procollagen mRNAs in cultured human skin fibroblasts and the effect of cortisol. J Biol Chem 260:720–725.

Jonat C, Rahmsdorf HJ, Park KK, Cato ACB, Gebel S, Ponta H, Herrlich P (1990): Antitumor promotion and antiinflammation: Down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell 62:1189–1204.

Katai H, Stephenson JD, Simkevich CP, Thompson JP, Raghow R (1992): An AP-1–like motif in the first intron of human proα1 (I) collagen gene is a critical determinant of its transcriptional activity. Mol Cell Biochem 118:119–129.

Kerppola TK, Luk D, Curran T (1993): Fos is a preferential target of glucocorticoid receptor inhibition of AP-1 activity in vitro. Mol Cell Biol 13:3782–3791.

Kim HT, Chen TL (1989): 1,25-dihydroxyvitamin D_3 interaction with dexamethasone and retinoic acid: Effects on procollagen messenger ribonucleic acid levels in rat osteoblast-like cells. Mol Endocrinol 3:97–104.

König H, Ponta H, Rahmsdorf HJ, Herrlich P (1992): Interference between pathway-specific transcription factors: Glucocorticoids antagonize phorbol ester–induced AP-1 activity without altering AP-1 site occupation in vivo. EMBO J 11:2241–2246.

Kratochwil K, Ghaffari-Tabrizi N, Holzinger I, Harbers K (1993): Restricted expression of Mov13 mutant alpha 1(I) collagen gene in osteoblasts and its consequences for bone development. Dev Dyn 198:273–283.

Landers JP, Spelsberg TC (1992): New concepts in steroid hormone action: Transcription factors, proto-oncogenes, and the cascade model for steroid regulation of gene expression. Crit Rev Eukaryot Gene Expr 2:19–63.

Lichtler A, Stover ML, Angilly J, Kream B, Rowe DW (1989): Isolation and characterization of the rat α1 (I) collagen promoter. J Biol Chem 264:3072–3077.

Liska DJ, Slack JL, Bornstein P (1990): A highly conserved intronic sequence is involved in transcriptional regulation of the α1 (I) collagen gene. Cell Regul 1:487–498.

Lukert BP, Raisz LG (1990): Glucocorticoid-induced osteoporosis: Pathogenesis and management. Ann Intern Med 112:352–364.

Määttä A, Penttinen RPK (1993): A fibroblast protein binds the 3′-untranslated region of pro-α1(I) collagen mRNA. Biochem J 295:691–698.

Määttä A, Bornstein P, Penttinen RP (1991): Highly conserved sequences in the 3′-untranslated region of the COL1A1 gene bind cell-specific nuclear proteins. FEBS Lett 279:9–13.

McCarthy TL, Centrella M, Canalis E (1988): Further biochemical and molecular characterization of primary rat parietal bone cell cultures. J Bone Miner Res 3:401–408.

Mukaida N, Morita M, Ishikawa Y, Rice N, Okamoto S, Kasahara T, Matsushima K (1994): Novel mechanism of glucocorticoid-mediated gene repression. J Biol Chem 269: 13289–13295.

Oakley RH, Cidlowski JA (1993): Homologous down regulation of the glucocorticoid receptor: The molecular machinery. Crit Rev Eukaryot Gene Expr 3:63–88.

Pavlin D, Lichtler AC, Bedalov A, Kream BE, Harrison JR, Thomas HF, Gronowicz GA, Clark SH, Woody CO, Rowe DW (1992): Differential utilization of regulatory domains within the α1(I) collagen promoter in osseous and fibroblastic cells. J Cell Biol 116:227–236.

Peterkofsky B, Diegelmann R (1971): Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. Biochemistry 10:988–994.

Raghow R, Gossage D, Kang AH (1986): Pretranslational regulation of type I collagen, fibronectin, and a 50-kilodalton noncollagenous extracellular protein by dexamethasone in rat fibroblasts. J Biol Chem 261:4677–4684.

Rao LG, Ng B, Brunette DM, Heersche JNM (1977): Parathyroid hormone- and prostaglandin E_1-response in a selected population of bone cells after repeated subculture and storage at −80°C. Endocrinology 100:1233–1241.

Ravazzolo R, Karsenty G, deCrombrugghe B (1991): A fibroblast-specific factor binds to an upstream negative control element in the promoter of the mouse α1(I) collagen gene. J Biol Chem 266:7382–7387.

Ray A, Prefontaine KE (1994): Physical association and functional antagonism between the p65 subunit of transcription factor NF-kB and the glucocorticoid receptor. Proc Natl Acad Sci USA 91:752–756.

Reid IR, Katz JM, Ibbertson HK, Gray DH (1986): The effects of hydrocortisone, parathyroid hormone and the bisphosphonate, APD, on bone resorption in neonatal mouse calvaria. Calcif Tissue Int 38:38–43.

Sambrook J, Fritsch EF, Maniatis T (1989): "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Press.

Subramaniam M, Colvard D, Keeting PE, Rasmussen K, Riggs BL, Spelsberg TC (1992): Glucocorticoid regulation of alkaline phosphatase, osteocalcin, and proto-oncogenes in normal human osteoblast-like cells. J Cell Biochem 50:411–424.

Vogt PK, Bos TJ (1990): Jun: Oncogene and transcription factor. Adv Cancer Res 55:1–35.

Wong GL, Cohn DV (1975): Target cells in bone for parathyroid hormone and calcitonin are different: Enrichment for each cell type by sequential digestion of mouse calvaria and selective adhesions to polymeric surface. Proc Natl Acad Sci USA 72:3167–3171.

Yun Tso J, Sun X-H, Reece KS, Wu R (1985): Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. Nucleic Acids Res 13:2485–2502.

Zandomeni R, Bunick D, Ackerman S, Mittleman B, Weinmann R (1983): Mechanism of action of DRB. Effect on specific in vitro initiation of transcription. J Mol Biol 167:561–574.