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Regulation of Collagenase-3 Gene Expression in Osteoblastic and Non-Osteoblastic Cell Lines

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Abstract Collagenase-3 expression in osteoblastic (UMR 106-01, ROS 17/2.8) and non-osteoblastic cell lines (BC1, NIH3T3) was examined. We observed that parathyroid hormone (PTH) induces collagenase-3 expression only in UMR cells but not in BC1 (which express collagenase-3 constitutively) or ROS and NIH3T3 cells. Since we know from UMR cells that the AP-1 factors and Cbfa1 are required for collagenase-3 expression, we analyzed the expression and PTH regulation of these factors by gel shift and Northern blot analysis in all cell lines. Gel mobility shift with a [^{32}P]-labeled collagenase-3 AP-1 site probe indicated the induction of c-Fos in osteoblastic cells upon PTH treatment. While *c-fos* was induced in UMR cells, both *c-fos* and *jun B* were induced in ROS cells. Since Jun B is inhibitory of Fos and Jun in the regulation of the rat collagenase-3 gene in UMR cells, it is likely that high levels of Jun B prevent PTH stimulation of collagenase-3 in ROS cells. When we carried out gel shift analysis with a [^{32}P]-labeled collagenase-3 RD (*runt* domain) site probe and Northern blot analysis with a Cbfa1 specific probe, we have observed the presence of Cbfa1 in both osteoblastic and non-osteoblastic cell lines, but there was no change in the levels of Cbfa1 RNA or protein in these cells under either control conditions or PTH treatment. From our studies above, it is evident that the expression of collagenase-3 and its regulation by PTH in osteoblastic and non-osteoblastic cells may be influenced by differential temporal stimulation of the AP-1 family members, especially c-Fos and Jun B along with the potential for posttranslational modification(s) of Cbfa1. J. Cell. Biochem. 79:182–190, 2000.

Key words: activator protein; *runt* domain; core binding factor-A; matrix metalloproteinase; collagenase-3

Matrix metalloproteinases (MMPs) are a family of secreted or transmembrane proteins that have been implicated in multiple physiological and pathological processes related to extracellular matrix turnover, such as normal growth and development, wound healing, angiogenesis, joint destruction in arthritis, and tumor invasion and metastasis [Birkedal-Hansen et al., 1993; Stetler-Stevenson et al., 1993]. In the MMP family, collagenase-3 (MMP-13) is thought to play a role in osteoclast-mediated bone resorption [Kahn and Partridge, 1987]. Collagenase-3 is strongly induced by bone resorbing hormones, such as parathyroid hormone (PTH) [Quinn et al., 1990]. PTH stimulates osteoclast-mediated bone resorption indirectly via the activation of the osteoblasts; however, the mechanism coupling these events is not clearly known. It has been suggested that collagenase could act as a coupling factor for activation of the osteoclast [Holliday et al., 1997]. It appears that expression of collagenase-3 during embryonic development is localized to bone and cartilage [Gack et al., 1995] and is modulated by bone specific regulators [Quinn et al., 1990; Scott et al., 1992; 1996; Rydziel et al., 1997], suggesting a possible role for collagenase-3 in bone resorption in both normal and pathological conditions. Recently the role of collagenase-3 in PTH-mediated bone resorption has been shown by mutating the type I collagen substrate [Zhao et al., 1999].

Earlier it was shown by our laboratory that PTH induces collagenase-3 synthesis and secretion in the rat osteosarcoma osteoblastic cell

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line, UMR 106-01 [Quinn et al., 1990; Omura et al., 1994] and rat and mouse interstitial collagenase-3 are identified as the homolog of human collagenase-3 [Freije et al., 1994; Pendas et al., 1997; Selvamurugan et al., 1998]. We have also demonstrated that UMR cell collagenase-3 induction by PTH is due to an increase in transcription and is a secondary response since it requires de novo protein synthesis [Scott et al., 1992]. Recently, we have shown that the AP-1 (activator protein-1) and RD (*runt* domain) sites in the collagenase-3 promoter are necessary for PTH responsiveness and there is increased binding of c-Fos and c-Jun proteins to the AP-1 site whereas there is no change in the abundance of Cbfa (core binding factor alpha)-related proteins binding to the RD site in UMR cells [Selvamurugan et al., 1998]. Cbfa is a mammalian homolog of the Drosophila genes, runt [Kania et al., 1990] and lozenge [Daga et al., 1996].

The expression of collagenase-3 is differentially regulated in cell cultures by cytokines and growth factors [Rydziel et al., 1997; Uria et al., 1998]. In the present study, we examine the expression of collagenase-3 in osteoblastic (UMR 106-01, ROS 17/2.8) and non-osteoblastic cell lines (BC1, NIH3T3). Since the expression of collagenase-3 by PTH in UMR cells appears to require the AP-1 and RD sites [Selvamurugan et al., 1998], it is likely that these sites and their transcription factors may be involved in collagenase-3 expression in other osteoblastic and non-osteoblastic cell lines. In this paper, we report that PTH induces collagenase-3 expression only in UMR cells and not in either BC1 (which express collagenase-3 constitutively) or ROS and NIH3T3 cells (which do not express collagenase-3 even in response to PTH). We also show that the expression of collagenase-3 in osteoblastic and non-osteoblastic cells may be influenced by differential temporal stimulation of the AP-1 family members, especially c-Fos and Jun B along with the potential for post-translational modification(s) of Cbfa1.

MATERIALS AND METHODS

Materials

Parathyroid hormone (rat 1-34) was purchased from Sigma Chemical Company (St. Louis, MO). Restriction endonucleases were products of New England BioLabs, Inc. (Beverly, MA) and radionuclides were obtained from DuPont-NEN (Boston, MA). Synthetic oligonucleotides were synthesized by Gibco BRL (Gaithersburg, MD). Tissue culture media and reagents were obtained from the Washington University Tissue Culture Center (St. Louis, MO). Fetal bovine serum was a product of JRH Biosciences (Lenexa, KS) and was also purchased through Washington University. All other chemicals were obtained from Sigma Chemical Company or Fisher Scientific (Pittsburgh, PA).

Antibodies and Plasmids

Anti-c-Fos and anti-c-Jun antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antisera for human Cbfa2 (AML-1B) and Cbf-β and rat cDNA clones for c-Fos and c-Jun were kindly provided by Drs. Scott Hiebert and Tom Curran, respectively. A Cbfa1 specific cDNA probe was a generous gift from Dr. Gerard Karsenty. The mouse Jun B cDNA clone was obtained from the American Tissue Type Culture Collection.

Northern Blot

Total RNA and poly(A)$^+$ mRNA from control and PTH-treated osteoblastic and non-osteoblastic cells were isolated using the Sigma TRI-Reagent and FastTrack kit (Invitrogen, La Jolla, CA), respectively. Ten μg of either total RNA or one μg of poly(A)$^+$ per lane was electrophoresed on a 1% agarose, 2.2 M formaldehyde gel in MOPS buffer (40 mM MOPS, pH 7.0, 10 mM sodium acetate, and 1 mM EDTA). RNA was transferred to Zeta-Probe GT-membrane (Bio-Rad, Richmond, CA) and hybridized in 50% formamide, 5× SSC, 10× Denhardts, 0.1% SDS, 0.05 M $NaPO_4$ and 100 μg/ml salmon sperm DNA at 42°C. cDNA probes used for hybridization were labeled with either random priming by Prime-a-Gene kit (Promega, Madison, WI) or by nick translation kit (Promega). Filters were washed two times for 15 min each with 2× SSC/0.1% SDS at room temperature, followed by two washes for 15 min each with 0.1 × SSC/0.1% SDS at 50°C. Northern blots were visualized by exposure to film and quantitated by exposure to phosphor screens and analysis in a PhosphorImager.

cAMP Assay

Osteoblastic (UMR 106-01 and ROS 17/2.8) cells were treated with control or PTH (10^{-8} M)-

containing phenol red free-MEM media with 1 mg/ml BSA, 25 mM Hepes, and IBMX (10^{-4} M) for 15 min. Then the cells were scraped in Gey's balanced salt solution (13.8 mM NaCl, 0.507 mM KCl, 0.1033 mM $MgCl_2$, 0.105 mM Na_2HPO4, 0.018 mM KH_2PO4, 1.1 mM glucose). The samples were boiled for 5 min followed by centrifugation. The pellet was dissolved in 0.1 N NaOH and used for protein estimation. The supernatant was used to measure cAMP level by a non-acetylation assay with an enzyme immunoassay (EIA) kit (Amersham, Arlington Heights, IL) according to the instructions of the manufacturer.

Gel Mobility Shift Assay

Total cellular lysates and nuclear extracts were prepared from control and PTH-treated osteoblastic and non-osteoblastic cells as described [Pearman et al., 1996; Selvamurugan et al., 1998]. Approximately 10 μg of lysate was incubated in a volume of 20 μl containing binding buffer (final concentrations: 4% glycerol, 1 mM $MgCl_2$, 0.5 mM DTT, 50 mM KCl, 10 mM Tris-HCl, pH 7.5), 100 ng/μl poly(dI-dC), and antisera or competitor DNA at room temperature for 15 min. [^{32}P]-labeled double stranded oligonucleotide was added to the reaction immediately following the above reagents. The incubation was carried out for 15 min at room temperature. The reaction was stopped by the addition of 2 μl of 10× gel loading dye. Electrophoresis was performed at 4°C on a 6% non-denaturing polyacrylamide gel in TGE buffer (25 mM Tris, 190 mM glycine and 1.1 mM EDTA, pH 8.5). The protein-DNA complexes were visualized by autoradiography. The sequences of the oligonucleotide probes were as follows: (RD, Cbfa, and AP-1 sites are underlined)

AP-1 site:	5′ CCAAGTGGTGACTCATCACTAT 3′ 3′ GGTTCACCACTGAGTAGTGATA 5′
RD site:	5′ CAAGACGGTGTTTGGTGTGCATGCT 3′ 3′ GTTCTGCCACAAACCACACGTACGA 3′
Cbfa consensus sequence:	5′ GCATAATTGGTGTTATGAGC 3′ 3′ CGTATTAACCACAATACTCG 3′

RESULTS

Expression and PTH Induction of Collagenase-3

To study the expression of collagenase-3 and its regulation by PTH in osteoblastic (UMR 106-01, ROS 17/2.8) and non-osteoblastic cell lines (BC1, NIH3T3), cells were treated with control or PTH (10^{-8} M)-containing media for 30 min and 4 h and total cellular RNAs were isolated and analyzed by Northern blot using a rat collagenase-3 cDNA probe [Quinn et al., 1990]. As shown in Figure 1, PTH induced collagenase-3 mRNA accumulation at 4 h in UMR cells. ROS cells did not produce collagenase-3 in either control or PTH treated conditions. Similarly, mouse fibroblast cells NIH3T3 also failed to express collagenase-3 even in response to PTH. In BC-1 cells, we have not seen PTH-induced collagenase-3 expression; but they express collagenase-3 constitutively. An equal amount of RNA loading and transfer was verified by detecting 18S ribosomal RNA on the same filter.

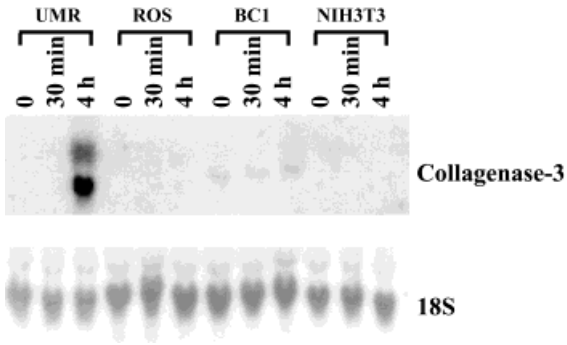

Fig. 1. Effect of PTH on collagenase-3 mRNA levels in osteoblastic and non-osteoblastic cells. Osteoblastic (UMR 106-01, ROS 17/2.8) and non-osteoblastic (BC1, NIH3T3) cells were treated with control or PTH (10^{-8} M)-containing media for 30 min and 4 h, and total RNA was isolated. Ten μg of RNA was resolved in a 1% agarose/formaldehyde gel and blotted onto a nylon membrane. Filter was hybridized with a rat collagenase-3 cDNA probe, stripped, and subsequently hybridized with a 18S ribosomal cDNA probe.

TABLE I. cAMP Synthesis by UMR and ROS Cells in Response to PTH*

Cell line	Treatment	cAMP (pmol/mg protein)
UMR 106-01	Control	18.6 ± 0.9
	PTH	39.7 ± 2.6[a]
ROS 17/2.8	Control	29.1 ± 1.6
	PTH	130.2 ± 7.5[b]

*UMR 106-01 and ROS 17/2.8 cells were treated either with or without PTH (10^{-8} M)-containing media for 15 min and the cAMP levels were then measured in the lysates using an EIA kit (Amersham). Data represent mean ± S.D. of three replicate plates. The statistical analysis was performed using student's *t*-test and SigmaStat 2.0.
[a]Significant difference compared with control UMR cells ($P < 0.001$).
[b]Significant difference compared with control ROS cells ($P < 0.001$).

Evaluation of PTH Response

Even though both UMR and ROS cells are osteoblastic in nature, PTH-induced collagenase-3 expression was found only in UMR cells. This raised the question of the PTH-responsiveness of ROS cells. This was determined in terms of cAMP production. Both UMR and ROS cells were treated with PTH for 15 min and cAMP accumulation was assayed. Table I clearly indicates that like UMR cells, ROS cells also responded to PTH and produced increased intracellular cAMP.

Regulation of the AP-1 Factors

Since we know from UMR cells that the AP-1 factors and Cbfa1 are required for collagenase-3 expression, we analyzed the PTH regulation of these factors by gel shift and Northern blot analysis in all cell lines. Total cellular lysates were prepared in osteoblastic and non-osteoblastic cell lines and used for gel mobility shift analysis with end labeled collagenase-3 AP-1 site probe (Fig. 2). To identify the proteins bound to the AP-1 site, antibodies to c-Fos or c-Jun were incubated with lysates of both control and PTH-treated cells, followed by the addition of labeled AP-1 probe. Since we found increased levels of c-Fos and c-Jun in UMR cells in response to PTH [Selvamurugan et al., 1998], we have used c-Fos and c-Jun antibodies in these studies. The antibodies to c-Fos and c-Jun caused reduction of protein binding when PTH-treated lysate was used in ROS cells; whereas inclusion of the antibodies did not change the gel mobility shift in BC1 cells.

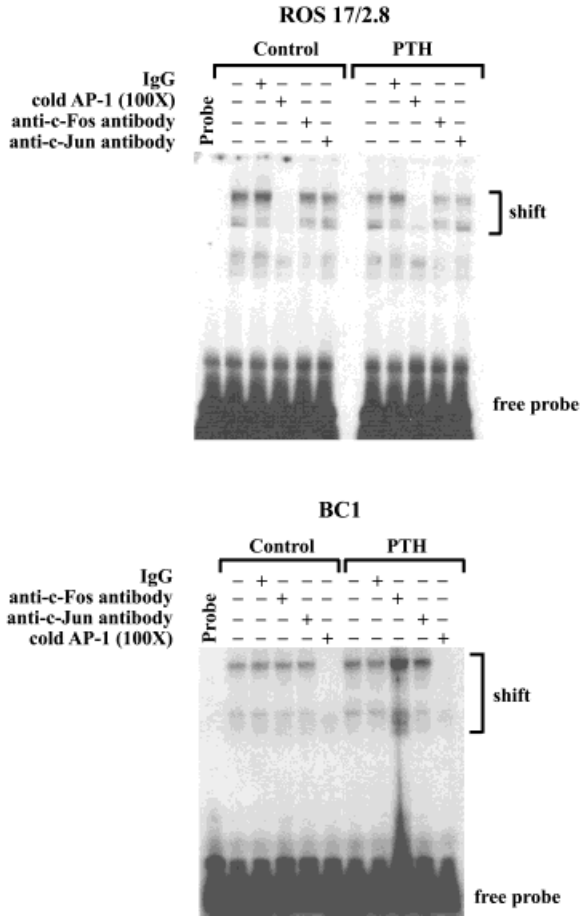

Fig. 2. Gel mobility shift analysis of the rat collagenase-3 AP-1 site binding proteins from osteoblastic and non-osteoblastic cell lysates. Total cellular lysates from osteoblastic (ROS 17/2.8) and non-osteoblastic (BC1) cells treated with control or PTH (10^{-8} M)-containing media for 1 h, were incubated with a labeled collagenase-3 AP-1 site probe. An antibody to c-Fos (2 μl), c-Jun (2 μl) or the same concentration of IgG was included in the incubation before adding labeled probe. Competition was performed with 100-fold molar excess of unlabeled collagenase-3 AP-1 site oligonucleotide.

Similarly, inclusion of antibodies to c-Fos and c-Jun elicited no change in the binding to the collagenase-3 AP-1 site in cellular lysates of control or PTH-treated NIH3T3 cells (data not shown). The specificity of protein binding to the AP-1 site was shown by competition with cold collagenase-3 AP-1 site probe.

In order to compare regulation of AP-1 gene expression by PTH in osteoblastic and non-osteoblastic cells, we carried out Northern blot analysis of total RNA using cDNA probes for c-*fos*, c-*jun*, and *jun* B. c-*fos* mRNA was detectable in osteoblastic cells that had been treated with PTH for 30 min (Fig. 3A). The stimulation

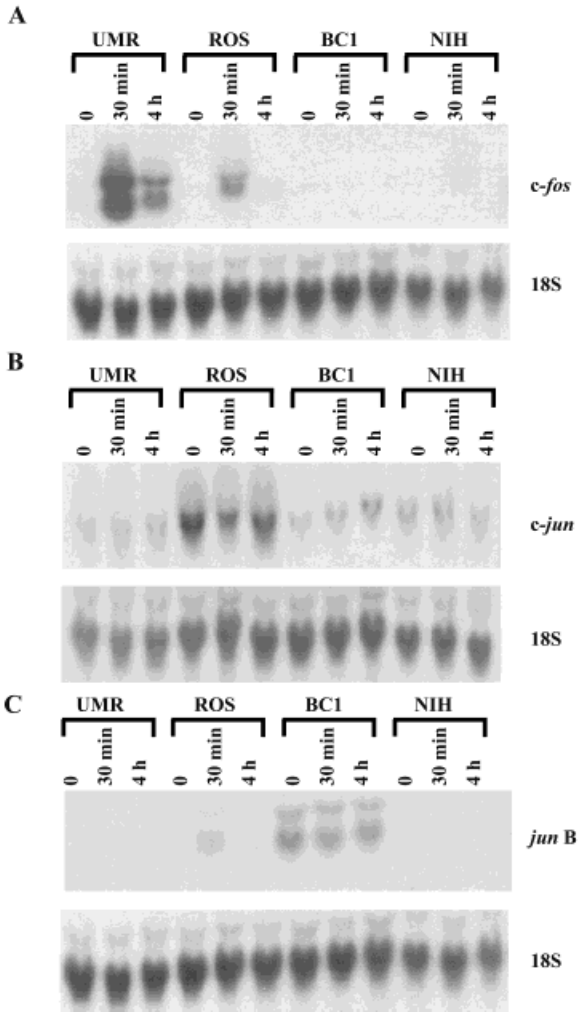

Fig. 3. Effect of PTH on expression of c-*fos*, c-*jun*, and *jun* B mRNA levels in osteoblastic and non-osteoblastic cells. Osteoblastic (UMR 106-01, ROS 17/2.8) and non-osteoblastic (BC1, NIH3T3) cells were treated with control or PTH (10^{-8} M)-containing media for 30 min and 4 h, and total RNA was isolated. RNA was analyzed by Northern blot with labeled probes for c-*fos* (**A**), c-*jun* (**B**), or *jun* B (**C**). Filters were hybridized consecutively with a labeled cDNA probe for 18S ribosomal RNA.

of c-*fos* by PTH was much greater in UMR cells than in ROS cells. In NIH3T3 cells, there was also a marginal stimulation of c-*fos* by PTH. When we used a c-*jun* cDNA probe, we observed a small PTH stimulation of c-*jun* mRNA levels in UMR and BC1 cells whereas there was inhibition of c-*jun* in ROS cells by PTH (at 30 min; Fig. 3B). In NIH3T3 cells, c-*jun* is expressed constitutively and was not changed by PTH. Since Jun B has previously been shown to be able to inhibit Fos/Jun regulation of collagenase-3 gene in UMR cells [Koe et al., 1997], *jun* B mRNA levels were determined in both osteoblastic and non-osteoblastic cells. Interestingly, we observed a PTH stimulation of *jun* B in ROS and NIH3T3 cells but not in either UMR or BC1 cells (Fig. 3C), although it was constitutively expressed in the latter cells. The RNA loading and transfer was verified by detecting 18S ribosomal RNA on the same filter.

Regulation of Cbfa1

To address the role of the Cbfa (AML) transcription factor family involved in collagenase-3 expression, we have carried out gel mobility shift and Northern blot analysis. For gel mobility shift analysis, nuclear extracts from osteoblastic and non-osteoblastic cells were used with end labeled collagenase-3 RD site probe. The RD site of collagenase-3 is identical to an osteoblast specific element-2 (OSE-2) and an AML (Cbfa) consensus binding sequence of mouse and rat osteocalcin genes, respectively [Geoffroy et al., 1995; Banerjee et al., 1996]. Nuclear extracts of both control and PTH-treated osteoblastic and non-osteoblastic cells were able to alter the mobility of the probe, producing identical shift patterns with no change in the abundance of protein binding (data not shown for UMR and NIH3T3). The binding specificity of the protein(s) to this site was examined by competing this protein-DNA complex with excesses of unlabeled RD site probe in BC1 and ROS (Fig. 4A) cells. The protein-DNA complex was similarly competed in both control and PTH-treated samples. As the RD site is identical to the Cbfa (AML) consensus sequence, cold cross-competition was performed in BC1 and ROS (Fig. 4B) cell nuclear extracts. The protein-DNA complex was significantly reduced when unlabeled Cbfa consensus binding site probe was added. The relationship between proteins binding to the collagenase-3 RD site was examined using polyclonal antisera raised against human Cbfa2 or Cbf-β (non-DNA binding partner protein of Cbfa members). The antiserum to human Cbfa2 also recognizes the mouse Cbfa1 and Cbfa3 [Bae et al., 1995; Selvamurugan et al., 1998]. The addition of either Cbfa or Cbf-β antisera decreased the shifted band in ROS and BC1 cells indicating the presence of Cbfa or Cbfa-related protein(s) in ROS and BC1 cells (Fig. 4C; data not shown for ROS cell).

Since it is clear from the gel mobility shift analyses that there was no change in the abundance of Cbfa protein(s) binding to the

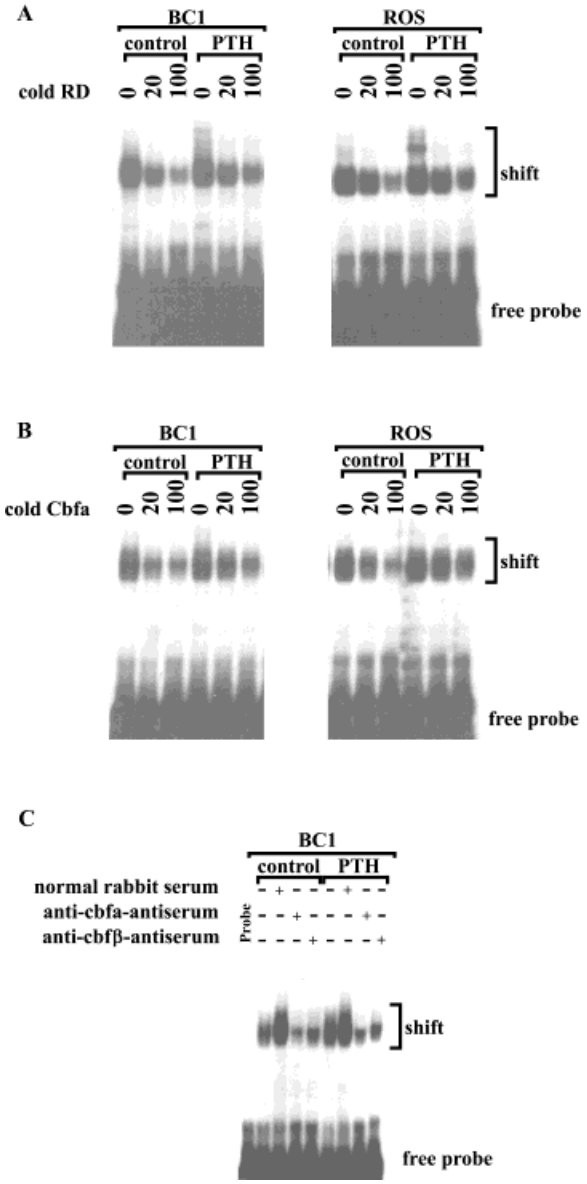

Fig. 4. Gel mobility shift analysis of the rat collagenase-3 RD site binding proteins from osteoblastic and non-osteoblastic cell nuclear extracts. Nuclear extracts from osteoblastic (ROS 17/2.8) and non-osteoblastic (BC1) cells treated with control or PTH (10^{-8} M)-containing media for 2 h, were incubated with a labeled collagenase-3 RD site probe. Competition was performed with 20- and 100-fold molar excess of unlabeled RD site oligonucleotide (**A**). Cross-competition was performed with 20- and 100-fold molar excess of unlabeled Cbfa (AML) consensus binding site oligonucleotide (**B**). Normal rabbit serum (2 μl), anti-Cbfa2 (2 μl), and anti-Cbf-β were included with the BC1 cell nuclear extracts before adding the RD site probe (**C**).

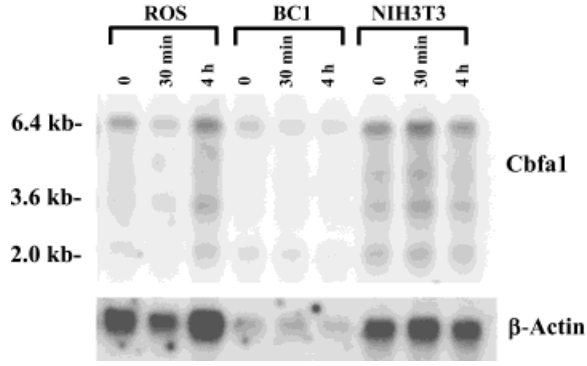

Fig. 5. Effect of PTH on expression of Cbfa1 in osteoblastic and non-osteoblastic cells. Osteoblastic (UMR 106-01, ROS 17/2.8) and non-osteoblastic (BC1, NIH3T3) cells were treated with control or PTH (10^{-8})-containing media for 30 min and 4 h, and poly $(A)^+$ mRNA was isolated. One μg of poly $(A)^+$ mRNA was resolved in a 1% agarose/formaldehyde gel and blotted onto a nylon membrane. Filter was hybridized with a mouse cDNA probe specific for Cbfa1, stripped, and subsequently hybridized with a cDNA probe for β-actin.

collagenase-3 RD site in response to PTH in both osteoblastic and non-osteoblastic cells, this suggested that different isomeric forms of Cbfa may be expressed through variation of mRNA splicing [Bae et al., 1993, 1994; Levanon et al., 1994]. To analyze the expression of Cbfa1 splice variants in osteoblastic and non-osteoblastic cells, we performed Northern blot analyses using a Cbfa1 specific cDNA probe with poly(A^+) mRNA isolated from all the cell lines. Three transcripts of the sizes of approximately 6.4 kb, 3.6 kb, and 2.0 kb were observed in both osteoblastic and non-osteoblastic cells (Fig. 5). But there were no significant changes in the abundance of the three Cbfa1 transcripts after PTH treatment. The filter was reprobed with a β-actin cDNA to verify the RNA loading and transfer.

DISCUSSION

In this paper, we show the differential expression of collagenase-3 and its regulation by PTH in osteoblastic and non-osteoblastic cell lines. Our earlier studies on the rat collagenase-3 gene promoter in UMR cells and in normal and differentiating osteoblasts indicated the requirement of both the AP-1 and RD sites for PTH-mediated collagenase-3 induction [Selvamurugan et al., 1998; Winchester et al., 2000]. Numerous cellular and viral genes contain AP-1 binding sites within their promoters and, accordingly, AP-1 has been shown to play a role in the regulation of basal and inducible transcription of these genes. The AP-1 transcription regulatory proteins function as dimers or multimers forming a complex with DNA to enhance gene activation [Buttice et al., 1996; Giese et al., 1995; Zhang et al., 1996].

AP-1 sites have been reported to mediate the response of growth factors on other genes such as osteocalcin [Banerjee et al., 1996], retinoic acid, and retinoid X receptors [Chen et al., 1996]. In some cases, an AP-1 site partially mediates the response to growth factors [Uria et al., 1998].

The selective expression of fos- and jun-related genes is functionally related to the stage of osteoblast growth and differentiation of cells in culture [McCabe et al., 1996]. The up-regulation of c-Fos and c-Jun could account for transactivation through an AP-1 site, leading to the basal collagenase-3 promoter activity in UMR cells [Selvamurugan et al., 1998]. The inhibitory effect by Jun B in Fos/Jun heterodimerization has been previously well documented [Chiu et al., 1989; Koe et al., 1997]. The enhancement of *jun B* in ROS and NIH3T3 cells induced by PTH suggests that AP-1 activity may be inhibited. Even though we observe PTH stimulation of *c-jun* and constitutive expression of *jun B* in BC1 cells, we have not seen any change in collagenase-3 expression suggesting that PTH stimulation of c-Fos may be essential for AP-1 activity. The ability of BC1 cells to express collagenase-3 in the presence of Jun B is not clear. It is possible that other members of the Fos family could also be involved in modulating the AP-1 site activity in BC1 cells. Fra-2 along with c-Jun suppresses the AP-1 transactivation of the collagenase gene whereas c-Fos along with c-Jun stimulates the transcriptional activity [Suzuki et al., 1991]. It has been suggested that high expression of Fra-1 also negatively regulates AP-1 activity, in a human breast cancer cell line treated with estradiol [Philips et al., 1998]. From our results, it is clear that differential temporal stimulation of AP-1 family members could either partially or completely account for collagenase-3 expression in osteoblastic and non-osteoblastic cells.

In recent studies, the role of Cbfa1 has been more clearly defined. Cbfa1, a bone specific transcription factor, is essential for the maturation of osteoblasts and for skeletal morphogenesis [Banerjee et al., 1997; Mundlos et al., 1997; Komori et al., 1997; Otto et al., 1997]. Multiple isoforms of Cbfa (AML) have been detected in T and B cell lines [Bae et al., 1993, 1994; Levanon et al., 1994]. Merriman et al. [1996] have also reported the presence of two transcripts in ROS 17/2.8 cells. The presence of three Cbfa1 transcripts in osteoblastic and non-osteoblastic cells could be a result of utilization of different transcription start sites and internal deletion of Cbfa1 by alternative splicing [Xiao et al., 1998]. From our gel shift analysis and Northern blot, however, it is evident that there is no change in the abundance of Cbfa protein(s) or RNA by PTH treatment in all cell lines suggesting the unlikelihood of altered expression of different isoforms of Cbfa. Previously we suggested that Cbfa or -related factor(s) may be modified post-translationally in response to PTH and that this modification is required to induce collagenase-3 promoter activity in UMR cells [Selvamurugan et al., 1998]. It has been shown that the ability of Cbfa to bind DNA is regulated by changes in the reduction-oxidation state of a conserved cysteine residue in its runt domain [Kurokawa et al., 1996]. Cbfa can also be phosphorylated by activation of extracellular signal-regulated kinase [Tanaka et al., 1996]. Recently we have found that PTH induces PKA-mediated post-translational modification of Cbfa1 for collagenase-3 promoter activity in UMR cells [Selvamurugan et al., 2000]. Thus, it is possible that a posttranslational modification constitutively occurs in BC1 cells, resulting in expression of collagenase-3. The constitutive expression of collagenase-3 in BC1 cells could also be due to the suppression of Groucho, a repressor protein of runt domain proteins [Aronson et al., 1997] or due to mutated p53 tumor suppressor protein. Recently it has been reported that human collagenase-1 is down-regulated by p53 tumor suppressor protein [Sun et al., 1999].

The effect of growth factors, hormones, and cytokines on regulation of collagenase-3 expression may be cell type-specific. It has been well documented that collagenase-3 expression is differentially regulated by TGF-β1. For example, TGF-β1 inhibits collagenase-3 in rat osteoblast culture [Rydziel et al., 1997] whereas it stimulates collagenase-3 expression in human fibroblasts and chondrosarcoma cells [Uria et al., 1998]. From our studies, it is likely that the regulatory mechanism(s) for PTH-induced collagenase-3 expression is cell type-specific. In summary, we report that the expression and regulation of collagenase-3 by PTH in osteoblastic and non-osteoblastic cells may be influenced by differential temporal stimulation of the AP-1 family members, especially c-Fos and Jun B along with the potential for posttranslational modification(s) of Cbfa1.

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