

Transcriptional Regulation of Osteopontin Production in Rat Osteoblast-like Cells by Parathyroid Hormone

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Abstract. Osteopontin (OP) or bone sialoprotein is a recently characterized extracellular matrix protein which is abundant in bone and is produced by osteoblasts. Parathyroid hormone (PTH) is a potent calcitropic hormone which regulates osteoblastic function including the synthesis of extracellular matrix proteins. This study examines the effect of human PTH (hPTH-[1-34]) on the expression of this novel protein in rat osteoblast-like cells. hPTH(1-34) significantly decreased the amount of OP in culture media of the rat osteoblastic osteosarcoma cell line, ROS 17/2.8, detected by Western immunoblot analysis. hPTH(1-34) also suppressed the steady-state level of OP mRNA two- to threefold with an ED₅₀ of $\sim 3 \times 10^{-10}$ M. This inhibition was detectable at 24 h, reached its nadir at 48 h, and lasted at least up to 96 h. The hPTH(1-34)

effects were mimicked by isobutylmethylxanthine, cholera toxin, 8-bromo-cAMP, forskolin, and isoproterenol. hPTH(1-34) suppressed by two- to threefold the rate of OP gene transcription, estimated by nuclear run-on assays. The suppression of OP mRNA levels by hPTH(1-34) was also seen when basal levels were increased by transforming growth factor type β , or 1,25-dihydroxyvitamin D₃, or were decreased by dexamethasone. A similar decrease in the steady-state level of OP mRNA by hPTH(1-34) was also observed in primary cultures of osteoblast-enriched cells from fetal rat calvaria. These findings indicate that hPTH(1-34) suppresses the production of the novel extracellular matrix protein, OP, in osteoblasts at least in part through transcriptional control.

OSTEOPONTIN (OP)¹ is a 44-kD glycoprotein which is rich in aspartic acid and glutamic acid and contains one phosphoserine and 12 phosphothreonine residues (Prince et al., 1987). OP is an abundant noncollagenous protein in bone (Franzen and Heinegard, 1985) and was immunohistochemically detected in bone matrix, osteoid, osteoblasts, osteocytes, preosteoblast-like cells, neurons, neurosensory cells, and the proximal convoluted tubules of the kidney (Mark et al., 1987a,b, 1988). OP messenger RNA was found in bone and kidney and at much lower levels in brain and lung (Yoon et al., 1987). In embryonic rat calvaria, OP mRNA expression was shown to increase between 15 and 21 d of gestation during development (Yoon et al., 1987). The amino acid sequence of OP predicted from a cDNA cloned from a rat osteosarcoma (ROS 17/2.8) cell library (Oldberg et al., 1986) was shown to be >92% identical to that of 2ar (Nomura et al., 1988). The 2ar gene was originally isolated on the basis of its enhanced expression in the mouse JB4 epidermal cell line in response to 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) (Smith and Denhardt, 1987). Since 2ar appears to be the product of a single copy

gene, it is probably the mouse homologue of rat OP (Nomura et al., 1988). In fact, 2ar mRNA, similar to rat OP, was expressed in mouse calvaria, vertebrae, and kidney (Nomura et al., 1988). In addition, 2ar mRNA was also expressed in placenta and decidua (Nomura et al., 1988). Although OP/2ar mRNA was expressed in cultured fibroblasts in response to TPA, neither the mRNA nor the antigen was detected in situ in rat or mouse skin (Mark et al., 1987b, 1988; Yoon et al., 1987; Nomura et al., 1988).

The cDNA-predicted amino acid sequence of OP contains the Arg-Gly-Asp-Ser(RGDS) motif (Oldberg et al., 1986), which is found in fibronectin and other cell-adhesion proteins, and was shown to interact with cell surface receptors (Pytela et al., 1986; Tamkun et al., 1986). OP also has high affinity to hydroxyapatite, possibly due to its nine consecutive aspartic acid residues (Oldberg et al., 1986; Prince et al., 1987). Based on these features, OP was proposed to participate in cell attachment to mineralized matrix (Oldberg et al., 1986). Indeed, OP promoted the attachment of rat osteosarcoma cells and human gingiva fibroblasts to plastic dishes, and this effect was inhibited by RGD-containing peptides (Oldberg et al., 1986; Somerman et al., 1987). The physiological function of OP is not yet known. The temporal and tissue-specific OP/2ar expression seen in vivo (Yoon et al., 1987; Nomura et al., 1988) indicates that this protein is

1. *Abbreviations used in this paper:* hPTH(1-34), human parathyroid hormone (1-34); OP, osteopontin; PTH, parathyroid hormone; TGF- β , transforming growth factor type β ; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

restricted to bone, kidney, neural tissue, and placenta and suggests a functional role in these tissues.

The steady-state level of OP mRNA or OP in osteoblast-like cells is increased by 1,25-dihydroxyvitamin D₃ (Yoon et al., 1987; Prince and Butler, 1987) and is reduced by glucocorticoids (Yoon et al., 1987). 2ar mRNA in epidermal cells and fibroblasts is induced by embryonic carcinoma-derived growth factor and basic fibroblast growth factor (Nomura et al., 1988) in addition to TPA (Smith and Denhardt, 1987). We have recently shown that transforming growth factor type β (TGF- β) enhances OP gene expression through transcriptional control in the rat osteoblast-like osteosarcoma cells, ROS 17/2.8, and in newborn mouse calvaria-derived osteoblast-like cells, MC3T3E1 (Noda et al., 1988a). These observations suggest that OP/2ar expression is regulated by factors which control growth and/or differentiation.

Parathyroid hormone (PTH) has pronounced effects on the production of many extracellular matrix proteins in osteoblastic cells (Luben et al., 1976; Wong et al., 1977; Kream et al., 1980, 1986; Majeska and Rodan, 1982; Hamilton et al., 1985; Noda et al., 1988b). Moreover, target cells of PTH correspond to OP-producing cells which include proximal tubule, distal tubule, and ascending loop of Henle cells (Klahr and Hruska, 1984), in addition to osteoblasts (Rodan and Rodan, 1984). This study examined the effects of PTH on OP gene expression in the osteoblastic osteosarcoma ROS 17/2.8 cells and in primary cultures of fetal rat calvaria cells. We found that PTH inhibited OP gene expression at least in part through transcriptional control.

Materials and Methods

Human PTH (1-34) (hPTH[1-34]) was purchased from Bachem Biochemical (Torrance, CA). Porcine TGF- β was obtained from R&D Systems, Inc. (Minneapolis, MN). 1,25-dihydroxyvitamin D₃ was a kind gift from Dr. Uskokovic at Hoffman-LaRoche (Nutley, NJ). Forskolin was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Cholera toxin, isobutylmethylxanthine, (-)-isoproterenol, and 8-bromo-cAMP were purchased from Sigma Chemical Co. (St. Louis, MO). Proteinase K and vanadyl ribonucleoside complex were obtained from Bethesda Research Laboratories (Gaithersburg, MD).

Cell Culture

ROS 17/2.8 cells were grown in 9.5-, 150-, or 500-cm² Costar (Cambridge, MA) or Nunc (Roskilde, Denmark) tissue culture dishes in modified Ham's F12 medium containing 5% FBS as described previously (Majeska et al., 1985; Noda and Rodan, 1987). Fetal rat osteoblast-enriched cells (fraction 3, F3 cells) were isolated by sequential enzymatic digestion of 19 d calvaria and were grown in modified Ham's F12 media supplemented with 2% FBS and 5% horse serum as described previously (Wong and Cohn, 1974; Noda et al., 1987a). To examine the effects of PTH and other agents, the media were replaced every 24 h unless described otherwise.

Western Immunoblot Analysis

ROS 17/2.8 cells were grown to confluence in media containing 5% FBS. The cultures were then rinsed three times with PBS and were cultured in serum-free modified F12 media containing 0.1% vol/vol vehicle (0.1% acetic acid, 0.1% bovine serum albumin) or 10⁻⁷ M hPTH(1-34) for 96 h. Then the media were collected and the cells were trypsinized and counted by a Coulter counter (Coulter Electronics, Inc., Hialeah, FL). The media were centrifuged at 12,000 g for 10 min at 4°C to remove cell debris, and the supernatants were concentrated 24-fold by Centricon-10 (Amicon Corp., Danvers, MA) at 4°C according to the manufacturer's instructions. Aliquots corresponding to equal numbers of cells were incubated with 5% β -mercap-

toethanol in the electrophoresis buffer and were analyzed by 10% SDS-PAGE (Laemmli, 1970). The proteins were electrophoretically transferred to nitrocellulose filters as described previously (Brunette, 1981). The antisera against rat osteopontin were described elsewhere (Mark et al., 1987b) and were kindly provided by Drs. C. W. Prince (University of Alabama, Birmingham, AL) and W. T. Butler (University of Texas Health Sciences Center, Houston, TX). The filters were incubated with the antisera and anti-rabbit IgG goat antibody conjugated with horseradish peroxidase (Bethesda Research Laboratories) followed by incubation with 4-chloro-1-naphthol for color development according to the manufacturer's protocol (Bethesda Research Laboratories).

RNA Isolation

ROS 17/2.8 cells or fetal calvaria-derived osteoblast-enriched (F3) cells were grown to confluence in 150- or 500-cm² dishes and were treated for indicated periods of time with hPTH(1-34) or other agents. Cytoplasmic RNA was extracted as described previously (Greenberg and Ziff, 1984; Noda and Rodan, 1987). Cells (2-6 \times 10⁷) were rinsed with cold PBS, scraped in PBS, and centrifuged at 500 g at 4°C. The cell pellets were resuspended in 1 ml of NP-40 lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5% vol/vol NP-40), incubated on ice for 5 min, and then spun at 500 g at 4°C for 5 min. The supernatants were mixed with vanadyl ribonucleoside complex (final concentration 10 mM), vortexed, and spun at 12,000 g at 4°C for 10 min. The supernatants were mixed with equal volume of digestion buffer (0.2 M Tris-HCl, pH 7.4, 0.44 M NaCl, 2% SDS, 25 mM EDTA) containing proteinase K (final concentration 200 μ g/ml) and were incubated at 37°C for 60 min, followed by phenol/chloroform (1:1) extraction, and ethanol precipitation.

Northern Blot Analysis

Total RNA (10 or 20 μ g) was subjected to electrophoresis through 1% agarose-formaldehyde (0.44 M) gel and electrophoretically transferred to nylon filters (Hybond N; Amersham Corp., Arlington Heights, IL) (Thomas, 1980). Complementary DNA probes for rat osteopontin and rat β -tubulin were kindly provided by Drs. K. Yoon (Merck, Sharp, and Dohme Research Laboratories, West Point, PA) (Yoon et al., 1987) and S. R. Farmer (Boston University, Boston, MA) (Bond et al., 1984), respectively. Rat alkaline phosphatase cDNA was described previously (Noda et al., 1987b). Rat actin cDNA was purchased from Stanford University (Palo Alto, CA).

The insert of each cDNA plasmid was purified and labeled with [α -³²P]dCTP (3,000 Ci/mmol, Amersham Corp.) by using random oligonucleotide primers (Amersham Corp.) and Klenow fragments as described by Feinberg and Vogelstein (1984) to a specific activity >10⁸ cpm/ μ g. Membranes with bound RNA were irradiated for 2 min by ultraviolet light to cross-link the RNA to the filters. Then the filters were prehybridized overnight at 42°C in 50% formamide, 5 \times SSC (1 \times SSC = 0.15 M NaCl/15 mM Sodium citrate), 5 \times Denhardt's solution, and 200 μ g/ml sonicated herring sperm DNA. Hybridization was carried out for 16-24 h in fresh prehybridization buffer to which each ³²P-labeled probe was added at 10⁶ cpm/ml. Filters were washed three times in 2 \times SSC, 0.1% SDS at room temperature for 5 min each; and once in 0.1 \times SSC, 0.1% SDS at 65°C; and were exposed to x-ray films (Eastman Kodak Co., Rochester, NY) with intensifying screens.

In Vitro Transcription (Nuclear Run-on) Assay

Isolation of nuclei, in vitro transcription, and hybridization were carried out essentially as described previously (McKnight and Palmiter, 1979; Groudine et al., 1981) with minor modifications. Nuclei (2-3 \times 10⁷) were isolated by gentle homogenization of cells on ice in a buffer containing 10 mM KCl, 10 mM Tris-HCl, pH 7.4, 1.5 mM MgCl₂, 0.25% vol/vol NP-40. The isolated nuclei were incubated at 25°C for 20 min in a buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM ammonium sulfate, 1.8 mM dithiothreitol, 1.8 mM MnCl₂, 80 U RNasin, 0.3 mM each ATP, GTP, and CTP, and 100 μ Ci of [α -³²P]UTP (800 Ci/mmol, Amersham Corp.) followed by sequential digestions with DNase I and proteinase K. RNA was extracted by phenol/chloroform and was ethanol precipitated. After centrifugation at 12,000 g for 10 min, the pellets were dissolved in 6 M guanidium hydrochloride followed by addition of 0.5 vol ethanol, and were reprecipitated at -20°C overnight. After centrifugation at 12,000 g for 10 min at 4°C, the pellets were rinsed with cold 80% ethanol, dried, and resuspended in TE

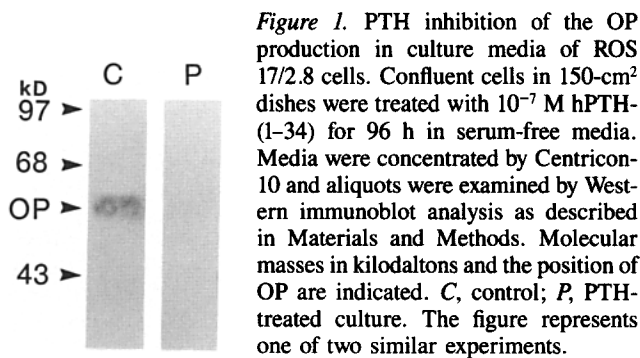


Figure 1. PTH inhibition of the OP production in culture media of ROS 17/2.8 cells. Confluent cells in 150-cm² dishes were treated with 10⁻⁷ M hPTH(1-34) for 96 h in serum-free media. Media were concentrated by Centricon-10 and aliquots were examined by Western immunoblot analysis as described in Materials and Methods. Molecular masses in kilodaltons and the position of OP are indicated. C, control; P, PTH-treated culture. The figure represents one of two similar experiments.

buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). Isolated ³²P-labeled transcripts (final concentration 2 × 10⁶ cpm/ml) were hybridized to plasmids (2 μg each) bound to nitrocellulose filter. Prehybridization (12-16 h) and hybridization (72 h) were carried out in 5× SSC, 50% formamide, 5× Denhardt's solution, 10 mM EDTA, 100 mM Tris-HCl, pH 7.4, 20 μg/ml tRNA, 10 μg/ml polyadenylic acid, 10 μg/ml sonicated herring sperm DNA, and 0.1% SDS at 42°C. Filters were washed three times in 2× SSC, 0.1% SDS at room temperature for 5 min each and once in 0.1× SSC, 0.1% SDS at 60°C for 20 min. The filters were then autoradiographed at -70°C using intensifying screens. Quantitation of the signals was carried out by densitometric scanning.

Results

The treatment of cells with 10⁻⁷ M hPTH(1-34) for 96 h greatly reduced OP levels in culture media, estimated by Western immunoblot analysis (Fig. 1). The specificity of the immunoblot analysis with the same antisera has previously been demonstrated (Noda et al., 1988a). Mobility of OP on SDS-PAGE was reported to depend on acrylamide concentration (45 kD in 15% and 75 kD in 5-15% gradient gels) (Prince et al., 1987). In 10% gels, OP migrated as a 60-kD band (Fig. 1).

hPTH(1-34) treatment also decreased the steady-state level of OP mRNA. This effect was dose-dependent between 10⁻¹¹ M and 10⁻⁸ M, which reduced OP mRNA levels by about threefold. The half-maximal effect dose (ED₅₀) was ~3 × 10⁻¹⁰ M (Fig. 2, A and B). Actin mRNA on the same filter was used as control (Fig. 2 C). The reduction of the steady-state level of OP mRNA produced by hPTH(1-34) was first detected at 24 h, reached its nadir at 48 h, and remained at that level at least up to 96 h (Fig. 3).

PTH stimulates adenylate cyclase in both osteoblast-like osteosarcoma (ROS 17/2.8) cells and primary cultures of osteoblast-enriched fetal rat calvaria cells (Majeska and Rodan, 1982; Rodan and Rodan, 1984). To test whether the PTH effect on OP mRNA was related to cAMP, ROS 17/2.8 cells were treated with 1 mM isobutylmethylxanthine, 0.3 μg/ml cholera toxin, 0.5 mM 8-bromo-cAMP, 10 μM forskolin, or 1 μM (-)-isoproterenol. Treatment with each of these agents for 48 h reduced the steady-state level of OP mRNA at least as much as 10⁻⁷ M hPTH(1-34) (Fig. 4). Isobutylmethylxanthine was the most potent inhibitor in this series. Alkaline phosphatase mRNA level was also reduced by these agents. β-Tubulin mRNA, used as control, was unchanged.

To study the mechanism for PTH regulation of OP mRNA, we examined the effect of 10⁻⁷ M hPTH(1-34) on the transcriptional rate of the OP gene by *in vitro* nuclear transcrip-

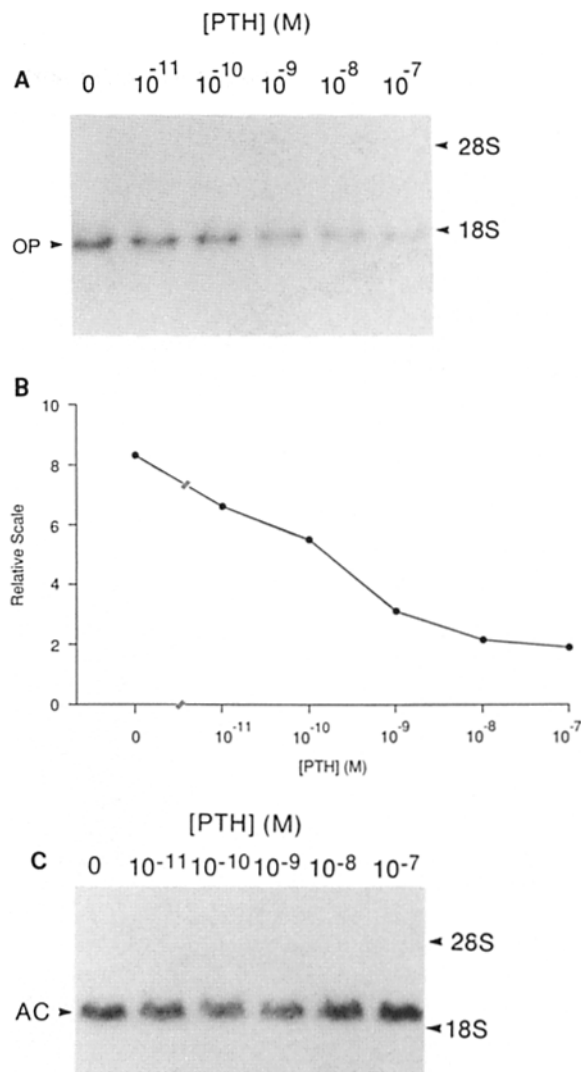


Figure 2. PTH suppression of the steady-state levels of OP mRNA. Confluent ROS 17/2.8 cells were treated with hPTH(1-34) at the indicated concentrations for 72 h. Northern blot analysis was carried out as described in Materials and Methods. The positions of OP (A), β-actin(AC) (C), 28-S and 18-S ribosomal RNA (A and C) are indicated. (A) Dose dependence of PTH effects on OP mRNA level. (B) Quantitation of OP mRNA bands in A. (C) β-actin mRNA levels on the same filter. The figures represent one of two similar experiments.

tion (run-on) assays and found it to be reduced by about threefold (Fig. 5). In the same experiment, the transcriptional rate of the β-actin gene, which served as the control, was not affected by hPTH(1-34) (Fig. 5). Treatment with 0.3 μg/ml cholera toxin also showed a similar suppression in the rate of OP gene transcription (data not shown).

Other factors which modulate OP gene expression include TGF-β which enhances the expression at least in part through transcriptional regulation (Noda et al., 1988a), dexamethasone which decreases OP mRNA levels, and 1,25-dihydroxyvitamin D₃ which also increases OP mRNA levels (Yoon et al., 1987). The combined effects of hPTH(1-34) and each of these agents on OP mRNA accumulation are shown in Fig. 6. When the cells were treated with saturation doses of

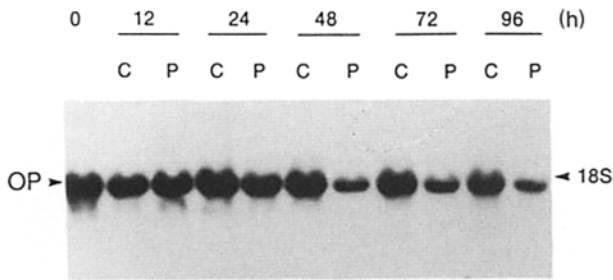


Figure 3. Time course of PTH effects on the steady-state levels of the OP mRNA. Confluent ROS 17/2.8 cells were cultured for the indicated periods of time (hours) in the absence (C) or the presence (P) of 10^{-7} M hPTH(1-34). Northern blot analysis of total RNA was carried out as described in Materials and Methods. The positions of OP and 18-S ribosomal RNA are indicated. The figure represents one of two similar experiments.

hPTH(1-34) (10^{-7} M) and TGF- β (4 ng/ml), the steady-state OP mRNA levels were similar to control. Together with a saturation dose of dexamethasone (10^{-7} M), hPTH(1-34) (10^{-7} M) caused further suppression of OP mRNA levels. 1,25-dihydroxyvitamin D₃ (10^{-8} M) increased OP mRNA about tenfold and hPTH(1-34) (10^{-7} M) partially inhibited this increase. As previously reported (Noda and Rodan, 1987), alkaline phosphatase mRNA level was increased by TGF- β and hPTH(1-34) also suppressed this effect.

PTH effect on osteopontin mRNA in osteoblast-like cells was also tested in osteoblast-enriched primary cultures of fetal rat calvaria-derived (F3) cells. Fig. 7 shows that the effect of hPTH(1-34) (10^{-7} M) on OP mRNA levels in fetal rat calvaria (F3) cells was similar to that on ROS 17/2.8 cells, indicating that PTH has this effect not only in osteoblastic osteosarcoma cells but also in nontransformed osteoblastic cells.

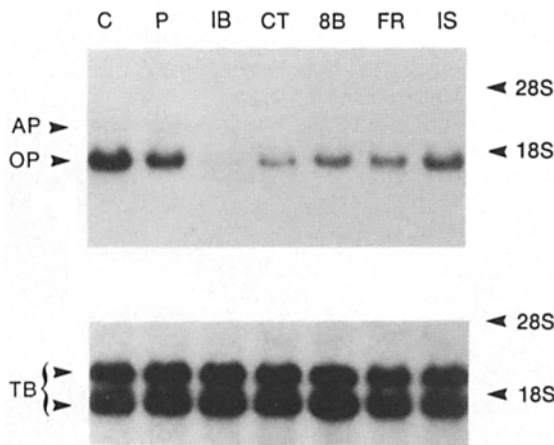


Figure 4. Effects of cAMP analog and agents which stimulate adenylate cyclase on OP mRNA accumulation. Confluent ROS 17/2.8 cells were treated for 48 h with 10^{-7} M hPTH(1-34) (P), 10^{-3} M isobutylmethylxanthine (IB), 0.3 μ g/ml cholera toxin (CT), 5×10^{-4} M 8-bromo-cAMP (8B), 5×10^{-5} M forskolin (FR), and 10^{-6} M isoproterenol (IS). Northern blot analysis of total RNA was carried out as described in Materials and Methods. Positions of OP, alkaline phosphatase (AP), β -tubulin (TB), and the 28-S and 18-S ribosomal RNA are indicated. The figure represents one of two similar experiments.

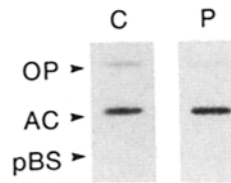


Figure 5. PTH suppression of OP gene transcription. Confluent ROS 17/2.8 cells were cultured for 36 h in the presence (P) or the absence (C) of 10^{-7} M hPTH(1-34) and an in vitro nuclear transcription (run-on) assay was carried out as described in Materials and Methods. The figure represents one of three similar experiments. AC, β -actin; pBS, plasmid vector (Bluescribe).

Discussion

The reduction in the rate of OP gene transcription by PTH indicates that PTH regulates OP expression at least in part via transcriptional control. The degree of the PTH effects on the steady-state levels of OP mRNA was similar to that on the rate of OP gene transcription. Preliminary studies also indicated that PTH had no significant effect on OP mRNA stability (data not shown). Taken together, these results suggest that transcriptional control is possibly the major regulatory pathway for PTH action on OP gene expression. PTH stimulates cAMP accumulation in ROS 17/2.8 cells, osteoblast-enriched fetal rat calvaria cells, and most target cells. The effects of cAMP-elevating agents and cAMP analogue on OP mRNA were very similar to those of PTH, suggesting the possible involvement of cAMP in this PTH action on OP expression. cAMP mediation has previously been implicated in the transcriptional control of genes encoding human proencephalin, human prolactin, corticotropin-releasing factor, and somatostatin (Cooke et al., 1981; Shibahara et al., 1983; Shen and Rutter, 1984; Comb et al., 1986). These genes contain short homologous *cis*-regulatory elements in their 5' flanking regions, the deletion of which abolishes modulation by cAMP (Comb et al., 1986). The data presented here suggest the presence of cAMP-responsive negative control element(s) in the regulatory region of the OP gene. *cis*-Regulatory elements which share the structure of the consensus sequence have been shown to mediate both positive and negative effects of glucocorticoid. The pathway(s) leading

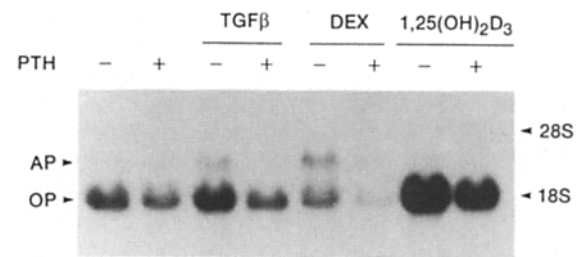


Figure 6. Modulation by hPTH(1-34) (10^{-7} M) of TGF- β 1 (4 ng/ml), dexamethasone (DEX) (10^{-7} M), and 1,25-dihydroxyvitamin D₃ (10^{-8} M); effects on the steady-state levels of OP mRNA. Confluent ROS 17/2.8 cells were treated for 48 h with combinations of hPTH(1-34) and other agents as shown in the figure. - and + indicate the absence and the presence of hPTH(1-34), respectively. Northern blot analysis was carried out as described in Materials and Methods. The figure represents one of two similar experiments. The positions of OP alkaline phosphatase (AP), and the 28-S and 18-S ribosomal RNA are indicated.

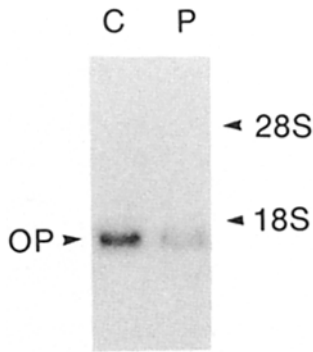


Figure 7. PTH suppression of the steady-state level of OP mRNA in fetal rat calvaria cells. Cells were prepared from 19 d fetus as described in Materials and Methods. Confluent cells were cultured for 48 h in the presence (P) or the absence (C) of 10^{-7} M hPTH(1-34). Northern blot analysis was carried out as described in Materials and Methods. The figure represents one of two similar experiments. The position of OP, and the 28-S and 18-S ribosomal RNA are indicated.

from cAMP elevation to gene regulation have not been elucidated. The *cis*-regulatory elements presumably interact with proteinaceous factors (Maniatis et al., 1987) which could be modulated by protein kinase A phosphorylation. A similar scheme was proposed for the regulation of collagenase gene promoter by TPA via protein kinase C phosphorylation of AP-1 (Angel et al., 1987).

In various cell types OP/2ar mRNA expression is stimulated by several other agents which modulate cell growth, including steroid hormone (Yoon et al., 1987; Prince and Butler, 1987) and peptide growth factors (Smith and Denhardt, 1987; Nomura et al., 1988; Noda et al., 1988a). The inhibition of OP expression by PTH in osteoblastic cells is probably not related to modulation of proliferation but rather to the effect of this hormone on phenotype-related proteins in these cells.

Inhibition of OP gene expression in osteoblast-like cells by PTH is consistent with the known catabolic effects of this hormone, which include inhibition of collagen (Luben et al., 1976; Kream et al., 1980, 1986) and of alkaline phosphatase (Luben et al., 1976; Wong et al., 1977; Majeska and Rodan, 1982) synthesis. The similar response of OP to PTH strengthens the relationship of this novel protein to the osteoblastic phenotype. Certain osteoblastic products such as plasminogen activator (Hamilton et al., 1985) and collagenase (Sakamoto and Sakamoto, 1986) are increased by PTH. However, these proteins may be involved in bone resorption and may participate in the catabolic effects of this hormone (Luben et al., 1976; Wong et al., 1977; Kream et al., 1980, 1986; Majeska and Rodan, 1982). In contrast, TGF- β , a possible anabolic agent on bone, enhances the expression of collagen (Centrella et al., 1987; Canalis et al., 1988), alkaline phosphatase (Harrod et al., 1985; Noda and Rodan, 1987; Pfeilshifter et al., 1987), and osteopontin (Noda et al., 1988a) in osteoblastic cells, thus having opposite effects to those of PTH. The TGF- β effects are probably not mediated by cAMP (Insogna et al., 1987). Further study will be necessary to elucidate the molecular details of these regulatory pathways.

Extrapolation of these findings to the *in vivo* situation remains to be established. The expression of OP and OP mRNA have been consistently observed in osteoblasts and bones *in vivo* in both rat (Yoon et al., 1987; Mark et al.,

1987a,b, 1988) and mouse (Nomura et al., 1988). This study suggests that OP expression would be reduced by PTH as part of its catabolic effects on bone.

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References

- Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin. 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated transacting factor. *Cell*. 49:729-739.
- Bond, J. F., G. S. Robinson, and S. R. Farmer. 1984. Differential expression of two neural cell specific beta-tubulin mRNAs during rat brain development. *Mol. Cell. Biol.* 4:1313-1319.
- Burnette, W. N. 1981. Western blotting: electrophoretic transfer of proteins from sodium-dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195-203.
- Canalis, E., T. McCarthy, and M. Centrella. 1988. Growth factors and the regulation of bone remodeling. *J. Clin. Invest.* 81:277-281.
- Centrella, M., T. L. McCarthy, and E. Canalis. 1987. Transforming growth factor beta is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. *J. Biol. Chem.* 262:2869-2874.
- Comb, M., N. C. Birnberg, A. Seasholtz, E. Herbert, and H. M. Goodman. 1986. A cyclic AMP- and phorbol ester-inducible DNA element. *Nature (Lond.)*. 323:353-356.
- Cooke, N. E., D. Coit, J. Shine, J. D. Baxter, and J. A. Martial. 1981. Human prolactin-cDNA structural analysis and evolutionary comparisons. *J. Biol. Chem.* 256:4007-4016.
- Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266-267.
- Franzen, A., and D. Heinegard. 1985. Isolation and characterization of two sialoproteins present only in bone calcified matrix. *Biochem. J.* 232:715-724.
- Greenberg, M. E., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the *c-fos* proto-oncogene. *Nature (Lond.)*. 311:433-438.
- Groudine, M., M. Peretz, and H. Weintraub. 1981. Transcriptional regulation of hemoglobin switching in chicken embryo. *Mol. Cell. Biol.* 1:281-288.
- Hamilton, J. A., S. R. Lingelbach, N. C. Partridge, and T. J. Martin. 1985. Regulation of plasminogen activator production by bone-resorbing hormones in normal and malignant osteoblasts. *Endocrinology*. 116:2186-2191.
- Harrod, J., D. J. Hill, and R. G. G. Russel. 1985. Effect of transforming growth factor β on osteoblast-like cells. *Calcif. Tissue Int.* 38(Suppl.):16s.
- Insogna, K., E. Weir, T. McCarthy, E. Burres, A. Broadus, and M. Centrella. 1987. Highly purified adenylate cyclase-stimulating activity from tumors associated with humoral hypercalcemia of malignancy is mitogenic in primary bone cell culture. *J. Bone Miner. Res.* 2(Suppl):88.
- Klahr, S., and K. Hruska. 1984. Effects of parathyroid hormone on the renal resorption of phosphorus and divalent cations. In *Bone and Mineral Research*. Vol. 2. W. Peck, editor. Elsevier Scientific Publishing Co., Amsterdam. 65-124.
- Kream, B. E., D. Rowe, S. C. Gworek, and L. G. Raisz. 1980. Parathyroid hormone alters collagen synthesis and procollagen mRNA levels in fetal rat calvaria. *Proc. Natl. Acad. Sci. USA*. 77:5654-5658.
- Kream, B. E., D. Rowe, M. D. Smith, V. Maher, and R. J. Majeska. 1986. Hormonal regulation of collagen synthesis in a clonal rat osteosarcoma cell line. *Endocrinology*. 120:1922-1928.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature (Lond.)*. 227:580-585.
- Luben, R. A., G. L. Wong, and D. V. Cohn. 1976. Biochemical characterization with parathormone and calcitonin of isolated bone cells: provisional identification of osteoclasts and osteoblasts. *Endocrinology*. 99:526-534.
- Majeska, R. J., and G. A. Rodan. 1982. Alkaline phosphatase inhibition by parathyroid hormone and isoproterenol in a clonal rat osteosarcoma cell line. Possible mediation by cyclic AMP. *Calcif. Tissue Int.* 34:59-66.
- Majeska, R. J., B. C. Nair, and G. A. Rodan. 1985. Glucocorticoids regulation of alkaline phosphatase in rat osteoblastic osteosarcoma cell line ROS 17/2.8. *Endocrinology*. 116:170-179.
- Maniatis, T., S. Goodburn, and J. A. Fischer. 1987. Regulation of inducible and tissue specific gene expression. *Science (Wash. DC)*. 236:1237-1245.

- Mark, M. P., C. W. Prince, S. Gay, R. L. Austin, M. Bhowan, R. D. Finkelman, and W. T. Butler. 1987a. A comparative immunocytochemical study on the subcellular distribution of 44kDa bone phosphoprotein and bone gamma-carboxyglutamic acid (Gla)-containing protein in osteoblasts. *J. Bone Miner. Res.* 2:337-346.
- Mark, M. P., C. W. Prince, S. Gay, R. L. Austin, and W. T. Butler. 1988. 44 KDalton bone phosphoprotein antigenicity at ectopic sites in newborn rats: kidney and nervous tissues. *Cell Tissue Res.* 251:23-30.
- Mark, M. P., C. W. Prince, T. Oosawa, S. Gay, A. L. J. J. Bronckers, and W. T. Butler. 1987b. Immunohistochemical demonstration of a 44-KD phosphoprotein in developing rat bones. *J. Histochem. Cytochem.* 35:707-716.
- McKnight, G. S., and R. D. Palmiter. 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormone in chick oviduct. *J. Biol. Chem.* 254:9050-9058.
- Noda, M., and G. A. Rodan. 1987. Type beta transforming growth factor (TGFbeta) regulation of alkaline phosphatase expression and other phenotype-related mRNA's in osteoblastic rat osteosarcoma cells. *J. Cell. Physiol.* 133:426-437.
- Noda, M., K. Yoon, C. W. Prince, W. T. Butler, and G. A. Rodan. 1988a. Transcriptional regulation of osteopontin production in rat osteosarcoma cells by type beta transforming growth factor. *J. Biol. Chem.* 263:13916-13921.
- Noda, M., K. Yoon, G. A. Rodan, and D. E. Koppel. 1987a. High lateral mobility of endogenous and transfected alkaline phosphatase: a phosphatidylinositol-anchored membrane protein. *J. Cell Biol.* 105:1671-1677.
- Noda, M., K. Yoon, and G. A. Rodan. 1988b. Cyclic AMP-mediated stabilization of osteocalcin mRNA in rat osteoblast-like cells treated with parathyroid hormone. *J. Biol. Chem.* 263:18574-18577.
- Noda, M., K. Yoon, M. Thiede, R. Buenaga, M. Weiss, P. Henthorn, H. Harris, and G. A. Rodan. 1987b. cDNA cloning of alkaline phosphatase from rat osteosarcoma (ROS 17/2.8) cells. *J. Bone Miner. Res.* 2:161-164.
- Nomura, S., A. J. Wills, D. R. Edwards, J. K. Heath, and B. L. M. Hogan. 1988. Developmental expression of 2ar (osteopontin) and SPARC (osteonection) RNA as revealed by in situ hybridization. *J. Cell Biol.* 106:441-450.
- Oldberg, A., A. Franzen, and D. Heinegard. 1986. Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Glu-Asp cell-binding sequence. *Proc. Natl. Acad. Sci. USA.* 83:8819-8823.
- Pfeilshifter, J., S. M. D'Sausa, and G. R. Mundy. 1987. Effects of transforming growth factor-beta on osteoblastic osteosarcoma cells. *Endocrinology.* 121:212-218.
- Prince, C. W., and W. T. Butler. 1987. 1,25-dihydroxyvitamin D₃ regulates the biosynthesis of osteopontin, a bone derived cell attachment protein, in clonal osteoblast-like osteosarcoma cells. *Collagen Relat. Res.* 7:305-313.
- Prince, C. W., T. Oosawa, W. T. Butler, M. Tomana, A. S. Bhowan, M. Bhowan, and R. E. Schrohenloher. 1987. Isolation, characterization and biosynthesis of a phosphorylated glycoprotein from rat bone. *J. Biol. Chem.* 262:2900-2907.
- Pytela, R., M. D. Rierschbacher, M. H. Ginsberg, E. F. Plow, and E. Rouslahti. 1986. Platelet membrane glycoprotein IIB/IIIa: member of a family of ARG-GLY-ASP specific adhesion receptors. *Science (Wash. DC).* 231:1559-1562.
- Rodan, G. A., and S. B. Rodan. 1984. Expression of the osteoblastic phenotype. *Bone Miner. Res.* 2:244-285.
- Sakamoto, S., and M. Sakamoto. 1986. Bone collagenase, osteoblasts and cell-mediated bone resorption. *Bone Miner. Res.* 4:49-102.
- Shen, L. P., and W. J. Rutter. 1984. Sequence of the human somatostatin I gene. *Science (Wash. DC).* 224:168-171.
- Shibahara, S., Y. Morimoto, Y. Furutani, M. Notake, H. Takahashi, S. Shimizu, S. Horikawa, and S. Numa. 1983. Isolation and sequence analysis of the human corticotropin-releasing factor precursor genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:775-779.
- Smith, J. H., and D. T. Denhardt. 1987. Molecular cloning of a tumor promoter-inducible mRNA found in JB4. Mouse epidermal cells: induction is stable at high but not low cell densities. *J. Cell Biochem.* 34:13-22.
- Somerman, M., C. W. Prince, J. J. Sauk, R. A. Foster, and W. T. Butler. 1987. Mechanism of fibroblast attachment to bone extracellular matrix: role of a 44 kilodalton bone phosphoprotein. *J. Bone Miner. Res.* 3:259-265.
- Tamkun, J. W., D. W. DeSimone, D. Fonda, R. S. Patel, C. Buck, A. F. Horowitz, and R. D. Hynes. 1986. Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. *Cell.* 46:271-282.
- Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201-5205.
- Wong, G. L., and D. V. Cohn. 1974. Separation of parathyroid hormone and calcitonin-sensitive cells from non-responsive bone cells. *Nature (Lond.).* 242:713-715.
- Wong, G. L., R. A. Luben, and D. V. Cohn. 1977. 1,25-dihydroxycholecalciferol and parathyroid hormone: effects on isolated osteoclast-like and osteoblast-like cells. *Science (Wash. DC).* 197:663-665.
- Yoon, K., R. Buenaga, and G. A. Rodan. 1987. Tissue specificity and developmental expression of rat osteopontin. *Biochem. Biophys. Res. Commun.* 148:1129-1136.