Signal Transduction Pathways Mediating Parathyroid Hormone Stimulation of Bone Sialoprotein Gene Expression in Osteoblasts*

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Bone sialoprotein is a major noncollagenous protein of bone. Parathyroid hormone (PTH) was shown to cause a 2–4-fold increase in the steady-state levels of bone sialoprotein mRNAs within primary cultures of embryonic osteoblasts. The induction could be mimicked by both forskolin and phorbol 12-myristate 13-acetate and was not inhibited by cycloheximide. Transient expression of a ~1200-base pair avian bsp promoter-reporter construct demonstrated similar inductions as mRNA levels. Co-transfection of an expression plasmid encoding heat-stable inhibitor of cAMP-dependent protein kinase, a peptide inhibitor of PKA, decreased both the basal and PTH-induced bsp transcription, while co-expression of the catalytic subunit of PKA-induced bsp expression 3-fold. Protein kinase C activation, on the other hand, did not appear to work through its activation of c-fos, since co-transfection of an expression clone for c-fos had no effect. Interestingly, heat-stable inhibitor of cAMP-dependent protein kinase also inhibited the phorbol 12-myristate 13-acetate induction, suggesting that the protein kinase C acts through some form of interaction with the cAMP/PKA pathway. A half-cAMP response element site in the bsp promoter was identified as the cis-acting element that mediated the PTH response by the transient transfections with reporter constructs containing nested deletions of the promoter or a heterologous promoter containing the cAMP response element. In conclusion, these data indicate that PTH stimulation of bsp gene expression is specific to osteoblasts and mediated by changing cellular cAMP/PKA levels. They further suggest that although protein kinase C is capable of stimulating the gene by itself, it plays a minimal role in mediating the PTH induction of bone sialoprotein.

PTH,† like a number of other peptide hormones, mediates its effects through interaction with its receptor, which modulates its activities through specific G proteins that activate or inhibit adenylyl cyclase production of cAMP. The levels of cAMP then control the activity of protein kinase A (PKA), which serves as the cAMP intracellular second signal transducer (1–3). There is now considerable evidence that the interaction of PTH with its receptor also leads to the activation of phospholipase C, which in turn causes intracellular calcium ion and diacylglycerol release and further activates members of the protein kinase C (PKC) family (4–7). Thus, both PKA and PKC may be activated by PTH, and at the nuclear level the actions of both PKA and PKC families of kinases through the phosphorylation of specific members of the leucine zipper family of transcription factors affect the transcriptional activity of specific genes. These transcription factors fall primarily into two broad classes, the cAMP response element binding protein family or CREB, which includes a variety of members of the CRE, CREM, and ATF classes of factors, and members of the AP1 family, whose primary members include fos, jun, and fra (8–11). In general, the action of PTK is mediated through the phosphorylation of members of the CREB family, while PKC appears to act on members of the AP1 family; however, phosphorylation may not be restricted to individual members of these families of factors or one type of kinase. These factors are active in dimeric form and members of both of these larger families share the ability of specific heterodimerization with each other (8, 11). The selective heterodimerization between members of both families of factors or between members within a given family leads to the diversity of actions by which different genes may be regulated in a tissue-specific fashion in response to common signals (12, 13). At the DNA level, the cis elements recognized by both families of transcription factors are also similar in that the palindromic sequences share common sequence features (12, 13). The consensus for the CREB family or cAMP response element (CRE) sequence is GTGACGTCA, while the AP1 family of cis elements or phorbol ester response elements (TRE) is TGACTCA (8, 11). There is considerable promiscuity in the recognition of these sequences in the sense that combinations of factors from either family can interact with these consensus sequences, thus diversity can be generated by selective interaction with given cis elements by various members of both families of transcription factor proteins. Within the CREB family there is also the capacity for half-sites of the palindrome to interact with the CREB family of transcription factors (14).

The role of PTHrP (parathyroid hormone-related peptide or hypercalcemia of malignancy factor) and/or PTH in controlling the embryonic development of skeletal tissues has been demonstrated by recent studies showing that the ablation of PTHrP gene leads to a postnatal lethal osteochondrodysplasia (15). An extensive amount of data has been accumulated to suggest that the leucine zipper family of transcription factors plays major
roles in the normal regulation of skeletal specific gene expression and skeletal tissue development. Initial studies in which the c-fos gene was introduced transgenically demonstrated that expression of c-fos within these animals generated osteosarcomas (16, 17). More recent data showed that the ablation of the normal expression of c-fos in transgenic animals led to animals having an osteochondrodysplasia (18). Unlike the PTHR-P-ablated animals, which had a premature replacement of the cartilage, c-fos ablation led to an overproduction of hypertrophic cartilage tissues and a failure of bone replacement, producing a conditions that appeared more like osteopetrosis (18, 19). Finally, there have been several studies that have shown an increased expression of c-fos in bone from patients with fibrous dysplasia, in which there is an overexpression or ectopic expression of bone formation (20). In a number of recent studies, members of the basic leucine zipper protein family, including c-fos (21–23), hXBP (24), and ATF2 (24) have been shown to be expressed selectively during normal skeletal tissue development. Finally, in recent studies, the ablation of one of these factors, ATF2, led to a defect in endochondral ossification, which has a similar histopathology to human hypochondrodysplasia (24). Thus these data suggest that the control of specific members of this large family of transcription factors has specific effects on skeletal development and tissue function.

In previous studies the avian form of the bone sialoprotein gene (bsp), an extracellular matrix gene that is expressed almost exclusively by skeletal cells (25–27), was shown to be stimulated transcriptionally 3–6-fold by PTH treatment (28). The studies presented here define the molecular mechanisms of signal transduction by which PTH mediates its effects on bone sialoprotein gene expression. These results indicate that PTH appears to directly regulate this gene through mainly the PKA side of the signal transduction pathway, and data based on the deletion and heterologous promoter studies suggest that the induction is mediated through a half-CRE site in this gene.

MATERIALS AND METHODS

Materials—All tissue culture supplies, forskolin, cycloheximide, and ionomycin were from Sigma. Phorbol 12-myristate 13-acetate (PMA) was from Sigma or Peninsula Laboratories, Inc., Belmont, CA. Bovine PTH-(1–34) and PTH-(2–34) were from LCLaboratories, Woburn, MA. Bovine PTH-(1–34) and PTH-(2–34) were 24 h after transfection. Drug and hormone doses were 10^−6 M for forskolin, 10^−6 M for PMA, 10^−7 M for cycloheximide, and 10^−8 M for ionomycin. All assays were performed in duplicate. A CAT assay was performed on equal aliquots of samples. CAT activity was assayed by liquid scintillation counting (42). Reactions were then counted at 5–10-min increments over a 2-h period. The counts per minute for each sample were plotted against time, and the slope of linear portion of the curve that was representative of the CAT activity was calculated. The final enzyme activities were expressed as counts per minute of converted [14C]chloramphenicol/min/μg of protein. CAT assay values were the averages of at least duplicate measurements from three separate transfections of each preparation of cells. All results presented represent the averages of assays from at least three separate preparations of cells.

RESULTS

Effect of PTH and Second Messenger Analogs on BSP mRNA Levels—Since activation of the PTH receptor triggers both adenylate cyclase and phospholipase C (6, 7), the potential exists that one or several known signal transduction pathways including PKA, PKC, or Ca^2+ transients could mediate a response to PTH. Initial experiments were directed at defining which of these transduction pathway(s) were used in the induction of the bsp gene expression and whether the bsp gene induction was transcriptionally controlled as a primary or secondary event in response to the signal transduction. These questions were addressed by first examining the temporal induction of the mRNA levels of Endo-stable RNA from primary calvaria osteoblasts over an 8-h period in response to PTH(1–34), PMA, which activates PKC, and forskolin, which directly activates adenylate cyclase (Fig. 1A–C). PTH increased...
Since forskolin and PMA mediated their effects primarily through either PKA or PKC, respectively, these results suggest that both the PKA and PKC arms of the signal transduction pathway may activate bsp gene expression. However, these experiments do not address whether the activation is at the transcription level or whether both PKA and PKC activation are necessary for the induction of the bsp gene.

The rapid increase (within 30 min) of the BSP mRNA levels after the addition of the various compounds, however, suggested that the induction was a primary response. In order to test whether the signal transduction process was independent of protein synthesis, cycloheximide was used to inhibit new protein translation. In these studies 50 μmol of cycloheximide was added to the cultures concurrently with the PTH, forskolin, or PMA. A similar fold of induction of BSP mRNA was observed despite the inhibition of translation, indicating that the stimulation was independent of protein synthesis (Fig. 1D).

**PTH Specifically Regulates Transcription of the bsp Gene—**
The regulation of bsp by PTH at the transcription level was examined using a bsp promoter construct containing −1.2 kilobases of the 5′ bsp promoter (pCAT-1239) which was transiently transfected into primary osteoblasts. In the first experiment, the specificity of the promoter activity in response to the activation of the PTH receptor was examined by treating the osteoblast cultures with either PTH-(1–34) or PTH-(3–34) peptides. While PTH-(1–34) increased the promoter activity by 3-fold, PTH-(3–34), the nonactive form of the PTH hormone that binds to the receptor but does not lead to receptor activation, decreased the bsp expression by 30% (Fig. 2A).

In the experiments depicted in Fig. 2B the tissue specificity of the bsp response to PTH was examined by comparing the actions of PTH on the bsp promoter activity in several different primary cell populations: osteoblasts prepared from 17-day embryonic calvaria, which are at a very mature state of differentiation; osteoblasts prepared from 12-day embryonic calvaria, which are at a very immature stage of differentiation (31); and embryonic skin fibroblasts, a nonskeletal cell. As shown in Fig. 2B, the PTH induction of the gene is specific to the fully differentiated osteoblasts, despite the fact that all the cell types tested contained PTH receptor. Furthermore, the specificity of PTH on the bsp promoter activity was examined by comparing the actions of PTH on the activity of a plasmid construct containing the promoter of opn, another extracellular matrix gene that is highly expressed by cells within the skeletal lineage. In comparing the relative activities of the opn to bsp gene promoters, the opn promoter was shown to be severalfold more active than the bsp promoter in 17-day embryonic osteoblasts. However, PTH had a moderately inhibitory effect on the opn promoter activity, indicating that PTH is not a general transcriptional activator in osteoblasts but is specific to the bsp promoter.

**Effects of Second Messenger Analogs Forskolin, PMA, and Ionomycin on bsp Transcription—**The results shown in Fig. 1 suggest that bsp transcriptional activation may be mediated by several different signal transduction pathways. A further examination of the transcriptional activation by these different pathways was carried out with the bsp promoter construct. Forskolin, PMA, and ionomycin were used to mimic the activation of PKA, PKC, and Ca2⁺ transients, respectively, in the primary osteoblasts, and the actions of these component parts of the signal transduction cascade on the transcriptional activation of the bsp promoter activity was examined (Fig. 3). Results similar to those of the Northern blot analysis depicted in Fig. 1 were obtained. Once again forskolin had the largest effect, generating a 4-fold increase in the bsp promoter activity.

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mediating the PTH transcriptional activation of the bsp, the high affinity peptide inhibitor of the catalytic subunit of PKA (PKI) was used to block the actions of PKA. An expression clone encoding PKI and a mutant form of PKI (PKImut) that is biologically inactive were co-transfected with the bsp promoter/cat construct (Fig. 4A). The transfection of PKI alone diminished the basal level of the bsp promoter activity and completely inhibited PTH and forskolin induction of the bsp promoter. The specificity of the PKI was further demonstrated, since the control transfections with the PKImut showed no effect on the stimulation of bsp in the presence of either PTH and forskolin. To further demonstrate that the actions of the PTH were indeed mediated through the activation of PKA, co-transfection experiments were carried out with an expression vector containing the catalytic subunit of PKA (Fig. 4B). In these experiments, expression of the PKA activity alone increased the bsp promoter activity to a similar level as was seen for the PTH stimulation.

Role of PKC in BSP Regulation—Although the experiments shown in Fig. 1 indicate that the activation of bsp transcription by PMA is a primary effect, PMA has been shown to induce c-fos in many cells (43). It has been reported that PTH is indeed capable of inducing c-fos in rat osteoblasts in vivo (22) and in UMR 106 osteosarcoma cells (44). To test whether c-fos plays a role in PTH activation of bsp gene, an expression vector containing c-fos was co-transfected with the bsp/cat plasmid (Fig. 5A). In these experiments overexpression of c-fos had no detectable effect on bsp gene expression, thus ruling out that an indirect effect through the activation of c-fos was mediating the transcriptional activation of the bsp gene. Considering the fact that PMA did increase the transcription of bsp and that the gene was regulated by cAMP/PKA level, this would suggest that PKC may activate bsp through some form of interaction with the PMA pathway. To test this possibility, primary osteoblasts were co-transfected with the bsp/cat and PKI expression plasmid and were treated with PMA. In these experiments the actions of PKI on the PMA activation of the bsp promoter activity were tested (Fig. 5B). Surprisingly, PKI also inhibited the PMA induction of the bsp promoter. To further test the specificity of the PKI activity on inhibiting PKA rather than on PKC or AP1 activity, its actions on the PMA induction of the

Fig. 2. Specificity of the transcriptional activation of bsp by PTH. A, relative activity of the bsp promoter/cat construct pCAT-1239 in the presence of 10^-8 M PTH-(1–34) and PTH-(3–34) peptide was measured in 17-day embryonic osteoblasts. Promoter activities in these experiments are expressed as a ratio to the control. B, relative basal activity and response of bsp promoter/reporter construct pCAT-1239 to 10^-8 M PTH-(1–34) treatment was determined in differentiated mature osteoblasts prepared from day 17 embryonic chicken calvaria, immature less differentiated osteoblasts prepared from day 12 embryo chicken calvaria, and skin fibroblasts prepared from day 12 embryo dermal tissue, which were used as a nonskeletal cell type. PTH specificity on the bsp promoter was examined by comparison of the actions of 10^-8 M PTH on the transcriptional activity of the avian osteopontin promoter within 17-day embryonic chicken calvaria osteoblast. Because different constructs and cell types were used in these studies, in order that direct comparisons of promoter activities could be made, the promoter activities were normalized as a relative value to the activity of the SV40 promoter which was measured in the same experiments and was set at 100%. Error bars, total range in variation seen in the relative values in three separate experiments.

Fig. 3. Effect of PTH second messenger analogs in bsp transcription. Relative activity of the bsp promoter/cat construct pCAT-1239 in the presence of various second messenger analogs was determined 24 h after the addition of the compounds. Activities are expressed relative to the control (Ct), which is set as 1. FS, forskolin; PMA, phorbol 12-myristate 13-acetate; Iono, ionomycin. Error bars, total range in variation seen in the relative values in three separate experiments.
opn promoter, which contains an AP1 element, was tested in skin fibroblasts. In these experiments PMA produced a moderate induction (2.0-fold) of the opn promoter, and the co-transfection of the PKI had no inhibitory effect on this induction (Fig. 5B). It is also interesting to note that co-expression of the c-fos with the opn promoter/cat construct did stimulate the expression of this promoter. These results were consistent with those of Basudev et al. (45), in which they reported PKI had no significant effect on Langerhans islet PKC activity in response to PMA. These data provided strong evidence that the PKC arm of the second messenger system activated the bsp transcription through an interaction with the PKA pathway.

Analysis of the PTH-responsive cis Elements in bsp Promoter—A series of nested deletions from the 5' end of the pCAT-1239 construct were used to identify the cis element(s) that was responsive to PTH. Plasmids were transfected into 17-day embryonic primary osteoblasts, followed by PTH, forskolin, and PMA treatment. Promoter activities for each of the constructs are depicted in Fig. 6A. The longest construct displayed the highest induction with PTH and forskolin, while all the other deletions showed only 1.5–2.0-fold increases with these compounds. In contrast, PMA activation of the various deletions revealed a pattern different from either forskolin or PTH, showing the greatest activation for the pCAT-620 construct, while longer or shorter promoter constructs had a lesser response to PMA stimulation. These results suggest that PTH uses the same cis element as forskolin, while the actions of the PMA are via a different cis element within the first 620 nt 5' to the transcriptional start site.

Sequence analysis of the bsp promoter identified a perfect half-CRE site in an inverted orientation at −1226 to −1230 nt, suggesting that this sequence is of primary functional consequence in the activation of the gene by PKA. Since the PMA response element is not co-localized with this CRE site, the interaction of the PMA element with the PKA pathway was further examined in the experiments depicted in Fig. 6B. Plasmid pCAT-620 that had the largest PMA stimulation was co-expressed with PKI. Contrary to the results for the pCAT-1239, PMA induction was no longer affected by the inhibition of PKA, suggesting that the cross-talk between PKC and PKA occurs at the level of transcription factor interaction, and the regulation of PMA is directly or indirectly under the control of the upstream CRE site.

To further test the involvement of the half-CRE site in the PTH regulation of the bsp, a heterologous promoter construct containing either forward or reverse orientation of the half-CRE element was placed in a position distal to the basal elements of the tk promoter and was transfected into the primary

FIG. 4. Role of PKA in the signal transduction of the induction for the bsp gene. A, relative activities of the bsp promoter pCAT-1239 in response to PTH, PMA, or forskolin in the presence of co-transfected expression vectors containing either the PKI or an inactive mutated form of the PKI (Mut). B, relative activity of the bsp promoter pCAT-1239 in response to either PTH addition or in the presence of the co-transfected expression vector containing the PKA catalytic subunit. Values are expressed relative to the control. Error bars, total range in variation seen in the relative values in three separate experiments.

FIG. 5. Role of PKC and c-fos in the signal transduction of the induction of bsp gene. A, comparison of the effects of PTH and c-fos on the bsp promoter activity. The c-fos expression clone was co-transfected with the bsp promoter/cat clone pCAT-1239. Parallel experiments with the bsp promoter construct in the presence of PTH additions were carried out with the same preparations of cells. Values are expressed relative to the control. B, comparison of the effect of PKI on the PMA induction of either the opn promoter or bsp promoter activities. The relative activities of the bsp promoter pCAT-1239 in osteoblasts and the opn promoter in skin fibroblasts were measured in the presence of co-transfected PKI and PKI (Mut). Values represent the ratios of PMA treatment to control. Error bar, total range in variation seen in the relative values in two separate triplicate experiments.

3 T. Uporova and L. C. Gerstenfeld, unpublished data.
In the studies presented here, the bsp gene was demonstrated to be transcriptionally up-regulated in response to PTH within primary cultures of embryonic chicken calvaria osteoblasts. The increased expression of bsp gene in the avian primary osteoblast culture system is consistent with the increased synthesis seen for the expression of BSP protein when the rat osteoblast-like UMR 106 cell line was treated with PTH (46). In the studies performed in the UMR 106 cells, the increase in BSP protein levels was modest (~25%); however, the protein levels were only indirectly assessed by measuring the 35S- and 3H-labeled glucosamine contents of BSP, and since no direct measurement of the BSP core protein or the BSP mRNAs were examined, the actual levels of new gene expression may have been much higher in these studies. The biological function of increased bsp expression in response to PTH can only be speculated, but the anabolic effect of low or intermittent doses of PTH on bone (47–49) would be consistent with the hypothesis that the BSP protein plays an important role in initiating or spatially directing mineral deposition during skeletal tissue formation (50).

While previous studies have demonstrated that the actions of PTH on specific genes in osteoblasts are mediated at the transcription level (51–53), the mechanism(s) of signal transduction by which PTH affects the transcription of a given gene within osteoblasts is still poorly understood. The present studies provide a detailed analysis of the signal transduction mechanism that controls the PTH activation of the bsp gene. Experiments presented here showed that PTH stimulation is not additive with forskolin and is completely inhibited by co-transfection of PKI, indicating the activation is mediated through the cAMP/PKA pathway. The identification of the PTH responsive half-CRE element within the promoter further suggests that a CREB-like transcription factor might be involved in the PKA mediation of the bsp activation. The inhibition of the basal level of bsp transcription by PKI indicates that the bsp transcription is coupled to the cellular cAMP/PKA levels. These results suggest that in osteoblasts bsp gene expression may be modulated by a wide variety of factors that alter the cellular cAMP levels. The control of the basal level of gene expression through maintenance of cAMP-mediated phosphorylation of specific transcription factors has been implicated for several cAMP-regulated genes in other cell types (54, 55). One observation which supports the hypothesis that bsp expression is controlled by the cellular levels of cAMP is that PTH-(3–34), a PTH antagonist, inhibited bsp gene expression (Fig. 2A). It is interesting to speculate, therefore, that in primary osteoblast cultures, the synthesis of PTHrP may serve as an autocrine or paracrine factor by which these cells maintain their basal cellular cAMP concentrations and thus bsp transcription at a certain level. Such a mechanism would be consistent with the inhibition of the basal level of bsp transcription by PTH-(3–34), since this peptide would compete with any endogenous peptide which activates the PTH receptor. The bsp gene transcription was shown to be activated as a primary response to PTH stimulation based on the absence of a requirement for new protein synthesis. These results suggest that bsp is activated through phosphorylation of a CREB or ATF transcription factor, and these activated factors interact with the half-CRE site within the bsp promoter, which appears to impart most of the inductive effect of PTH. This mechanism is, however, in contrast to...
the activation of the collagenase gene by PTH, which does require new protein synthesis (53).

It is interesting to note that the bsp, c-fos, and c-jun genes were all capable of being induced by PMA, suggesting that the PTH induction of all of these genes also involves the PKC pathway. This in and of itself is not surprising, since PTH, besides activating adenylate cyclase, also mediates its actions through phospholipase C, which causes an increased production of diacylglycerol, which in turn activates PKC (1). However, data presented here demonstrate that PKC by itself played a minimal role in the PTH activation of bsp. The additive effects of PTH and PMA together in activating bsp transcription suggest that the activation of PKC by PTH in osteoblast is at a relatively low level, so that it is not a determining factor for the transcription of the bsp gene. Indeed, inhibition of PKC by prolonged treatment with PMA or chelerythrine did not inhibit PTH stimulation of the gene (data not shown). Although the activation of PKC is not a major pathway used by PTH induction of the gene, it does provide the cells with a possible mechanism for long term regulation of bsp gene expression from other potential environmental or intrinsic signals.

The understanding of how PKC and PKA signal transduction pathways converge to activate the same gene is much harder to resolve mechanistically than are the isolated pathways. Some clues as to how these two pathways are integrated may be drawn from the comparison of the ability of the various deletion constructs to be activated by either forskolin/PTH or PMA. These data demonstrated that the PMA response element is within 620 nt of the promoter, and it is distinct from the CRE site. Co-transfection with the specific PKA inhibitor PKI produced the most surprising and perhaps the most informative results, in which PKI was shown to inhibit the PMA transcriptional activation of the longest bsp construct while having no effect on pCAT-620 construct which did not contain the CRE element. These data taken together suggest that although there is a distinct PKC response element in the bsp promoter, the PKC regulation of the gene is ultimately controlled by the cellular cAMP/PKA level in osteoblasts. The data further suggest that the cross-talk between the PKC and PKA pathways happens at the level of gene transcription. This interpretation of the data, however, is dependent on the specificity of the PKI inhibitor. Data which suggest that PKI is indeed specific for PKA come from the fact that it does not inhibit the PMA transcriptional activation of the osteopontin promoter. Previous studies of the avian osteopontin promoter demonstrated that the primary actions of PMA were mediated through an AP1 cis element (56).2 These data are also supported by the results from the cycloheximide study, which demonstrate that activation of an AP1 complex by PKC is probably not involved in the stimulation of bsp gene expression. In other systems in which the PKI expression clone has been used, variable results have been obtained for the inhibitory actions of PKI on the PKC signal transduction system. In the original studies in which the development of PKI expression clone was described, PKI inhibited both cAMP- and PKA-stimulated activation of the prolactin gene promoter (39); however, in studies with the collagenase gene, PKI had no effect on the PMA stimulation of this promoter’s activities (54). In studies of the tyrosine hydroxylase gene PKI also had no effect on the PMA stimulation of its activity but was a potent inhibitor of forskolin-mediated activation (55, 57).

In previous studies c-fos has been implicated in the transcriptional activation of collagenase within osteoblast-like cells. This conclusion was based on the demonstration that the transcriptional activation of this gene in osteoblasts was by an indirect mechanism requiring new protein synthesis (53). In subsequent studies PTH was shown to directly activate c-fos, and since in other cell types it had been demonstrated that the transcription factor mediated collagenase activation, it was hypothesized that this mechanism was also operative in osteoblasts (44). In the studies presented here c-fos was shown not to be involved in the activation of the bsp promoter. This conclusion was based on the demonstration that the bsp promoter was directly activated independent of new protein synthesis and that co-transfection of a c-fos expression clone had no effect on the activity of the bsp promoter. Indeed an inconsistency in the conclusions drawn about the role of c-fos in the regulation of the collagenase gene in osteoblasts resides in the demonstration that PMA will activate c-fos expression in osteoblasts, but PMA is incapable of activating collagenase itself (53). In other studies from this laboratory c-fos is shown to have an inhibitory role on both the basal expression of osteopontin and the PMA-stimulated expression of this gene in immature osteoblasts. Thus while it is clear that c-fos plays an important role in skeletal tissue development, it may be speculated that perhaps another member of the AP1 family may also be involved in mediating the effects of PTH and PMA. In conclusion, the data presented here demonstrate that the transcriptional regulation of bsp by PTH occurs through a direct mechanism that is mediated primarily by the actions of PKA via a CRE element in the bsp promoter.

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