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Signal Transduction Pathways Mediating Parathyroid Hormone Regulation of Osteoblastic Gene Expression

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Abstract Parathyroid hormone (PTH) plays a central role in regulation of calcium metabolism. For example, excessive or inappropriate production of PTH or the related hormone, parathyroid hormone related protein (PTHrP), accounts for the majority of the causes of hypercalcemia. Both hormones act through the same receptor on the osteoblast to elicit enhanced bone resorption by the osteoclast. Thus, the osteoblast mediates the effect of PTH in the resorption process. In this process, PTH causes a change in the function and phenotype of the osteoblast from a cell involved in bone formation to one directing the process of bone resorption. In response to PTH, the osteoblast decreases collagen, alkaline phosphatase, and osteopontin expression and increases production of osteocalcin, cytokines, and neutral proteases. Many of these changes have been shown to be due to effects on mRNA abundance through either transcriptional or post-transcriptional mechanisms. However, the signal transduction pathway for the hormone to cause these changes is not completely elucidated in any case. Binding of PTH and PTHrP to their common receptor has been shown to result in activation of protein kinases A and C and increases in intracellular calcium. The latter has not been implicated in any changes in mRNA of osteoblastic genes. On the other hand activation of PKA can mimic all the effects of PTH; protein kinase C may be involved in some responses. We will discuss possible mechanisms linking PKA and PKC activation to changes in gene expression, particularly at the nuclear level.

Key words: parathyroid hormone, signal transduction, osteoblasts, cAMP, gene expression, activator protein-1

GENES REGULATED BY PTH

Hypercalcemia presents relatively commonly in medical practice [1–2 per 1,000 population, Bilezikian, 1987a]. The majority of these cases (at least 75%) are either due to over production of PTH [primary hyperparathyroidism, Bilezikian, 1987b] or improper secretion of PTHrP by a tumor [humoral hypercalcemia of malignancy, Suva et al., 1987]. Parathyroid hormone and PTHrP both mediate their skeletal effects by binding to the same receptor on osteoblasts [Jüppner et al., 1991]. The actions of the two hormones are multiple, including indirect activation of the osteoclast resulting in increased bone resorption, as well as many direct changes in the functions of the osteoblast. The latter involve a switch in the phenotype of the osteoblast from one of bone formation to one of matrix degradation and active participation in the resorption process. Thus, bone resorbing hormones such as PTH have been shown to cause a decrease in collagen synthesis and mRNA in both bone organ cultures and cultures of osteoblastic cells [Dietrich et al., 1976; Kream et al., 1980, 1986; Partridge et al., 1989]. Several other osteoblastic genes involved in bone matrix formation also decline in response to PTH, for example, alkaline phosphatase [Luben et al., 1976; Majeska et al., 1982] and osteopontin [Noda and Rodan, 1989]. PTH can also affect the growth and morphology [Miller et al., 1976] of osteoblasts. In some systems, PTH decreases DNA synthesis in osteoblastic cells [Partridge et al., 1985] which may be linked to a concomitant decrease in expression of an H-*ras*-related gene [Scott et al., 1992a]. All of these effects result in a generalized decrease in classic osteoblastic function, i.e., bone formation. This is attended by other changes in osteoblastic gene expression which promote bone resorption.

Osteoblasts exposed to PTH are stimulated to produce unidentified factor(s) which can recruit osteoclasts and their precursors [McSheehy and

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Chambers, 1986]. Parathyroid hormone also increases the synthesis of cytokines such as IL-6 by the osteoblast which then activate osteoclast functions [Feyen et al., 1989; Lowik et al., 1989; Greenfield et al., 1993]. Apart from its role in transmitting a signal to osteoclasts, we and others have shown that the osteoblast also responds to PTH by secretion of metalloproteinases, particularly collagenase [Partridge et al., 1987; Meikle et al., 1992], and enhanced activity of tissue plasminogen activator [tPA; Hamilton et al., 1985; Pfeilschifter et al., 1990]. These enzymes may then participate in the removal of nonmineralized matrix and assist osteoclastic bone breakdown. Osteocalcin is one other osteoblastic gene which has been shown to be positively regulated by PTH as part of the resorption process [Noda et al., 1988].

PTH RECEPTOR AND SIGNAL TRANSDUCTION

In osteoblasts and renal tubular cells, it has been known for a long time that the interaction of PTH and its receptor results in activation of adenylate cyclase, a rapid increase in intracellular cAMP and activation of cAMP-dependent protein kinase (PKA) [Chase and Aurbach, 1970; Partridge et al., 1981]. PTH also causes stimulation of phospholipase C with subsequent production of diacylglycerol and inositol 1,4,5-trisphosphate [Civitelli et al., 1988]. These second messengers then mediate the activation of protein kinase C [PKC; Iida-Klein et al., 1989] and the release of calcium from intracellular organelles [Reid et al., 1987], respectively. In addition, PTH directs a rapid influx of extracellular calcium via activated calcium channels [Yamaguchi et al., 1987]. All of these signal transduction pathways may mediate the alterations of gene expression observed in PTH-responsive cells, but the nuclear mechanisms and transcription factors involved have not been identified and are not understood.

In many of the osteoblastic responses detailed above, cAMP appears to be the major mediator of PTH action. This is a demonstrated pathway for the decreases in collagen synthesis [Harrison et al., 1988], osteopontin mRNA [Noda and Rodan, 1989], and DNA synthesis [Reid et al., 1988]. Similarly, agents which mimic or augment activation of protein kinase A (PKA) can reproduce the stimulatory effect of PTH on expression of collagenase [Civitelli et al., 1989; Scott et al., 1992b], tPA [Hamilton et al., 1985], and osteocalcin [Noda et al., 1988]. In contrast, agents which mimic the other two possible signal transduction pathways elicited by PTH, protein kinase C (PKC) activation, and an increase in intracellular Ca^+ are not able to decrease cell growth [Reid et al., 1988] nor stimulate collagenase production [Delaisse et al., 1988; Civitelli et al., 1989] by UMR 106-01 cells. In fact, PTH is able to increase collagenase mRNA levels in UMR cells in the absence of the PKC pathway [Clohisy et al., 1992], suggesting it has a limited role in this cell line for regulating the collagenase gene. In some other osteoblastic systems, the protein kinase C activator, phorbol myristate acetate (PMA) inhibits collagen expression. (Note that we are using the terminology of PMA for 12-*O*-tetradecanoyl-phorbol-13-acetate rather than TPA since tPA is a standard abbreviation for tissue plasminogen activator.) However, generally the PTH-mediated signal transduction pathway involved in inhibition of collagen gene expression appears to also be via cAMP [Kream et al., 1993; Bogdanovich et al., 1993].

PTH REGULATION OF mRNA

PTH has proven to be mechanistically versatile at the level of gene regulation. PTH increases the abundance of osteocalcin mRNA in ROS 17/2.8 osteosarcoma cells by increasing the stability of osteocalcin transcripts [Noda et al., 1988]. In the latter cells, steady-state levels of osteopontin mRNA are decreased at the transcriptional level by PTH [Noda and Rodan, 1989]. Transcriptional effects of PTH on the Type I collagen gene have been difficult to demonstrate, requiring establishment of transgenic mice bearing the rat α_1I procollagen promoter. Calvariae from these animals were then incubated in the presence of PTH and a decline in collagen synthesis, CAT activity, and endogenous collagen mRNA was observed [Kream et al., 1993], thus showing transcriptional regulation by PTH. Studies with cultured cells, either osteosarcoma lines or calvarial-derived cells, have not shown such clear-cut results. In fact, we have obtained data suggesting that this hormone caused a decrease in α_1I procollagen mRNA levels in UMR 106-01 cells by lessening the stability of the transcripts [Partridge et al., 1991]. In these same cells, PTH increases collagenase mRNA levels dramatically by transcriptional induction of the collagenase gene [Scott et al., 1992b]. Thus, PTH regulates the abundance

of mRNAs in osteoblastic cells at both the transcriptional and post-transcriptional levels.

Notwithstanding, scant information has accrued to connect the immediate signal transduction events (second messengers) to events occurring at the transcriptional or post-transcriptional level. All of the second messengers must lead to activation of protein kinases, either PKA, PKC, or Ca^+-calmodulin-dependent protein kinase with subsequent phosphorylation of substrate proteins which either exert their effects in the nucleus or extranuclearly. There is evidence that the catalytic subunit of PKA is able to translocate to the nucleus after activation [Nigg et al., 1985] and phosphorylate substrate proteins such as cAMP response element binding protein (CREB) in that site. This would then be a mechanism connecting signaling at the cell surface with the nucleus. Data has also been reported that PKC is able to translocate to nuclear membranes after activation [Hocevar and Fields, 1991]. Nevertheless, it is not clear what the nuclear substrate proteins may be for PKC phosphorylation which then regulate gene expression.

The time for PTH to exert its effects on the genes discussed above may give us some clues on the mechanisms responsible for those changes. Collagenase mRNA reaches maximal levels 4 h after PTH treatment and this response clearly requires protein synthesis and is, thus, a secondary effect [Scott et al., 1992b]. For those other genes for which transcriptional or post-transcriptional regulation by PTH has been identified, α_1I procollagen, osteopontin and osteocalcin, maximal changes in mRNAs are seen 24–48 h after treatment. Only limited investigations have been conducted to determine whether these were secondary responses [e.g., collagen appears to be; Bogdanovich et al., 1993]. However, we can perhaps extend from our work on collagenase and suggest that this is likely, given the delayed nature of the effects and signal transduction through phosphorylation. Thus, we would like to suggest that PTH elicits its effects on mRNAs for these genes through synthesis of other proteins, for instance transcription factors such as the activator protein-1 (AP-1) family which are immediate early genes. This is not to say that there may not be other transcription factors whose synthesis is affected by PTH, including, perhaps, osteoblast-specific transcription factors.

AP-1 AND THE OSTEOBLAST

We have chosen to examine PTH regulation of the collagenase (matrix metalloproteinase-1, MMP-1) gene as a model system to elucidate a pathway operating in the osteoblast for this hormone from cell surface to nucleus. We have previously demonstrated that the PTH induction of MMP-1 mRNA in UMR 106-01 cells is due to activation of transcription of the rat MMP-1 gene [Quinn et al., 1990] and is dependent upon protein synthesis [Scott et al., 1992b]. The latter observation suggested that other genes, perhaps immediate early genes, need to be expressed for transcription of collagenase to occur in rat osteoblastic cells. Studies with the human collagenase gene in fibroblasts have shown that there is a PMA-responsive element (TRE) 73 base pairs upstream of the transcriptional start site of the gene [Angel et al., 1987a] which binds transcription activating proteins, the AP-1 complex, in a PMA-inducible fashion [Angel et al., 1987b]. The AP-1 complex can be composed of either a homodimer of Jun or a heterodimer of Fos and Jun or other members of this gene family [Halazonetis et al., 1988], which, upon binding to the collagenase AP-1 binding site, confers increased transcriptional activity upon the collagenase gene [Angel et al., 1987b]. Experiments co-transfecting various constructs of c-*fos* (either overexpressed or antisense) established the absolute requirement for Fos in the activation of the human collagenase TRE [Schonthal et al., 1988].

AP-1 consensus binding sequences are also seen in the promoters of other osteoblastic genes, e.g., alkaline phosphatase, osteocalcin, and α_1I collagen genes [Owen et al., 1990]. However, it is not presently apparent that these sequences, or AP-1, are involved in regulation of these genes by PTH. For instance, in the α_1(I) collagen promoter, the AP-1 binding sequence is at −2918 [Owen et al., 1990], while the PTH-responsive element appears to lie between −2296 and −1695 [Kream et al., 1993]. Other transcription factors, such as NF-IL6 [Bogdanovich et al., 1993], may instead mediate regulation of these genes.

The AP-1 complex, as outlined above, is composed of dimers of the *fos* and *jun* families. Of these two, Fos has been implicated in several ways in the regulation of bone cell function. It was first identified as an oncogene, v-*fos*, from mice with osteosarcomas [Curran et al., 1982],

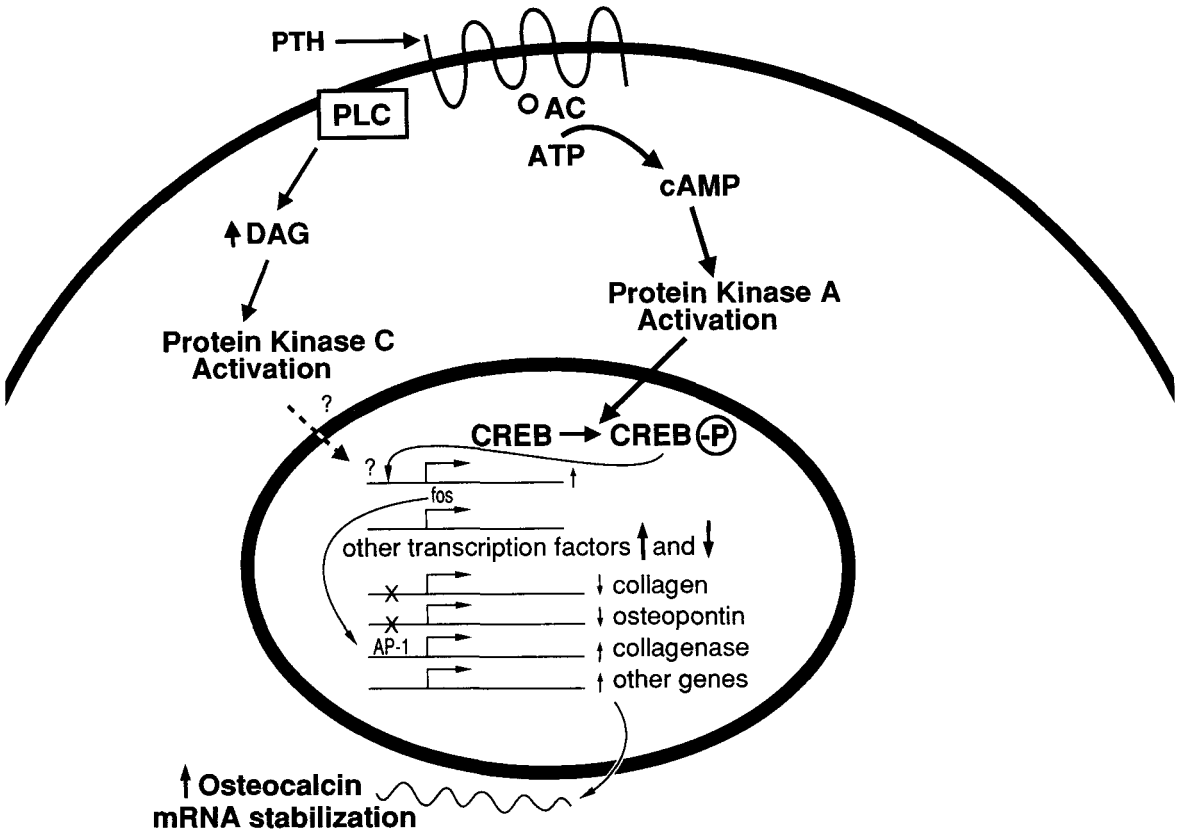

Fig. 1. Proposed model of PTH regulation of osteoblastic gene expression. AC, adenylate cyclase; DAG, diacylglycerol; CREB, cAMP response element binding protein; AP-1, activator protein-1 complex.

suggesting that its overexpression in the osteoblast may lead to deranged growth of that cell. Similarly, overexpression of c-*fos* in transgenic mice leads to bone adenocarcinomas [Ruther et al., 1987]. In normal development, c-*fos* has been detected in abundance in perichondrial growth regions of fetal mouse bone and differentiating areas of mesodermal web tissue [Dony and Gruss, 1987]. Its importance in normal bone development has been confirmed with the recent observations of osteopetrosis in mice with a null mutation in c-*fos* [Johnson et al., 1992; Wang et al., 1992].

Studies in vitro have shown many agents regulate c-*fos*, including activators of PKC, growth factors, transforming oncogenes, and agents and hormones acting through cAMP or Ca^{2+} [see review by Angel and Karin, 1991]. Regulation by growth factors or through PKC appears to entail the serum response element [SRE, centered at −310 bp upstream of the cap site; Treisman, 1986] and post-translational modification of the serum response factor [Graham and Gilman, 1991]. The response element for agents acting through increases in cAMP [Boutillier et al., 1991] and Ca^{2+} [Auwerx et al., 1990] is thought to mainly involve the cAMP response element (CRE) −65 bp upstream of the transcriptional start site. However, hormonal regulation through PKA may also involve non-CREB action through the SRE [Boutillier et al., 1992] as well as other CRE-like sites around the SRE [Berkowitz et al., 1989]. We and others have shown that calciotropic hormones stimulate c-*fos* expression in osteoblastic cells. This has included action through PKA by PTH and PGE_2 [Clohisy et al., 1992; Fang et al., 1992] as well as epidermal growth factor [Fang et al., 1992] and $1,25(OH)_2D_3$ [Candeliere et al., 1991]. Thus, agents operating through several distinct mechanisms can increase expression of this gene in osteoblastic cells.

cAMP AND CREB

As presented above, PTH appears to mainly regulate osteoblastic function through the PKA

pathway and, in our hands, stimulation of transcription of the collagenase gene seems to be primarily via this route and also requires induction of another gene as a tertiary messenger, c-*fos* as one possibility. Thus, PTH causes activation of PKA which may then phosphorylate proteins which stimulate transcription of the c-*fos* gene. There is a family of proteins now identified which bind to cAMP response elements [CREs, Habener, 1990] but only a few of these are phosphorylated by activated PKA [CREB, CREMτ, and ATF-1; Yamamoto et al., 1988; Foulkes et al., 1992; Hurst et al., 1991]. Of these, only CREB has a generalized physiological role showing enhanced DNA binding and transcriptional activity following phosphorylation by PKA [Foulkes et al., 1991, 1992; Flint and Jones, 1991]. Thus, at the present time, CREB is the best candidate to mediate the cAMP pathway leading to increased transcription of a gene. Until the nuclear transcription factors or primary response genes for PTH regulation of osteoblastic genes are identified, we will be unable to determine whether these are, indeed, controlled by phosphorylated CREB.

CONCLUSIONS

The majority of the evidence indicates that PTH predominantly regulates osteoblastic gene expression via activation of PKA. In addition, data from our own lab and that of others tends to support the notion that PTH regulation of osteoblastic gene expression is a secondary effect, implying the induction of some other gene(s). Taken together, we propose that PKA activation following PTH stimulation leads to phosphorylation of CREB-like nuclear transcription factors which then induce transcription of other genes. These could be transcription factors such as the AP-1 members, Fos and Jun, or genes which mediate post-transcriptional regulation. This hypothesis is illustrated in Figure 1.

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