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Butyrate response factor 1 is regulated by parathyroid hormone and bone morphogenetic protein-2 in osteoblastic cells

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Abstract

Parathyroid hormone (PTH) exerts potent and diverse effects in bone and cartilage through activation of type 1 PTH receptors (PTH1R) capable of coupling to protein kinase A (PKA) and PKC. We have used macroarrays to identify zinc finger protein butyrate response factor-1 (BRF1) as a novel PTH regulated gene in clonal and normal osteoblasts of human and rodent origin. We further demonstrate that in human osteoblast-like OHS cells, biologically active hPTH(1–84) and hPTH(1–34) stimulate BRF1 mRNA expression in a dose- and time-dependent manner, while the amino-terminally truncated hPTH(3–84) which does not activate PTH1R has no effect. Moreover, using specific stimulators or inhibitors of PKA and PKC activity, the PTH-elicited BRF1 mRNA expression is mediated through the PKA signaling pathway. In mouse calvarial osteoblasts, BRF1 mRNA levels are upregulated by PTH(1–84) and reduced in response to bone morphogenetic protein 2 (BMP-2). Hence, our data showing that BRF1 is expressed in osteoblastic cells and regulated by PTH and BMP-2, suggest an important role for BRF1 in osteoblasts within the molecular network of PTH-dependent bone remodeling.

Keywords: TIS11; BRF-1; Osteoblast; PTH; BMP-2; PTH1R; PKA; PKC

PTH plays multiple roles of importance for normal embryonic skeletal development and, most notably as a regulator of bone remodeling and calcium homeostasis in adult life. For example, PTH receptor 1 (PTH1R) null mutant mice display severe defects in the epiphyseal growth plates associated with increased rate of chondrocyte differentiation [1]. Furthermore, PTH increases the number of osteoblasts which in turn stimulates hematopoietic stem cells in stromal cultures and in vivo [2]. Full-length PTH(1–84) and amino-terminal PTH(1–34), but not N-terminally truncated analogues such as PTH (3-34), can activate the PTH1R present on the surface of chondrocytes, osteoblasts, and renal epithelial cells [3]. The PTH signal is transduced [4] via stimulation of effector systems that include cAMP-dependent adenylate cyclase (cAMP/AC) and Ca-dependent phospholipase C (Ca/PLC), leading to activation of protein kinase A (PKA) and protein kinase C (PKC), respectively [5]. Recently, PTH signaling has been shown to be modulated by Na(+)/H(+) exchanger regulatory factors (NHERF) which interact with the PTH1R [6]. While the classical PTH1R has been studied in most detail, other classes of PTH receptors are known to be present in osteoblasts and osteocytes [7–9], e.g., a "C-PTH" receptor, which unlike PTH1R, recognizes only the

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C-terminal part of the hormone [10–12]. However, the biochemical properties and biological significance of these other receptors remain to be elucidated.

BRF1 is a member of the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced sequences (TIS)11 family, which is known to comprise early response genes whose expression is rapidly and transiently induced independent of protein synthesis upon treatment with agonists like TPA, polypeptide mitogens such as epidermal growth factor (EGF), or insulin and insulin-like growth factor I (IGF-I) [13]. BRF1 is also known as EGF response factor-1 (ERF-1) in humans, TIS11b in mice, and cMG1 in rats. BRF1 consists of 338 amino acids coded by two exons [14].The amino-acid sequences differ only by 1% between the human versus rat or mouse BRF1, and amino acid substitutions are conservative [14]. The TIS11 subfamily is characterized by a highly conserved consensus sequence of (X^+)YKTEL C(X^+) X_5GXC(X^+)YGX(X^+)CXFXH that is repeated and contains a potential zinc finger motif [15,16]. TIS11-like proteins are capable of binding to AU-rich elements (AREs) of a number of cytokine mRNAs and regulate their stability [17,18]. Furthermore, continuous expression of TIS11 proteins at physiological levels causes apoptotic cell death in a number of cell types (3T3, HeLa, 293, U2OS, SAOS2 as well as in primary cultured human foreskin keratinocytes and mouse embryo fibroblasts) analogous to the effect of oncoproteins such as c-Myc, E1A, and E2F-1 [19].

The role(s) of PTH as a major regulator of osteoblast development and function is well recognized, and is likely to be mediated by an ensemble of downstream target genes under direct influence of PTH. However, detailed knowledge of the molecular mechanisms responsible for PTH action in responsive cells is only beginning to be understood and involves genes that belong to various protein families such as transcription factors, growth factors and their receptors, matrix proteins, etc. The time-dependent changes in the expression levels of these genes are likely to differ depending on whether early (i.e., transcriptional) or later (secondary to cell proliferation and differentiation; e.g., matrix proteins) effects are considered. In the process of characterizing the molecular mechanisms regulating PTH-dependent processes in bone, we previously identified the transcription factor Sox4 as a PTH-regulated gene in osteoblasts and characterized its expression pattern in vivo [20].

In the present study we have identified BRF1 as a novel PTH-regulated gene in osteoblast-like cells with kinetic characteristics of an immediate-early response gene with prolonged expression. We find that PTH increases BRF1 mRNA levels via a cAMP/PKA-dependent mechanism. Furthermore, in normal mouse calvarial osteoblasts, its expression is regulated by PTH and BMP-2, two proteins with central roles in skeletal development and maintenance. These data imply for the first time BRF1 as an important mediator in PTH regulated osteoblast activities.

Materials and Methods

Materials. ExpressHyb were from Clontech (Palo Alto, CA). Recombinant hPTH(1–84) and hPTH(3–84) were prepared in our laboratory as described [21,22], hPTH(1–34), TPA (12-*O*-tetradecanoylphorbol-13-acetate) and H89 {*N*-[2-((bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide, HCl} were purchased from Sigma (St. Louis, MO). hBMP-2 and human Sonic Hedgehog (hSHH) were gifts from the Genetics Institute (Cambridge, MA) and Ontogeny (Cambridge, MA), respectively. Human osteoblast-like OHS, KPDXM, and KRIB cell lines were obtained from Dr. Ø. Bruland (The Norwegian Radium Hospital, Oslo). Human osteoblast-like MNNG-HOS and SaOS-2 cell lines, and GAPDH cDNA were from ATCC. The origin and properties of the cell lines are reviewed in [23]. Collagenase type I and type II were purchased from Worthington Biochemical (Lakewood, NJ).

Northern blot analysis. Total RNA was isolated using the TRIZOL reagent (Life Technologies, Rockville, MD), and poly(A)$^+$ RNA was prepared from total RNA using Dynabeads (DYNAL, Oslo, Norway), according to the manufacturer's instructions. Northern blot analysis was performed as described previously [24] using Hybond N+ filters (Amersham, Arlington Heights, IL). In brief, filters were prehybridized, hybridized with a radiolabeled BRF1 cDNA fragment, and washed, and signals were quantified with direct nuclear counting on Instant Imager (Packard, Meriden, CT). Values for BRF1 mRNA signals were normalized to glyceraldehyde 3- phosphate dehydrogenase (GAPDH) or β-actin to correct for variations in loading. Data are presented as fold stimulation in PTH-treated cultures relative to untreated controls. The cDNAs used as probes for hBRF1 and mBRF1 were the IMAGE clones 1881361 and 764675, respectively, purchased from UK-HGMP (Cambridge, UK). Human bone-specific alkaline phosphatase (ALP) and GAPDH were purchased from ATCC.

Cell culture. Normal human osteoblasts (NO) were prepared from bone biopsies of patients undergoing hip surgery and cultured as described [25]. OHS, KPDXM, and SaOS-2 cells were cultured in RPMI-1640 medium with 10% fetal calf serum (FCS), L-glutamine (0.3 mg/ml), penicillin (5×10^5 IU/L), and streptomycin (50 mg/L). The MNNG-HOS and ROS 17/2.8 cell lines were cultured according to instructions from ATCC. All cells were sub-cultured weekly and medium was changed every 3–4 days. Cells always received fresh medium containing 5% FCS 24 h before the experiments were started. Full-length PTH(1–84), C- and N-terminally truncated PTH peptides at indicated concentrations, or vehicle (acetic acid, 1.7×10^{-5} M final concentration), were added to the culture medium of cells that had just reached confluence, and the cells were harvested at the given time points (see figure legends). Primary cultures of mouse calvarial osteoblasts were prepared from 2- to 3-day-old mice by sequential collagenase digestion and cultured according to a published protocol [26].

Results

BRF1 mRNA is expressed in normal and clonal human osteoblastic cell lines

As part of our strategy to characterize genes that are regulated by PTH(1–84) in human osteoblasts, we have used macroarrays to identify the zinc finger protein

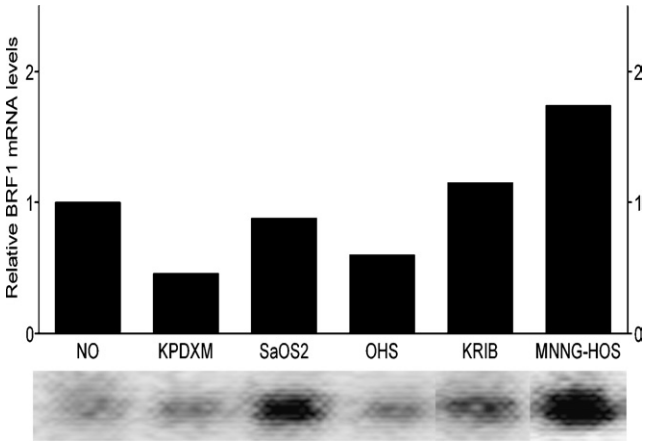

Fig. 1. Northern blot analysis demonstrating BRF1 mRNA expression in human and rodent osteosarcoma cell lines. Total RNA from MNNG-HOS, KRIB, OHS, SaOS-2, KPDXM, and primary cultures of normal human osteoblast derived trabecular bone (NO), as well as rat ROS 17/2.8 cells were subjected to Northern blot analysis. Levels of BRF1 mRNA expression are relative to those in NO (=1).

butyrate response factor-1 (BRF1) as a novel PTH regulated gene. Northern blot analysis showed that BRF1 mRNA migrates as a 4 kb species (Fig. 1), in agreement with previous findings in humans and mice [27,15]. BRF1 mRNA is expressed in clonal osteoblasts of both human and rodent origin, including human MNNG-HOS, KRIB, OHS, SaOS-2, and KPDXM, as well as in primary cultures of human and mouse osteoblasts (Fig. 1).

Dose- and time-dependent effects of PTH on BRF1 mRNA levels in human OHS cells

Fig. 2A shows that BRF1 mRNA levels increased dose-dependently in response to treatment with PTH(1–84) at doses from 5×10^{-11} M PTH(1–84) to 5×10^{-9} M for 24 h, with a maximum of 2-fold stimulation at a dose of 5×10^{-9} M. Time-course experiments showed that levels of BRF1 mRNA were rapidly increased, reaching a maximum already at 1 h of treatment with PTH(1–84) (10^{-7} M) (Fig. 2B), and levels were maintained for at least 48 h relative to untreated controls.

The effect of PTH(1–84) on BRF1 mRNA levels is mediated by activated PTH1R coupled to cAMP-dependent PKA in OHS cells

BRF1 mRNA levels in OHS cells increased relative to untreated controls at 24 h using PTHs with intact N-terminal (i.e., PTH(1–84) and PTH(1–34)), which both bind and activate the PTH1 receptor. In contrast PTH(3–84), which binds to the receptor [21] but is unable to stimulate cAMP production, was without any effect. These data indicate that the effect of PTH on BRF1 mRNA is conveyed by activated PTHiR (Fig. 3A).

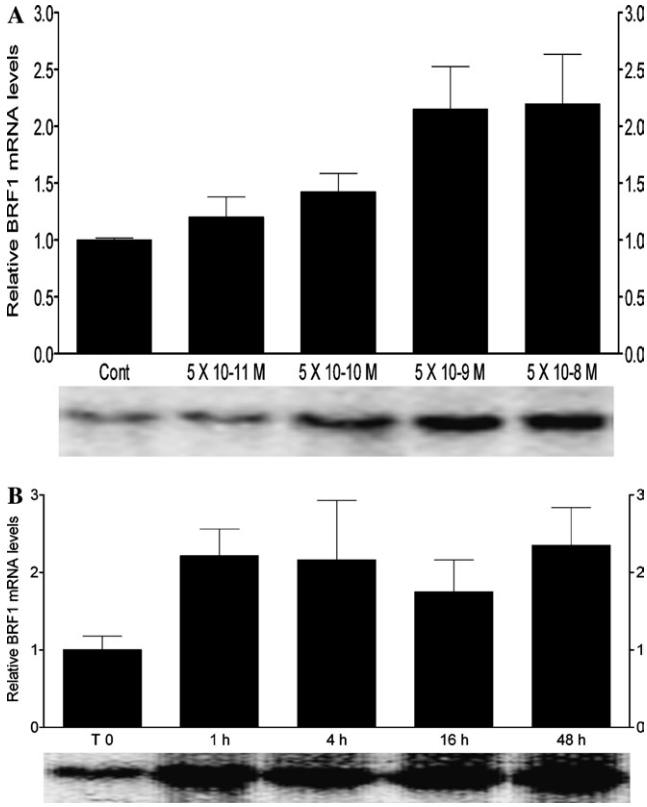

Fig. 2. Northern blot analysis demonstrating dose- and time-dependent effects of hPTH(1–84) on BRF1 mRNA expression. (A) Effect of indicated doses of PTH on BRF1 mRNA in OHS cells at 24 h. (B) Time-course effect of PTH (1×10^{-8} M) on BRF1 mRNA in OHS cells. Two independent experiments each with 2–3 replicates were performed. SEM is given as error bars.

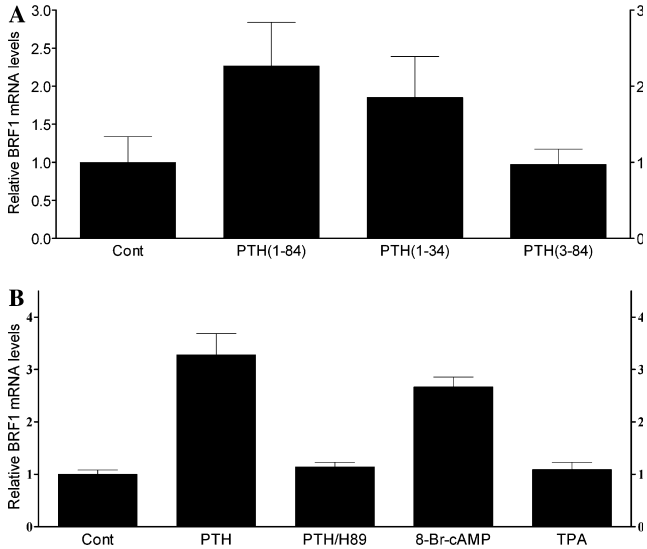

Fig. 3. Northern blot analysis. (A) Effect of intact and truncated forms of PTH (1×10^{-8} M) on BRF1 mRNA at 24 h in OHS cells. (B) Effects of PTH (1×10^{-8} M), 8-Br-cAMP (2.5×10^{-3} M) or TPA (1×10^{-8} M), alone or in combination with the PKA inhibitor H89 (2×10^{-5} M), on BRF1 mRNA in OHS cells. Data are representative of 2–4 independent experiments with 2–4 replicates. SEM is given as error bars.

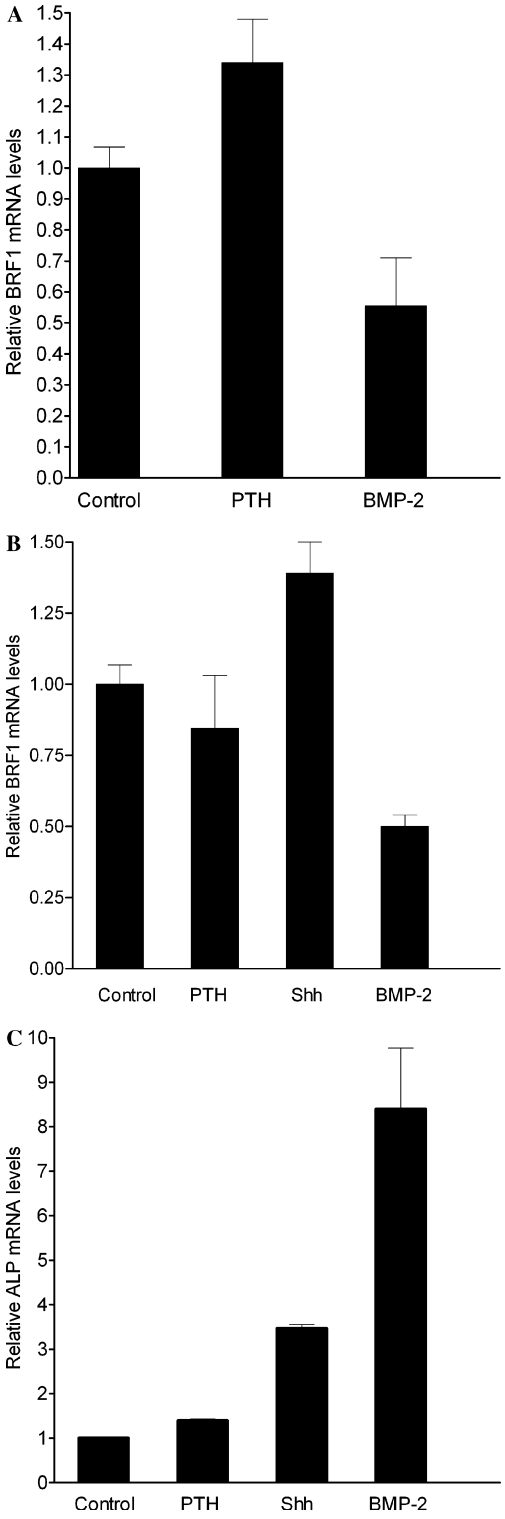

Fig. 4. Northern blot analysis demonstrating effects of various osteogenic factors on BRF1 mRNA and alkaline phosphatase mRNA expression (relative levels) in mouse calvarial osteoblasts. Osteoblasts were incubated with hPTH (5×10^{-8} M), hBMP-2 (100 ng/ml), hSHH (100 ng/ml) or vehicle (control) (see Materials and methods). (A) Osteoblasts treated with hPTH, hBMP-2, or vehicle (control) for 24 h. (B,C) Osteoblasts treated with hPTH, hBMP-2, hShh or vehicle (control) for 8 days, BRF1 mRNA levels and ALP mRNA levels, respectively. Error bars represent SEM from two experiments with 2–4 replicates.

In order to elucidate the importance of PKA and PKC signaling pathways in mediating the effect of PTH on BRF1 mRNA levels in more detail, OHS cells were treated with agonist or antagonist for these pathways under conditions as indicated in the figure legend. Whereas the PKA activator 8-Br-cAMP was equipotent to PTH(1–84), the PKC specific activator TPA had no effect on BRF1 mRNA levels (Fig. 3B). Furthermore, PTH-stimulated BRF1 mRNA expression was blunted by the PKA inhibitor H89 (Fig. 3B). Taken together, these data clearly show that the enhanced expression of BRF1 mRNA in response to PTH is mainly mediated by activated PTH1Rs coupled to cAMP/PKA.

Effect of osteogenic factors on BRF1 mRNA levels in mouse calvarial osteoblasts

In addition to PTH, BMP-2, and Sonic Hedgehog (SHH) are also known to promote anabolic processes in bone which in part may be mediated by common downstream components. To investigate whether BRF1 is similarly regulated by BMP-2 and PTH in osteoblastic cells, primary cultures of mouse calvarial osteoblasts (see Materials and methods) were stimulated with the respective recombinant proteins for 24 h. PTH led to increased BRF1 mRNA levels also in primary osteoblast cultures, consistent with our results in the clonal OHS cell line (Fig. 4A). In contrast, BRF1 mRNA levels decreased in response to BMP-2 treatment.

We next investigated the effects of PTH, BMP-2 or SHH on BRF1 mRNA levels in cultured mouse calvarial cells after extending the incubation period to 8 days. The differentiation status of cells was scored in parallel cultures by monitoring mRNA for alkaline phosphatase (ALP), which is characteristically expressed by cells of the osteoblastic lineage and is present at higher levels in more mature cells as compared to more immature cells [28]. Under these conditions, we found no effect of PTH on BRF1 mRNA levels, and only small increases (1.5-fold) in ALP mRNA expression levels. However, treatment with BMP-2 reduced the BRF1 mRNA levels to approximately 50% (Fig. 4B), concomitant with increased ALP mRNA levels (about 8-fold) relative to untreated controls (Fig. 4C). Whereas SHH enhanced ALP mRNA levels (about 3-fold), only a modest effect was seen on BRF1 mRNA levels.

Discussion

In this study we demonstrate for the first time that BRF1 is widely expressed in various normal and clonal osteoblastic cells of both human and rodent origin, and that BRF1 mRNA is regulated by PTH via a PTH1R-dependent signaling pathway. Our results indicate that type 1 PTH receptors in the plasma membrane mediate

the effects of PTH on BRF1 mRNA levels in osteoblasts for the following reasons: (i) PTH(1–84) and PTH (1–34) increase BRF1 mRNA levels, while PTH(3–84) which binds to PTH1R but does not elicit a cAMP response, has no effect. (ii) The dose-dependent increases of BRF1 mRNA in OHS cells in response to PTH are similar to what has been previously shown upon PTH-stimulated cAMP production in human OHS cells (unpublished). (iii) Stimulation of BRF1 mRNA expression by the cAMP analogue 8-Br-cAMP mimics the effect of PTH, and the PKA inhibitor H89 abolishes the effects of PTH on BRF1 mRNA. Only the PKA system seems to be involved since no alterations of BRF1 mRNA are observed in response to TPA, a PKC activator, during these culture conditions.

BRF1 was upregulated by PTH(1–84) in OHS cells in a dose- and time-dependent manner, with rapid kinetics. Thus, our findings are similar to several previous reports on regulation of the Tis11 family of genes in other cell types, in which it has been demonstrated that induction by agonists like TPA, EGF, NGF, FGF or insulin leads to a rapid increase in the BRF1 mRNA levels [13,15,16]. However, in contrast to earlier reports in which the stimulatory effect of other factors tends to subside early [13], high BRF1 mRNA levels were sustained for at least 48 h after PTH treatment. The rapid kinetics upon stimulation with PTH is indicative of a role for BRF1 in the early stage of PTH action.

Also, we found that under conditions in which BMP-2 is believed to promote osteblast differentiation, as evidenced by increased ALP mRNA levels, BRF1 mRNA is decreased approximately 50%. Thus, our results suggest that decreases in BRF1 mRNA levels induced by BMP-2 are not dependent on the stage of osteoblast differentiation/proliferation. However, SHH, which also is known to promote osteoblast differentiation and increased ALP mRNA approximately 3-fold, tended to stimulate rather than reduce BRF1 mRNA levels.

The relatively modest effect of PTH treatment for 24 h in cultured calvarial osteoblasts as compared to the situation in OHS cells may be due to phenotypic alterations during in vitro conditions and due to the fact that normal osteoblasts are in various phases of differentiation/proliferation. However, the data demonstrate that PTH causes enhanced BRF1 mRNA levels also in normal primary osteoblasts.

Although the functional significance of BRF1 in osteoblasts is unknown, proposed roles for BRF1 mRNA in other cell types range from effects on mRNA stability of cytokines and growth factors to a function in apoptosis. These processes are believed to be of central importance for bone development and maintenance, and executed by hormones such as PTH and PTH related protein (PTHrP). These mRNAs contain ARE in their 3′ untranslated region mRNA [29,30] and thus have the potential of being regulated by BRF1, and the present results indicate the possible existence of a feedback regulatory loop. Moreover, apoptosis is involved in determining the life-span of bone cells, including osteoblasts and osteoclasts, and important regulators of apoptosis include PTH and corticosteroids. For example, PTH protects against apoptosis in cultured osteoblasts [31–34]. BMP-2 appears to have a dual role in activating apoptosis as well as in inducing differentiation in osteoblasts [35,36]. While further studies are necessary to elucidate the precise functional role(s) of BRF1 in bone, our study identifies BRF1 as a previously unrecognized factor in osteoblasts where its regulation by PTH and BMP-2 suggests important role(s) for this protein in skeletal tissue.

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References

[1] B. Lanske, A.C. Karaplis, K. Lee, A. Luz, A. Vortkamp, A. Pirro, M. Karperien, L.H. Defize, C. Ho, R.C. Mulligan, A.B. Abou-Samra, H. Juppner, G.V. Segre, H.M. Kronenberg, PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth, Science 273 (1996) 663–666.

[2] L.M. Calvi, G.B. Adams, K.W. Weibrecht, J.M. Weber, D.P. Olson, M.C. Knight, R.P. Martin, E. Schipani, P. Divieti, F.R. Bringhurst, L.A. Milner, H.M. Kronenberg, D.T. Scadden, Osteoblastic cells regulate the haematopoietic stem cell niche, Nature 425 (2003) 841–846.

[3] E. Schipani, H. Karga, A.C. Karaplis, J.T. Potts, Jr., H.M. Kronenberg, G.V. Segre, A.B. Abou-Samra, H. Juppner, Identical complementary deoxyribonucleic acids encode a human renal and bone parathyroid hormone (PTH)/PTH-related peptide receptor, Endocrinology 132 (1993) 2157–2165.

[4] A.B. Abou-Samra, H. Juppner, T. Force, M.W. Freeman, X.F. Kong, E. Schipani, P. Urena, J. Richards, J.V. Bonventre, J.T. Potts Jr., Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium, Proc. Natl. Acad. Sci. USA 89 (1992) 2732–2736.

[5] N.C. Partridge, D. Alcorn, V.P. Michelangeli, B.E. Kemp, G.B. Ryan, T.J. Martin, Functional properties of hormonally responsive cultured normal and malignant rat osteoblastic cells, Endocrinology 108 (1981) 213–219.

[6] M.J. Mahon, M. Donowitz, C.C. Yun, G.V. Segre, Na(+)/H(+) exchanger regulatory factor 2 directs parathyroid hormone 1 receptor signalling, Nature 417 (2002) 858–861.
[7] F. De Miguel, P. Martinez-Fernandez, C. Guillen, A. Valin, A. Rodrigo, M.E. Martinez, P. Esbrit, Parathyroid hormone-related protein (107–139) stimulates interleukin-6 expression in human osteoblastic cells, J. Am. Soc. Nephrol. 10 (1999) 796–803.
[8] A. Valin, A. Garcia-Ocana, F. De Miguel, J.L. Sarasa, P. Esbrit, Antiproliferative effect of the C-terminal fragments of parathyroid hormone-related protein, PTHrP-(107–111) and (107–139), on osteoblastic osteosarcoma cells, J. Cell. Physiol. 170 (1997) 209–215.
[9] M. Nasu, T. Sugimoto, H. Kaji, J. Kano, K. Chihara, Carboxyl-terminal parathyroid hormone fragments stimulate type-1 procollagen and insulin-like growth factor-binding protein-5 mRNA expression in osteoblastic UMR-106 cells, Endocr. J. 45 (1998) 229–234.
[10] H. Takasu, H. Baba, N. Inomata, Y. Uchiyama, N. Kubota, K. Kumaki, A. Matsumoto, K. Nakajima, T. Kimura, S. Sakakibara, T. Fujita, K. Chihara, I. Nagai, The 69–84 amino acid region of the parathyroid hormone molecule is essential for the interaction of the hormone with the binding sites with carboxyl-terminal specificity, Endocrinology 137 (1996) 5537–5543.
[11] N. Inomata, M. Akiyama, N. Kubota, H. Juppner, Characterization of a novel parathyroid hormone (PTH) receptor with specificity for the carboxyl-terminal region of PTH-(1–84), Endocrinology 136 (1995) 4732–4740.
[12] P. Divieti, N. Inomata, K. Chapin, R. Singh, H. Juppner, F.R. Bringhurst, Receptors for the carboxyl-terminal region of pth(1–84) are highly expressed in osteocytic cells, Endocrinology 142 (2001) 916–925.
[13] A.N. Corps, K.D. Brown, Insulin and insulin-like growth factor I stimulate expression of the primary response gene cMG1/TIS11b by a wortmannin-sensitive pathway in RIE-1 cells, FEBS Lett. 368 (1995) 160–164.
[14] S.A. Bustin, X.F. Nie, R.C. Barnard, V. Kumar, J.C. Pascall, K.D. Brown, I.M. Leigh, N.S. Williams, I.A. McKay, Cloning and characterization of ERF-1, a human member of the Tis11 family of early-response genes, DNA Cell Biol. 13 (1994) 449–459.
[15] B.C. Varnum, Q.F. Ma, T.H. Chi, B. Fletcher, H.R. Herschman, The TIS11 primary response gene is a member of a gene family that encodes proteins with a highly conserved sequence containing an unusual Cys-His repeat, Mol. Cell. Biol. 11 (1991) 1754–1758.
[16] M. Gomperts, A.N. Corps, J.C. Pascall, K.D. Brown, Mitogen-induced expression of the primary response gene cMG1 in a rat intestinal epithelial cell-line (RIE-1), FEBS Lett. 306 (1992) 1–4.
[17] W.S. Lai, E. Carballo, J.M. Thorn, E.A. Kennington, P.J. Blackshear, Interactions of CCCH zinc finger proteins with mRNA. Binding of tristetraprolin-related zinc finger proteins to Au-rich elements and destabilization of mRNA, J. Biol. Chem. 275 (2000) 17827–17837.
[18] G. Stoecklin, M. Colombi, I. Raineri, S. Leuenberger, M. Mallaun, M. Schmidlin, B. Gross, M. Lu, T. Kitamura, C. Moroni, Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover, EMBO J. 21 (2002) 4709–4718.
[19] B.A. Johnson, M. Geha, T.K. Blackwell, Similar but distinct effects of the tristetraprolin/TIS11 immediate-early proteins on cell survival, Oncogene 19 (2000) 1657–1664.
[20] S. Reppe, E. Rian, R. Jemtland, O.K. Olstad, V.T. Gautvik, K.M. Gautvik, Sox-4 messenger RNA is expressed in the embryonic growth plate and regulated via the parathyroid hormone/parathyroid hormone-related protein receptor in osteoblast-like cells, J. Bone Miner. Res. 15 (2000) 2402–2412.
[21] O.K. Olstad, S. Reppe, O.P. Løseth, R. Jemtland, K.M. Gautvik, Binding and cyclic AMP stimulation by N-terminally deleted human PTHs (3–84 and 4–84) in a homologous ligand receptor system, J. Bone Miner. Res. 12 (1997) 1348–1357.
[22] O.S. Gabrielsen, S. Reppe, O. Saether, O.R. Blingsmo, K. Sletten, J.O. Gordeladze, A. Høgset, V.T. Gautvik, P. Alestrøm, T.B. Øyen, Efficient secretion of human parathyroid hormone by *Saccharomyces cerevisiae*, Gene 90 (1990) 255–262.
[23] O.K. Olstad, V.T. Gautvik, S. Reppe, E. Rian, R. Jemtland, C. Ohlsson, O.S. Bruland, K.M. Gautvik, Molecular heterogeneity in human osteosarcoma demonstrated by enriched mRNAs isolated by directional tag PCR subtraction cloning, Anticancer Res. 23 (2003) 2201–2216.
[24] E. Rian, R. Jemtland, O.K. Olstad, J.O. Gordeladze, K.M. Gautvik, Synthesis of human parathyroid-hormone-related protein(1-141) in *Saccharomyces cerevisiae*. A correct amino-terminal processing vital for the hormone's biological activity is obtained by an ubiquitin fusion protein approach, Eur. J. Biochem. 213 (1993) 641–648.
[25] P.J. Marie, M.C. de Vernejoul, D. Connes, M. Hott, Decreased DNA synthesis by cultured osteoblastic cells in eugonadal osteoporotic men with defective bone formation, J. Clin. Invest. 88 (1991) 1167–1172.
[26] P. Divieti, B. Lanske, H.M. Kronenberg, F.R. Bringhurst, Conditionally immortalized murine osteoblasts lacking the type 1 PTH/PTHrP receptor, J. Bone Miner. Res. 13 (1998) 1835–1845.
[27] A.N. Corps, J.C. Pascall, K.M. Hadfield, K.D. Brown, Identification of a functional promoter element in the 5′-flanking region of the rat cMG1/TIS11b gene, Biochem. J. 311 (1) (1995) 251–258.
[28] J.E. Aubin, F. Liu, L. Malaval, A.K. Gupta, Osteoblast and chondroblast differentiation, Bone 17 (1995) 77S–83S.
[29] R. Kilav, O. Bell, S.Y. Le, J. Silver, T. Naveh-Many, The parathyroid hormone mRNA 3′-untranslated region AU-rich element is an unstructured functional element, J. Biol. Chem. 279 (2004) 2109–2116.
[30] L.J. Suva, G.A. Winslow, R.E. Wettenhall, R.G. Hammonds, J.M. Moseley, H. Diefenbach-Jagger, C.P. Rodda, B.E. Kemp, H. Rodriguez, E.Y. Chen, A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression, Science 237 (1987) 893–896.
[31] N. Kimura, R. Matsuo, H. Shibuya, K. Nakashima, T. Taga, BMP2-induced apoptosis is mediated by activation of the TAK1-p38 kinase pathway that is negatively regulated by Smad6, J. Biol. Chem. 275 (2000) 17647–17652.
[32] T. Bellido, M. Han, S. Mocharla, S. Said, C. O'Brien, R.L. Jilka, S. Manolagas, PTH prevents glucocorticoid induced apoptosis of osteoblasts and osteocytes in vitro: direct interference with a private death pathway upstream from caspase-3, Bone 23 (2004) 518.
[33] R.L. Jilka, R.S. Weinstein, T. Bellido, P. Roberson, A.M. Parfitt, S.C. Manolagas, Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone, J. Clin. Invest. 104 (1999) 439–446.
[34] S.C. Manolagas, Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis, Endocr. Rev. 21 (2000) 115–137.
[35] E. Hay, J. Lemonnier, O. Fromigue, H. Guenou, P.J. Marie, Bone morphogenetic protein receptor IB signaling mediates apoptosis independently of differentiation in osteoblastic cells, J. Biol. Chem. 279 (2004) 1650–1658.
[36] S. Ebara, K. Nakayama, Mechanism for the action of bone morphogenetic proteins and regulation of their activity, Spine 27 (2002) S10–S15.