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# Identification of a PTH Regulated Gene Selectively Induced In Vivo During PTH-Mediated Bone Formation

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**Abstract** The biological activities of parathyroid hormone (PTH) on bone are quite complex as demonstrated by its catabolic and anabolic activities on the skeleton. Although there have been many reports describing genes that are regulated by PTH in osteoblast cells, the goal of this study was to utilize a well-established in vivo PTH anabolic treatment regimen to identify genes that mediate bone anabolic effects of PTH. We identified a gene we named PTH anabolic induced gene in bone (PAIGB) that has been reported as brain and acute leukemia cytoplasmic (BAALC). Therefore, using the latter nomenclature, we have discovered that BAALC is a PTH-regulated gene whose mRNA expression was selectively induced in rat tibiae nearly 100-fold (maximal) by a PTH 1–34 anabolic treatment regimen in a time-dependent manner. Although BAALC is broadly expressed, PTH did not regulate BAALC expression in other PTH receptor expressing tissues and we find that the regulation of BAALC protein by PTH in vivo is confined to mature osteoblasts. Further in vitro studies using rat UMR-106 osteoblastic cells show that PTH 1–34 rapidly induces BAALC mRNA expression maximally by 4 h while the protein was induced by 8 h. In addition to being regulated by PTH 1–34, BAALC expression can also be induced by other bone forming factors including $PGE_2$ and 1,25 dihydroxy vitamin $D_3$. We determined that BAALC is regulated by PTH predominantly through the cAMP/PKA pathway. Finally, we demonstrate in MC3T3-E1 osteoblastic cells that BAALC overexpression regulates markers of osteoblast differentiation, including downregulating alkaline phosphatase and osteocalcin expression while inducing osteopontin expression. We also demonstrate that these transcriptional responses mediated by BAALC are similar to the responses elicited by PTH 1–34. These data, showing BAALC overexpression can mimic the effect of PTH on markers of osteoblast differentiation, along with the observations that BAALC is induced selectively with a bone anabolic treatment regimen of PTH (not a catabolic treatment regimen), suggest that BAALC may be an important mediator of the PTH anabolic action on bone cell function. J. Cell. Biochem. 98: 1203–1220, 2006.


**Key words:** osteoblast; osteoporosis; bone anabolic; bone mineral density; BAALC

Bone remodeling occurs throughout life to maintain the structural integrity of bone and to provide a source of extracellular calcium. Bone remodeling is a coordinated process involving many cell types including bone forming osteoblasts and bone resorbing osteoclasts [Rodan, 1996]. The activities of these cells are regulated by many growth factors/cytokines and hormones including bone morphogenic proteins (BMPs), transforming growth factor beta (TGF-β), insulin-like growth factor (IGF), prostaglandins ($PGE_2$), receptor activator of NF-κB ligand (RANKL), interleukins (IL-1, IL-6), glucocorticoids, estrogen, 1,25 dihydroxy vitamin $D_3$, and parathyroid hormone (PTH)

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[Bonewald and Dallas, 1994; Canalis, 1996; Rosen et al., 1996; Hofbauer et al., 2000; Manolagas, 2000]. The coupling between the bone formation and the bone resorption processes can be negatively altered, resulting in bone loss and osteoporosis [Riggs and Melton, 1986; Manolagas, 2000]. This skeletal disorder is characterized by low bone mass and a deterioration of the bone microarchitecture. Antiresorptive therapies including estrogen replacement therapy, calcitonin, bisphosphonates, and selective estrogen receptor modulators (SERMS) can abrogate further bone loss, whereas PTH 1–34 therapy stimulates bone formation [Tashjian and Chabner, 2002].

PTH is an 84 amino acid polypeptide which binds to the G-protein coupled type 1 PTH receptor (PTH-R1) found in osteoblast cells [Abou-Samra et al., 1992; Goltzman, 1999; Juppner, 1999]. A second PTH receptor has been reported, however, its expression and role in the skeleton are not known [Goltzman, 1999; Hoare and Usdin, 2001]. Similar to the full-length protein, the N-terminal 1–34 fragment retains full functional activity [Habener et al., 1984; Goltzman, 1999; Swarthout et al., 2001]. It is well known that PTH can signal through multiple signaling pathways. Two of the most commonly studied are the cAMP-dependent protein kinase A (PKA) pathway and the phospholipase C-dependent protein kinase C (PKC) pathway [Dempster et al., 1993; Swarthout et al., 2002]. Furthermore, the biological activity of PTH is quite complex as PTH can elicit both catabolic and anabolic activity on bone. It is now well established based on human and animal studies that low dose intermittent administration of PTH results in increased bone mass while continuous high dose administration results in increased bone resorption and subsequent bone loss [Tam et al., 1982; Podbesek et al., 1983; Dempster et al., 1993; Whitfield et al., 1999; Tashjian and Chabner, 2002]. Studies have suggested that the anabolic action of PTH may be due to the abilities of PTH to increase IGF-I and IGF-II in osteoblasts, activate bone lining cells into active osteoblasts and increase the lifespan of the osteoblast by decreasing cell apoptosis [Dempster et al., 1993; Manolagas, 2000]. In contrast, the PTH catabolic action may be mediated by the ability of PTH to increase expression of collagenase, IL-6, IL-11, and RANKL, and decrease osteoprotegerin expression, thereby resulting in an increase in osteoclast formation [Manolagas, 2000; Ma et al., 2001; Qin et al., 2004].

Recombinant PTH 1–34 treatment (teriparatide, Forteo$^{TM}$) is currently the only bone anabolic therapy approved by the Food and Drug Administration [Tashjian and Chabner, 2002]. However, the molecular mechanisms underlying the PTH anabolic activity are not well understood. Thus exploring the PTH bone forming activity may provide identification of novel molecules and mechanisms that may prove to be advantageous for osteoporosis treatment. To learn more about how PTH elicits its anabolic action and to identify new bone anabolic targets, differential display PCR experiments were performed. Specifically, transcripts were identified that were differentially regulated from rat tibiae RNA following treatment with an anabolic (intermittent treatment) or a catabolic (continuous treatment) regimen of PTH. Here we describe the identification of a gene we designated PTH anabolic induced gene in bone (PAIGB) that was reported as brain and acute leukemia, cytoplasmic (BAALC) [Tanner et al., 2001]. For nomenclature consistency, this gene will be referred to herein as BAALC. We find that BAALC was selectively induced in bone-forming cells with a PTH anabolic treatment and we provide evidence that regulation of BAALC by PTH is mediated predominantly through a PKA/cAMP-dependent signaling pathway. Furthermore, we demonstrate that overexpression of BAALC in MC3T3-E1 cells regulates markers of osteoblast differentiation including alkaline phosphatase, osteocalcin, and osteopontin expression similar to the actions of PTH in these cells. We therefore believe that BAALC plays an important role in mediating the anabolic responses of PTH on bone cell function.

## MATERIALS AND METHODS

### Reagents

Human PTH (1–34) and PTH (3–34) were purchased from Bachem (Torrance, CA). $PGE_2$ was purchased from Cayman Chemical Company (Ann Arbor, MI). The adenylate cyclase inhibitor SQ 22536, the PKC inhibitor 19–27 and 1,25-dihydroxy vitamin $D_3$ (1,25-$(OH)_2$-$D_3$) were obtained from Calbiochem (San Diego, CA). Forskolin, cyclohexamide, and Nonidet P40 were purchased from Sigma (St. Louis, MO). Dulbecco's Modified Eagle medium

(DMEM), and DMEM with Nutrient mixture F-12 (DMEM/F12), alpha minimum essential medium (αMEM), OptiMEM, penicillin, streptomycin, Glutamax, sodium pyruvate, Lipofectamine 2000, Trizol, and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). FuGENE was purchased from Roche Diagnostic (Indianapolis, IN). Bovine serum albumin (BSA) was obtained from Serologicals Proteins, Inc. (Kankakee, IL).

### Cell Culture

Rat osteoblastic ROS 17/2.8 cells were cultured in DMEM/F12. The rat osteoblastic UMR-106 cells were cultured in DMEM and mouse osteoblastic MC3T3-E1 cells were cultured in αMEM. All media were supplemented with 1% penicillin, 1% streptomycin, 1% Glutamax, and 10% heat inactivated fetal bovine serum. The αMEM media was also supplemented with 1 mM sodium pyruvate. To investigate the regulation of BAALC mRNA expression by PTH 1–34, ROS 17/2.8 cells were seeded at $2.2 \times 10^6$ cells per 100 mm dish. UMR-106 cells were seeded at $8.5 \times 10^5$ cells per 100 mm dish or $1.42 \times 10^5$ cells per well in a 6-well dish. The cells were cultured in growth media for 3 days until confluent. The media were then removed and replaced with fresh growth media and incubated for 2 h prior to treatment. The cultures were then treated with various agents including PTH 1–34, PTH 3–34, dexamethasone, 17β-estradiol, 1,25-$(OH)_2$-$D_3$, $PGE_2$, BMP 2, and BMP6 at different time points (as described in the figure legends). For cyclohexamide experiments, cells were treated with 10 μg/ml cyclohexamide at the same time as PTH 1–34 (10 nM) treatment. In other experiments in which the adenylate cyclase inhibitor and PKC inhibitor were used, the inhibitors were added to the cultures 2 h prior to PTH 1–34 treatment. To investigate the regulation of AP, osteocalcin and osteopontin expression, MC3T3-E1 cells were plated at 17,000 cells per well (24-well plate) and then cultured for 3 days. Cells were then either infected with recombinant BAALC adenovirus, control LacZ virus (QBI), or were treated daily with a range of doses of PTH 1–34 and were harvested at the indicated times. The RNA from each of these experiments was then harvested and DNased using the RNeasy kit (Qiagen, Valencia, CA) and RNase free DNase (Qiagen) as described by the manufacturer.

### Animal Studies

All animals were produced, bred, and housed in facilities accredited by the American Association for Accreditation of Laboratory Animal Care in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. The local Institutional Animal Care and Use Committees of Wyeth Research approved the study protocols.

To perform the RADE experiments, mature female Sprague–Dawley rats (2.5–3-month-old, 225–250 g) were ovariectomized (OVX) and 3 weeks post OVX they were treated with vehicle (0.1% BSA, 0.01% acetic acid) or human PTH 1–34, 40 μg/kg of body weight for 8 days. The total BMD was measured prior to and following OVX to determine if osteopenia was established. Subcutaneous (s.c.) injection of PTH once a day represented intermittent administration of PTH, while an osmotic pump with a release rate of 40 μg/kg/day implanted under the skin was used as a continuous regimen of PTH administration. Animals were sacrificed and samples were collected for further analysis 6 h after the final treatment. Each treatment group contained eight animals.

The tibiae were dissected free of soft tissue, and the trabecular bone and marrow cavities were flushed with ice-cold sterile PBS to remove the marrow and the clean bone was placed in liquid nitrogen. A Bessman tissue pulverizer (Fisher Scientific, Pittsburgh, PA) was used to reduce four tibiae at a time to a powder that was then transferred to 50 ml tubes containing 4 ml Trizol reagent (Invitrogen) for subsequent homogenization. The final RNA isolation was performed according to the manufacturer's protocol. RNA was also isolated from brain, heart, and kidney using Trizol.

To perform the PTH time course experiments, OVX rats (as described above) were treated with 40 μg/kg human PTH (1–34) once daily by s.c. injection. Six hours after the last injection the animals were sacrificed (6 h, 2, 4, and 8 days) and tibiae were collected for RNA isolation.

### Bone Mineral Density (BMD) Measurements

Prior to the start of treatment (baseline), and at the end of the treatment period (post-treatment), volumetric BMD (vBMD, $mg/cm^3$) of the proximal tibiae was evaluated in anesthetized rats by

peripheral quantitative computed tomography (pQCT) using an XCT-960M instrument (XCT Research, Stratec Medizintechnik, Pforzheim, Germany). A 1 mm thick pQCT slice obtained 3.4 mm distal from the proximal end of the tibia was used to compute total and trabecular density for the proximal tibial metaphysis. The tomographic slice had an in-plane voxel (three-dimensional pixel) size of 0.140 $mm^3$. After acquisition, the image was displayed and the region of interest including tibia but excluding fibula was outlined. The soft tissue was automatically removed using an iterative algorithm, and the density of the entire bone (total density, $mg/cm^3$) in the slice was determined. For trabecular density determination, the outer 55% of the bone slice was then peeled away in a concentric spiral and the value of the trabecular density was reported in $mg/cm^3$. The change in bone density was calculated for each animal by subtracting the post-treatment from the baseline bone density values.

### Differential Display PCR

Differential display PCR was performed by rapid analysis of differential expression (RADE) technology [Liang et al., 1994; Sampath et al., 2001] that was licensed through Millenium Pharmaceuticals (Cambridge, MA). Total RNA was isolated and then treated with 20 U of DNase (Ambion, Austin, TX). The samples were split into two reverse transcription (RT) reactions. Each RT reaction contained 4 μg RNA, 1,000 U SuperScript II RT (Invitrogen), 100 μM dNTPs and 0.2μM anchor primer (containing the sequence (5′ → 3′) $T_{11}A$, $T_{11}C$, or $T_{11}G$) in a final volume of 100 μl. For differential display PCR, each cDNA sample was subjected, in duplicate, to 80 PCR reactions using the appropriate 15 bp anchor primer and one of 80 arbitrary 13 bp primers (HAPs 1–80; Genhunter, Nashville, TN). PCR reactions contained 20 ng cDNA, 2 μM dNTPs, 0.2 μM anchor primer, 0.2 μM arbitrary primer, 1 U Taq polymerase (Perkin-Elmer, Boston, MA), and 2.5 μCi [α-$^{33}$P]dATP (2,000 Ci/mmole) in a final volume of 20 μl. PCR conditions were as follows: 1 cycle of 92°C for 2 min, 40 cycles of 92°C for 15 s, 40°C for 2 min, 72°C for 30 s, and 1 cycle of 72°C for 5 min. PCR samples were then loaded onto a 50 cm 6% crylamide sequencing gel and were run at 2,000 V. Differentially expressed bands were excised from the dried gel. Identical primers and PCR conditions as in the original reaction were used to re-amplify DNA samples recovered from the differential display gels. Products were ligatedin to pGem-T Easy (Promega, Madison, WI).

### BAALC cDNA Cloning

A 700 bp BAALC cDNA fragment was $^{32}$P labeled and used as a probe to screen a Lambda ZAP II rat brain cDNA library (Stratagene, La Jolla, CA) for the full-length BAALC clone. Plaques were selected for two additional rounds of screening and once the final clones were selected they were subjected to DNA sequence analysis. To clone the mouse BAALC cDNA, the first strand cDNA was obtained using 0.1 μg of mouse brain polyA + mRNA (Clontech, Palo Alto, CA) and a Takara RNA PCR kit (Takara Biomedicals, Shiga, Japan) as described by the manufacturer. The cDNA was then subjected to PCR using the forward primer 5′-GCAGCAGC-CACAGCCGCAAG-3′ and the reverse primer 5′-CGGCAAAGGAGAAGACATAC-3′. The PCR product was subsequently cloned into pCR Blunt II TOPO vector (Invitrogen) and DNA sequence verified. The human full-length BAALC cDNA clone was obtained from the Genescope clone collection (Invitrogen) using the rat BAALC cDNA as a query sequence. PCR amplification of the human ORF was performed using human brain total RNA (Clontech, Mountain View, CA) with forward primer 5′-GAA-GATCTCCACCATGGGCTGCGGCGGGAGC-3′ and reverse primer 5′-GAAGATCTCTAGTT-GACACAGTTCT-TTG-3′.

### 5′ RACE Analysis

To extend the 400 bp BAALC DNA fragment obtained from the RADE analysis and to ensure that we obtained the entire coding sequence for human, mouse, and rat BAALC, 5′ RACE experiments were performed using SmartRACE (Clontech) as described by the manufacturer. Briefly, 0.1 μg of either human, rat, or mouse brain polyA + mRNA (Clontech) was mixed with Smart II A oligo and oligo dT primers and incubated at 72°C for 2 min and then placed on ice for 2 min. The buffer, including a dNTP mix and PowerScript reverse transcriptase, was added to the sample and incubated at 42°C for 1.5 h to allow the synthesis of RACE ready first strand cDNA. The RACE ready cDNA was amplified using Oligo II specific primers and BAALC 5′ specific primers including rat

5′-GTCGGTGTAGGTGAGCCAGGTGGACTC-3′, rat 5′-GATTCCACTGCAATGGTTGGTCCT-3′, rat 5′-AACCGGGATGGTCGTCACCGCGTG-3′, rat 5′-CTGTCCATCTGCCGGATATTC-TC-TG-3′, human 5′-CGGTGTAGGTGAGCCAG-GTGGATTCTG-3′, and mouse 5′-GTCGG-TGTAGGTGAGCCAGGTGGACTC-3′ in the presence of GC melt (Clontech). The PCR products were directly cloned into the TOPO TA cloning vector (Invitrogen) followed by DNA sequence analysis.

### BAALC Polyclonal Antibody Production

The BAALC polyclonal antibody was developed for these studies by Invitrogen. The antibody was raised against amino acid sequence RADAIEPRYYESWTRE that recognizes human, rat, and mouse BAALC protein. Briefly, New Zealand white rabbits (ranging from 3 to 9 months of age) were immunized with the KLH conjugated peptide mixed with an equal volume of Freund's Adjuvant. The rabbits were immunized with four subcutaneous dorsal injections over a period of 8 weeks with 0.1 mg of peptide per immunization. The serum was collected following the last immunization and the titer was determined by ELISA using peptide bound to a microtiter plate.

### BAALC Western Blot Analysis

UMR-106 cells ($2 \times 10^6$) were cultured in growth medium in 100-mm dishes overnight, then washed with PBS followed by a fresh serum-free DMEM (containing 0.25% BSA) medium replacement for 24 h. The cells were then treated with 100 nM PTH and harvested at the indicated times following treatment. Cytosolic and membrane protein extracts were obtained using the Qproteome Cell Compartment Kit (Qiagen) as described by the manufacturer. The membrane fraction contains proteins from the plasma membrane and organelle membranes (e.g., the ER and mitochondria). Protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, IL) as described by the manufacturer. Equal amounts of protein were separated using a NuPAGE 4%–12% Bis-Tris reduced gel (Invitrogen) followed by Western blotting using the BAALC polyclonal antibody and WesternBreeze kit (Invitrogen) as described by the manufacturer.

### BAALC Recombinant Adenovirus Development

The full-length coding sequence of Human BAALC with a 5′ Kozak sequence (CCACC) was amplified by RT-PCR, with the oligonucleotide forward primer 5′-GAAGATCTCCACCATGG-GCTGCGGCGGGAGC-3′ and reverse primer 5′-GAAGATCTCTAGTTGACACAGTTCTTTG-3′. The resulting 443 bp PCR product was then cloned into the Adenovirus transfer vector pQBI-AdCMV5-IRES-GFP (Qbiogene, Irvine, CA) and co-transfected into 293A cells with QBI-Viral DNA backbone. Multiple plaques were screened for BAALC overexpression by Western analysis using the BAALC polyclonal antibody. BAALC expressing viruses were purified by three rounds of plaque screening.

### BAALC mRNA Expression Analysis

The regulation of BAALC mRNA expression in rat tibiae was determined by Northern blot analysis. Ten micrograms of total tibiae RNA was separated on a 1.2% agarose gel in 0.02 M MOPS buffer containing 2.2 M formaldehyde. The RNA was then transferred to a nylon membrane (Schleicher & Schuell, Keene, NH) using Turbo-blot (Schleicher & Schuell) as described by the manufacturer. The membrane was probed with either the 400 bp RADE fragment or with a probe to rat cyclophilin B (to account for RNA loading differences). Hybridization with $^{32}$P-labeled probes was performed using ExpressHyb (Sigma) at 68°C overnight. The blots were washed two times at room temperature for 15 min in low stringency buffer containing 2× SSC, 0.1% SDS and once at 65°C for 15 min in high stringency buffer containing 0.1× SSC, 0.1% SDS. The blots were exposed overnight using Kodak film. To determine the mRNA expression pattern of BAALC, Northern blot analysis was also performed on mouse multiple tissue blots (Clontech).

In addition, BAALC mRNA expression was assessed using a PCR-based method with both mouse and human Rapid Scan panels (OriGene Technologies, Rockville, MD). Briefly, 35 cycles of PCR were performed using eLONGase DNA polymerase (Invitrogen), Advantage GC buffer mix (Clonetech), and either the human primers, forward 5′-ATGGGCTGCGGCGGGAGC-3′ and reverse 5′-GATCAACTGTGTCAAGAAAC-3′ or mouse primers, forward 5′-CCCCGCTACTAC-GAGAGTTG and reverse 5′-CTACGTCAAGAA-CCACTGGG-3′. Where indicated, densitometry

was performed using a BioRad Fluro-S Multi-Imager.

### Real Time Quantitative RT-PCR (Taqman™)

Quantitative analysis of BAALC, alkaline phosphatase (AP), osteocalcin, and osteopontin mRNA expression was determined by Taqman™ using an AmpliTaq Gold Polymerase reagent kit and a Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Briefly, 100 ng of total RNA is used in a one step reaction mixture containing 1× Taqman™ buffer A, 5.5 mM $MgCl_2$, 0.3 mM dNTP's, 0.025 U AmpliTaq Gold, 0.25 U Multiscribe, 0.05 U RNase inhibitor, 500 nM BAALC rat forward primer 5′-CCATGCTCTGATATGGACCCTT-3′, 500 nM BAALC rat reverse primer 5′-TCAAACTCAGGCTGTGCCATAC-3′, 200 nM BAALC rat probe 6FAM-5′-CCCACATTCCTAAACACATCCTCCTGCAA-3′, or 40 nM of the rodent forward and reverse GAPDH primers and 50 nM of the GAPDH probe (Applied Biosystems). The mouse alkaline phosphatase primers and probe include 500 nM forward primer 5′-GAGACCCACGGTGGAGAAGA-3′, 500 nM reverse primer 5′-GGAGGCATACGCCATCACAT-3′, and 200 nM probe 6FAM-5′-CGGCGTCCATGAGCAGAACTACATTCC-3′. The mouse osteopontin primers and probe include 500 nM forward primer 5′-CCCTCGATGTCATCCCTGTT-3′, 500 nM reverse primer 5′-CTTTCCGTTGTTGTCCTGATCA-3′, and 200 nM probe 6FAM-5′-CCCAGCTTCTGAGCATGCCCT-3′. The mouse osteocalcin primers and probe include 500 nM forward primer 5′-CGGCCCTGAGTCTGACAAA-3′, 500 nM reverse primer 5′-GCCGGAGTCTGTTCACTACCTT-3′, and 200 nM probe 6FAM-5′-CCTTCATGTCCAAGCAGGAGGG-3′. Extracellular matrix gene expression was normalized to Cyclophilin B (PPIB) using 40 nM forward primer 5′-TGGAGAGCACCAAGACAGACA-3′, 40 nM reverse primer 5′-TGCCGGAGTCGACAATGAT-3′ and 50 nM probe 6FAM-5′-CCGGGACAAGCCACTGAAGGATGT-3′. The thermocycler was programmed to run at 48°C for 30 min to generate the cDNA, followed by 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min.

### Immunohistochemical Analysis of BAALC Expression In Vivo

Swiss Webster mice were treated with 20 μg/kg/day PTH 1–34 subcutaneously applied onto the calvaria for 18 days. Following this PTH anabolic treatment [Zhao et al., 2001], the calvariae were harvested and BAALC protein expression was analyzed using the BAALC polyclonal antibody. Briefly, the mouse calvariae were gently dissected and fixed in 10% phosphate-buffered formalin. After fixation, the calvariae were dissected perpendicular to the sagittal suture through the central portion of the parietal bones parallel to the lambdoidal and coronal sutures. The samples were then decalcified in TBD-2 decalcifying agent (Shandon Lipshaw, Pittsburgh PA) for 7–8 h, dehydrated in graded alcohol and embedded in paraffin. Four to six 5-μm thick representative, non-consecutive step sections were cut. The bone samples were then deparafinized in xylene and rehydrated in graded ethanol and PBS. Sections were treated with 0.3% $H_2O_2$/methanol for 30 min at room temperature and then digested with 1:1,000 diluted proteinase K (Invitrogen) for 30 min at 37°C. After blocking with normal horse serum for 30 min, the sections were incubated overnight at 4°C with the BAALC rabbit polyclonal antibody. Sections were then washed three times each for 10 min with PBS. The binding of BAALC antibody was assayed using a biotinylated secondary antibody and an avidin-linked peroxidase Vectastain kit (Vector Laboratories, Burlingame, CA). Controls included samples incubated with the antibody-peroxidase kit but without BAALC polyclonal antibody. The peroxidase was detected using peroxidase substrate kit DAB (Vector Laboratories).

### Statistical Analysis

The results are presented as means ± SD or SEM of three or more replicates for each experiment and are representative of a minimum of two–three similar experiments unless otherwise indicated. The data were analyzed for statistical significance by unpaired Student's *t*-test or one-way ANOVA as indicated in the figure legends.

## RESULTS

### BAALC Expression Induced Only With an Anabolic PTH Treatment Regimen

We determined that intermittent PTH treatment of OVX Sprague–Dawley rats induced significant increases in total BMD of already osteopenic bones, with Δ BMD of 80 mg/cm$^3$ ($P < 0.01$ compared to vehicle), while continuous PTH administration did not induce significant

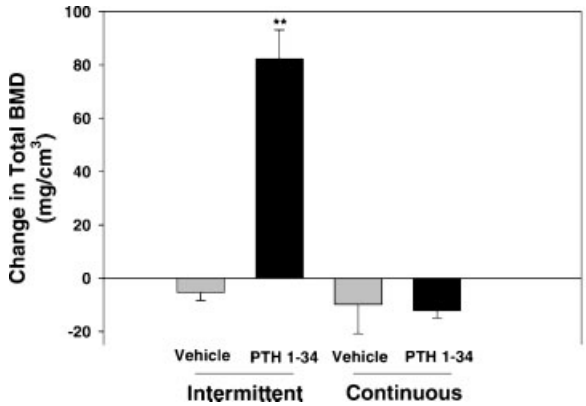


**Fig. 1.** The effects of 8 days of intermittent subcutaneous (s.c.) injection or continuous infusion (pump) with vehicle or PTH 1–34 (40 μg/kg/day) treated ovariectomized rats on tibial BMD. The change in bone density was calculated for each animal by subtracting post-treatment from baseline bone density values. $^{**}P < 0.01$ compared to corresponding vehicle (one-way ANOVA).

changes (Fig. 1). RNA from bone samples after intermittent and continuous administration was isolated and analyzed by differential display PCR using RADE technology. One differentially regulated gene we identified as PAIGB, which is reported as BAALC, was induced in the samples from bones after intermittent administration of PTH, while continuous PTH injection did not induce any changes in expression (Fig. 2A). PCR analysis of the isolated band revealed a 400 bp DNA product. Using the 400 bp isolated DNA fragment as a probe for Northern blotting, we demonstrated that there is low endogenous expression of BAALC in the rat tibiae and we confirmed that BAALC was induced only with an anabolic treatment regimen of PTH (Fig. 2B). In addition, we show that PTH 1–34 induced two BAALC mRNA transcripts approximately 2.4 and 3 kb in size (Fig. 2B).

### Full-Length BAALC is Highly Conserved

Using the 400 bp RADE nucleotide sequence in conjunction with additional sequence obtained from 5′ RACE experiments, the 2.2 kb rat BAALC cDNA was cloned from a rat brain cDNA library. The cDNA revealed a 438 bp ORF that encodes a 145 amino acid protein that has no known functional motifs. Using the rat cDNA as a query, sequence analysis of the public, and Celera databases (Celera Discovery System and Celera associated databases) identified mouse and human homologues showing 95% and 87% DNA sequence identity within the ORF, respectively, and 97% and 82% amino acid similarity, respectively (Fig. 3). We obtained the full-length human cDNA from a Genescope library, while the mouse full-length cDNA was cloned by RT-PCR. The murine BAALC cloning efforts revealed a splice variant missing the second of three exons encoding the full-length ORF, which was consistent with the original BAALC report [Tanner et al., 2001]. Splicing of this second coding exon creates a stop codon at the end of exon 1 resulting in a truncated 54 amino

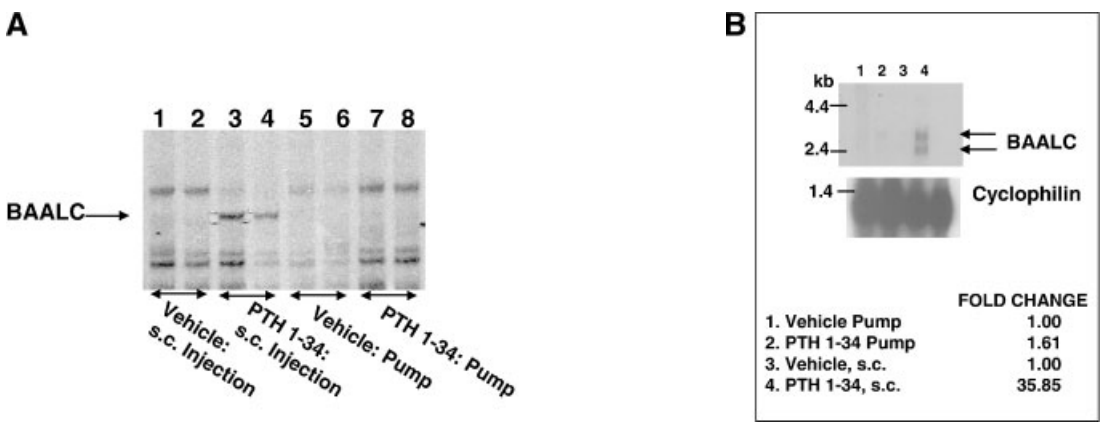


**Fig. 2.** Analysis of BAALC mRNA expression from rat tibiae RNA samples. Ovariectomized rats were treated for 8 days with a single daily (intermittent) s.c. injection or continuous administration with vehicle or PTH 1–34 (40 μg/kg/day). **Panel A** shows the RADE analysis of replicate RNA samples identifying BAALC mRNA induction with intermittent PTH 1–34 administration (**lanes 3–4**) compared to continuous PTH 1–34 administration (**lanes 7–8**) or vehicle control samples (1–2, 5–6). **Panel B** represents the Northern Blot analysis of BAALC mRNA expression of tibiae RNA samples taken from the vehicle or PTH 1–34-treated rats.

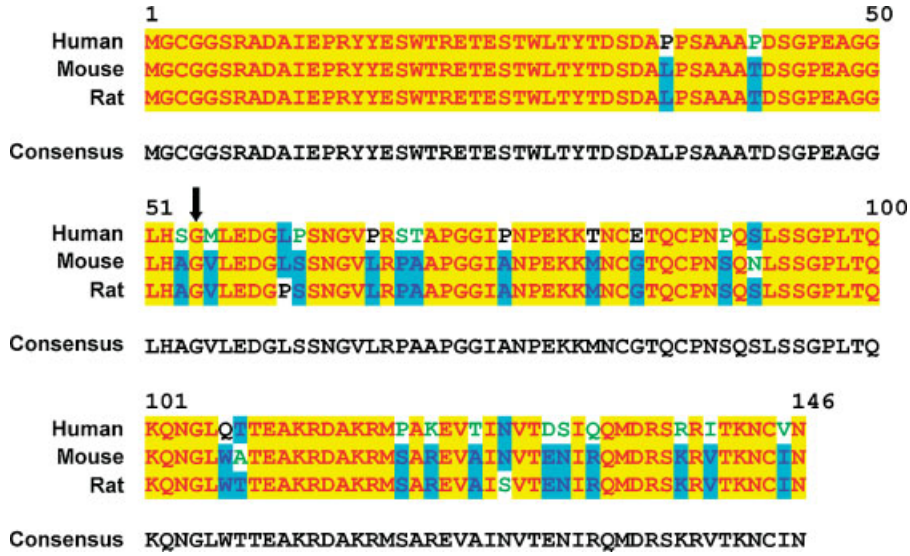


**Fig. 3.** Comparison of the full-length BAALC amino acid sequence between human, mouse, and rat species. The sequences in red/yellow represent 100% sequence identity, green indicates similar, blue indicates conserved, and black indicates non-similar. The arrow indicates the location of the C-terminal amino acid of the 54 amino acid BAALC splice variant.

acid protein. Using the coding sequence to the human BAALC sequence, we were also able to clone by RT-PCR the same splice variant that was expressed in mouse.

### BAALC is Broadly Expressed

Northern blot analysis was performed to characterize the expression pattern of BAALC in various tissues. Although our Northern blot data from rat tibiae showed little to no endogenous levels of BAALC, a mouse multiple tissue blot (Fig. 4A) demonstrated that BAALC is highly expressed in brain and to a much lesser extent in heart, lung, and kidney. Furthermore, the Northern blot demonstrated two distinct mRNA transcripts in these tissues, which is consistent with the rat tibiae RNA Northern blot data and our cloning results. However, a larger 4 kb transcript was also found in mouse brain and is likely yet another splice variant of the gene [Tanner et al., 2001]. A more extensive survey of BAALC expression using a PCR-based Rapid Gene Scan analysis (Fig. 4B) of human tissues revealed the most pronounced expression of BAALC to be in the brain, heart, adrenal gland, prostate, skin, uterus, placenta, and fetal brain. Using this methodology, BAALC could be detected in the testis and muscle where it was undetected in the mouse multiple tissue Northern. In addition, we find expression of both the full length and splice variant in U2OS and UMR-106 osteoblasts (data not shown). A mouse Rapid Gene Scan panel showed a similar expression profile and additionally demonstrated BAALC expression in the mouse embryo as early as day 8.5 (data not shown).

### BAALC Regulation by PTH Appears to be Bone Specific

OVX rats were treated with intermittent PTH injections (40 μg/kg body weight) and tibiae were collected at 6 h, 2, 4, and 8 days. The data demonstrate a small but significant induction of BAALC mRNA expression after 6 h of PTH treatment ($P < 0.05$ compared to control) followed by an 80-fold increase in BAALC expression by 2 days post-treatment (Fig. 5A). We also analyzed BAALC expression after intermittent PTH treatment in other PTH target tissues including brain, kidney, and heart. As shown in Figure 5B, intermittent administration of PTH did not result in changes in BAALC expression in any of the tissues tested suggesting that the effect of PTH on BAALC expression may be bone specific.

### Regulation of BAALC Protein in Calvarial Osteoblasts In Vivo

Additional studies were performed using a mouse calvarial model to investigate the regulation of BAALC at the protein level in vivo. We observed an induction of BAALC protein by

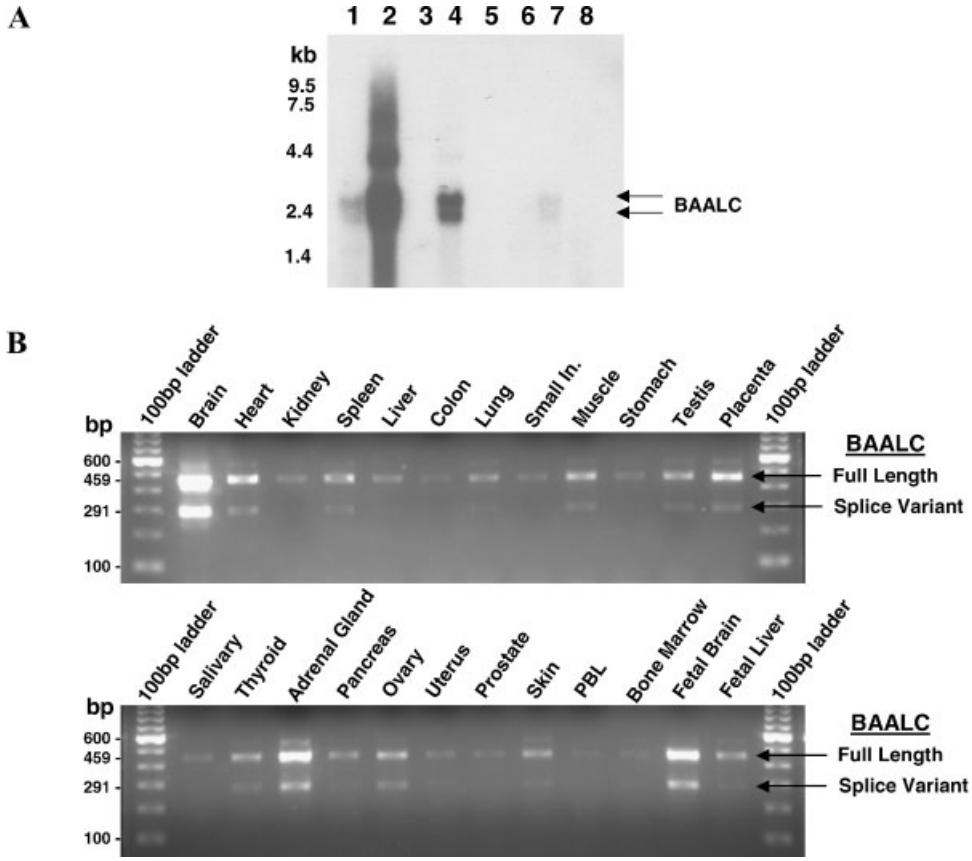


**Fig. 4.** **Panel A**: Multiple tissue Northern Blot analysis demonstrating the endogenous expression of BAALC mRNA in various mouse tissues. The tissues include heart (1), brain (2), spleen (3), lung (4), liver (5), skeletal muscle (6), kidney (7), and testis (8). **Panel B**: Rapid Gene Scan analysis of endogenous BAALC mRNA expression in various human tissues. PCR primers that would amplify both the full length and splice variant forms of BAALC were utilized.

PTH in the periosteum and endosteum of the calvarial bone at a dose that was anabolic as demonstrated by a 7% ($P < 0.05$) increase in calvarial thickness compared to vehicle-treated calvariac (data not shown). Figure 6 demonstrates that BAALC protein expression was induced by PTH in the periosteal osteoblasts adjacent to the bone surface (Fig. 6B,D). In addition, BAALC protein expression was also induced by PTH in endosteal osteoblasts but not in the stromal cells of the bone marrow cavity (Fig. 6F,H).

### BAALC Regulation by PTH is Dependent on Protein Expression and is Mediated Predominantly via PKA/cAMP Signaling

To further characterize the regulation of BAALC by PTH in vitro, a time course study in UMR-106 cells, using 10 nM PTH, showed that BAALC mRNA expression was induced maximally (250-fold) after 4 h of treatment and then declined to untreated levels by 48 h (Fig. 7A). PTH regulation of BAALC was also observed in ROS17/2.8 cells (Fig. 7C,D) and U2OS osteoblastic cells (data not shown) but the fold induction (2–6-fold) was significantly less than that observed in UMR-106 cells. Furthermore, we demonstrate that the induction of BAALC mRNA expression by PTH in UMR-106 cells was attenuated by cyclohexamide at all time points where PTH was shown to regulate BAALC (Fig. 7A). We also demonstrate from the membrane fraction of UMR-106 cells, that PTH modestly but reproducibly, induces (1.6–1.8-fold) BAALC protein expression maximally 8 h following treatment (Fig. 7B) and is back to control levels by 24 h (data not shown). No regulation was observed in the cytoplasmic fraction at any time points analyzed (data not shown).

To examine which PTH signaling pathway is involved in the regulation of BAALC expression by PTH, we utilized ROS 17/2.8 cells that were

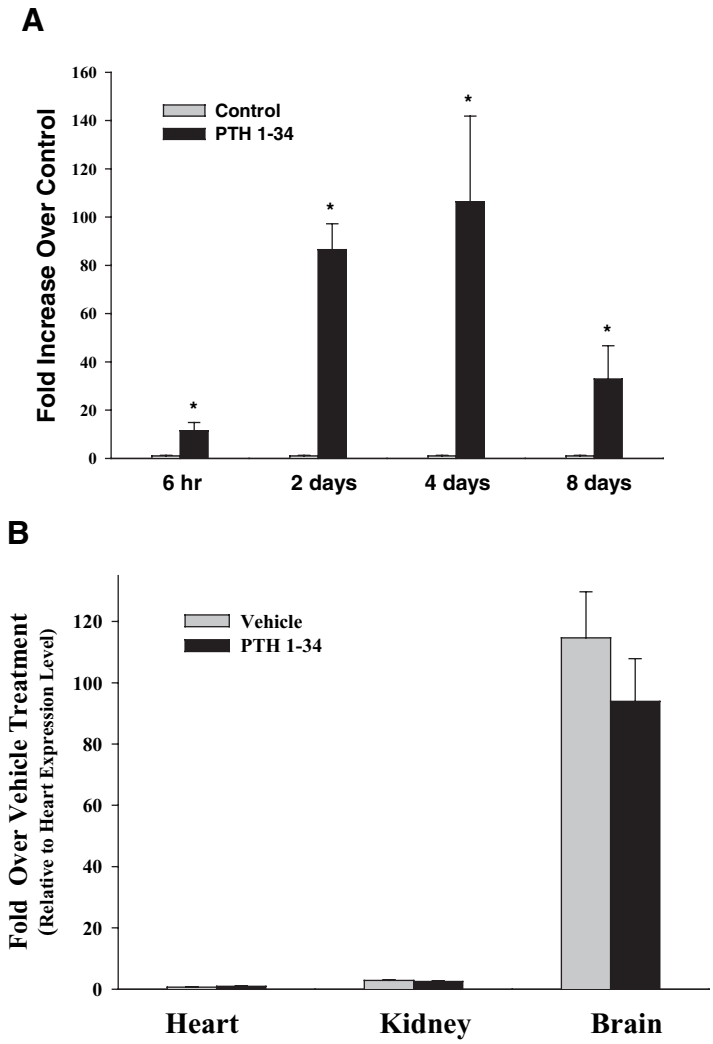


**Fig. 5.** Quantitative analysis of BAALC mRNA expression from tibiae samples taken from ovariectomized rats treated with an intermittent regimen of PTH 1–34. **Panel A** represents a time course of BAALC mRNA induction by PTH 1–34 (40 μg/kg/day), $^*P < 0.05$ compared to control (Student's *t*-test). **Panel B** illustrates the effects of intermittent PTH 1–34 treatment on BAALC mRNA expression in non-bone tissues. Rats were treated with intermittent (s.c.) PTH 1–34 (40 μg/kg/day) for 8 days. Quantitative RT-PCR results were compared to the control for each tissue and then normalized to the expression levels found in heart.

treated with 10 nM PTH in the presence or absence of 200 μM SQ 22536 (an adenylyl cyclase inhibitor) and Protein Kinase C inhibitor 19–27 (10 μM). Cells pretreated for 2 h with SQ22536 followed by 4 h of PTH treatment completely blocked the regulation of BAALC mRNA expression by PTH (Fig. 7C). Consistent with these results, treatment of ROS 17/2.8 cells with PTH 3–34, which has been reported to have minimal effects on PKA activity [Cole et al., 1989; Chakravarthy et al., 1990; Fujimori et al., 1992; Armamento-Villareal et al., 1997], was not able to induce BAALC expression. Furthermore, forskolin, an activator of adenylate cyclase, significantly induced BAALC expression ($P < 0.05$ compared to vehicle control). Conversely, when we treated cells with PKC-signaling inhibitors, there was very little effect on PTH-mediated stimulation of BAALC expression, suggesting that PTH exerts its action on BAALC expression predominantly through PKA/cAMP signaling (Fig. 7D).

### BAALC is Also Regulated by $PGE_2$ and $1,25\text{-}(OH)_2\text{-}D_3$

Evaluation of other factors that influence bone metabolism demonstrated that 100 nM $PGE_2$ and $1,25\text{-}(OH)_2\text{-}D_3$ induced BAALC

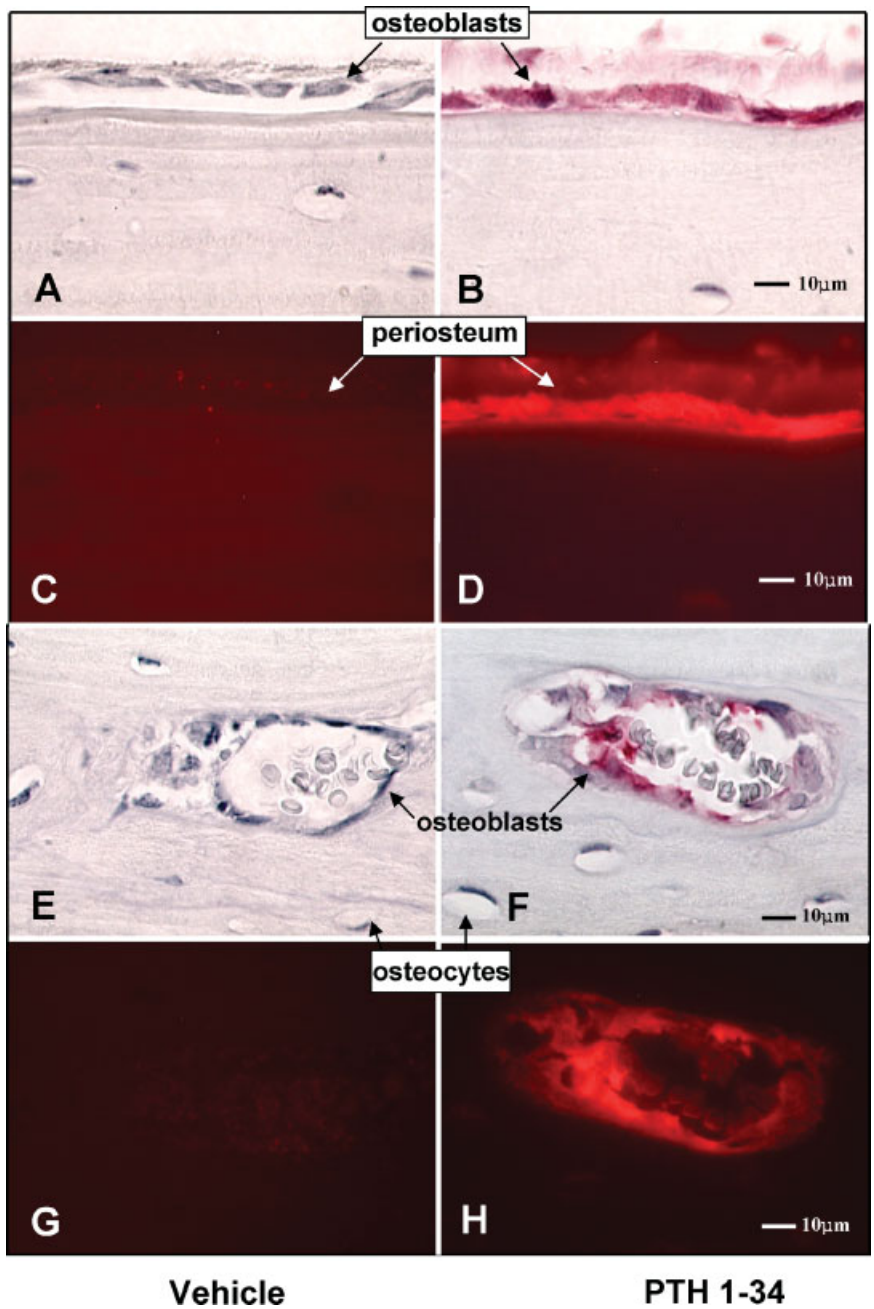


**Fig. 6.** Regulation of BAALC protein expression by PTH 1–34 in mouse calvariae. Mice were treated s.c. with either vehicle (**Panels A**, **C**, **E**, **G**) or 20 μg/kg/day PTH 1–34 (**Panels B**, **D**, **F**, **H**) directly onto the calvaria for 18 days. Induction of BAALC protein expression by PTH 1–34 is demonstrated in osteoblasts of the periosteum (Panels B, D) and the endosteum (Panels F, H) as illustrated by bright field images (Panels B, F) and dark field images (Panels D, H).

mRNA expression 75- and 50-fold, respectively (Fig. 8). Other agents including 17β-estradiol, BMP2, and BMP6 had minimal to no effect on BAALC expression in UMR-106 cells even after 24 h of treatment (Fig. 8). Similar effects were observed in ROS 17/2.8 cells (data not shown), however, the relative magnitude of regulation by these factors was significantly less in the ROS 17/2.8 cells compared to the UMR-106 cells.

### BAALC Overexpression Regulates Markers of Osteoblast Differentiation

To investigate the effects of BAALC expression on osteoblast function, overexpression experiments were performed with MC3T3-E1

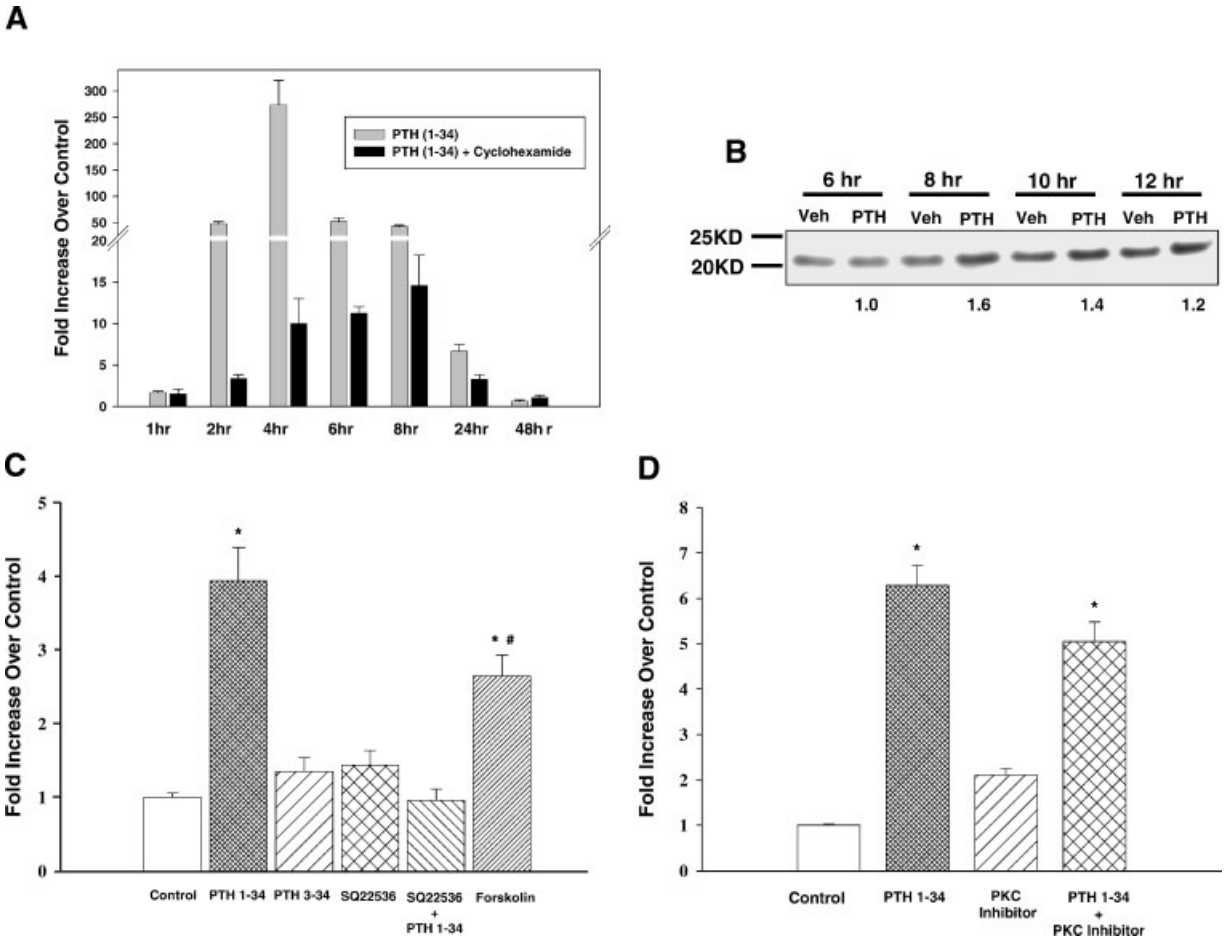


**Fig. 7.** **Panel A**: A time course of BAALC mRNA induction in UMR-106 cells by 10 nM PTH 1–34 in the presence or absence of 10 μg/ml of cyclohexamide. **Panel B** represents a Western blot illustrating the effect of PTH 1–34 (100 nM) on BAALC protein expression at the indicated time points. Values below the PTH treated lanes represent the fold change relative to vehicle treated cultures as assessed by densitometry (data represent two independent experiments). **Panel C** demonstrates the effects of 200 μM adenylyl cyclase inhibitor (SQ 22536) alone or in combination with PTH 1–34 (10 nM), 10 μM forskolin or PTH 3–34 (10 nM) compared to 10 nM PTH 1–34 in ROS 17/2.8 cells. **Panel D** demonstrates the effects of the PKC inhibitor (10 μM) alone or in combination with 10 nM PTH 1–34 compared to 10 nM PTH 1–34 treatment alone in ROS 17/2.8 cells. In all experiments, the expression of BAALC is normalized to the vehicle control treated cells. $^{*}P < 0.05$ compared to control values and $^{\#}P < 0.05$ compared to PTH 1–34 values (one-way ANOVA).

cells to assess the effects on osteoblast marker expression. Figure 9 demonstrates that BAALC overexpression in MC3T3-E1 cells downregulates the expression of AP and osteocalcin mRNA expression by 20%–60% (Fig. 9A,B, respectively), while osteopontin was induced 3–14-fold (Fig. 9C) compared to treatment with the control virus. PTH 1–34 treatment of MC3T3-E1 cells causes similar changes in the expression of these extracellular matrix genes (Fig. 9D,E,F).

## DISCUSSION

Since PTH plays such an important role in maintaining skeletal homeostasis via its anabolic and catabolic activities, there have been many studies aimed at elucidating the mechanisms underlying these opposing activities. In addition, due to the therapeutic implications of PTH as a bone anabolic treatment for osteoporosis, many laboratories have focused their efforts on characterizing this anabolic activity by assessing the effects of PTH on osteoblast differentiation, gene expression, cell proliferation, and apoptosis [Dempster et al., 1993; Ishizuya et al., 1997; Adams et al., 1999; Jilka et al., 1999; Swarthout et al., 2001, 2002; Qin et al., 2003].

The goal of this study was to identify genes that mediate only the bone anabolic actions of PTH. Although there have been many in vitro

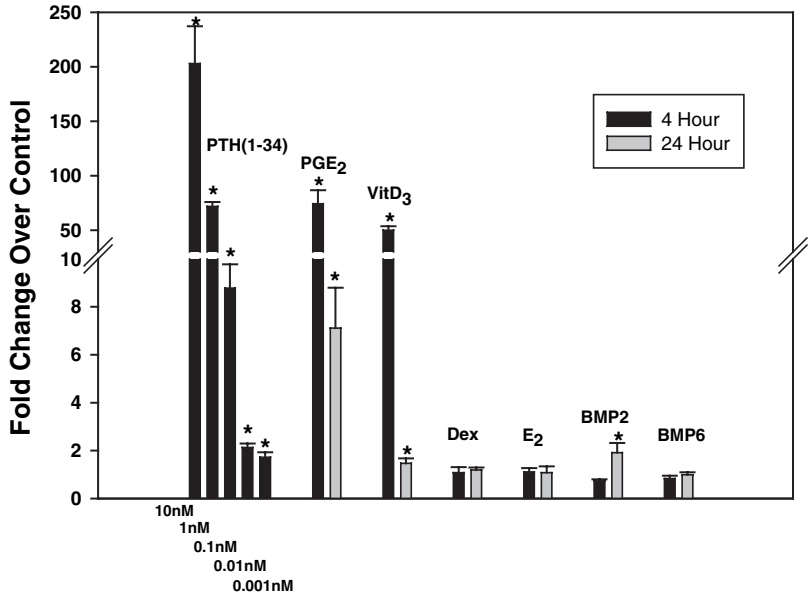


**Fig. 8.** The effects of bone related factors on regulation of BAALC mRNA expression in UMR-106 cells as determined by quantitative RT-PCR. Cells were treated for either 4 h or 24 h as indicated with either PTH 1–34, $PGE_2$ (100 nM), 1,25-$(OH)_2$-Vit $D_3$ (100 nM), 100 nM dexamethasone (Dex), 100 nM 17β-estradiol ($E_2$), BMP2 (300 ng/ml) or BMP6 (300 ng/ml). BAALC expression was normalized to the vehicle control samples. *$P < 0.05$ compared to control values (Student's *t*-test).

studies to determine which genes are regulated in osteoblasts by PTH, there are no clearly defined in vitro PTH treatment regimens that can be exploited to identify genes that are specifically associated with its anabolic activity. Furthermore, the gene responses in vitro have been shown to vary depending on the state of differentiation of the osteoblast, the time of exposure, the amount of PTH administered and the mode of administration [Hall and Dickson, 1985; Jongen et al., 1993; Isogai et al., 1996; Ishizuya et al., 1997; Schiller et al., 1999]. Therefore, since the in vivo PTH anabolic treatment regimens are well established, we combined an in vivo treatment approach with differential display PCR technology to identify genes that modulate the bone anabolic effects of PTH.

Here we demonstrate that BAALC is induced in rat tibiae only under PTH anabolic treatment conditions. We show that the PTH mediated induction of BAALC mRNA in the tibiae occurred 6 h following PTH treatment, the earliest time point evaluated. In vitro analysis with UMR-106 cells showed induction of BAALC mRNA as early as 2 h, and since the protein inhibitor cyclohexamide was able to attenuate this induction, expression of another factor(s) must be required for BAALC regulation. Although an extensive PTH gene array study was reported by Qin et al. [2003] in UMR-106 cells and by Onyia et al. [2005] from rat bone, BAALC was not identified in these studies because the rat Affymetrix RG_U34A oligonucleotide array that was used did not contain the BAALC probe set sequences.

We have evaluated the regulation of BAALC protein in a mouse calvarial model involving the direct administration of PTH subcutaneously in an anabolic fashion. Here we demonstrated that BAALC was induced in the mature osteoblasts most adjacent to the bone surface within the periosteum and endosteum of the calvariae. We observed minimal regulation of BAALC in the stromal cells within the marrow compartment adjacent to the endosteum. This pattern of BAALC regulation is highly consistent with the reported pattern of the PTH receptor in bone, as the highest levels of receptor were reported to be expressed in the active osteoblasts closest to the bone surface while the marrow cells express very little receptor [Fermor and Skerry, 1995]. This report also noted expression of the PTH receptor in the osteocytes, however, the expression was only observed in a portion of the osteocytes. This may

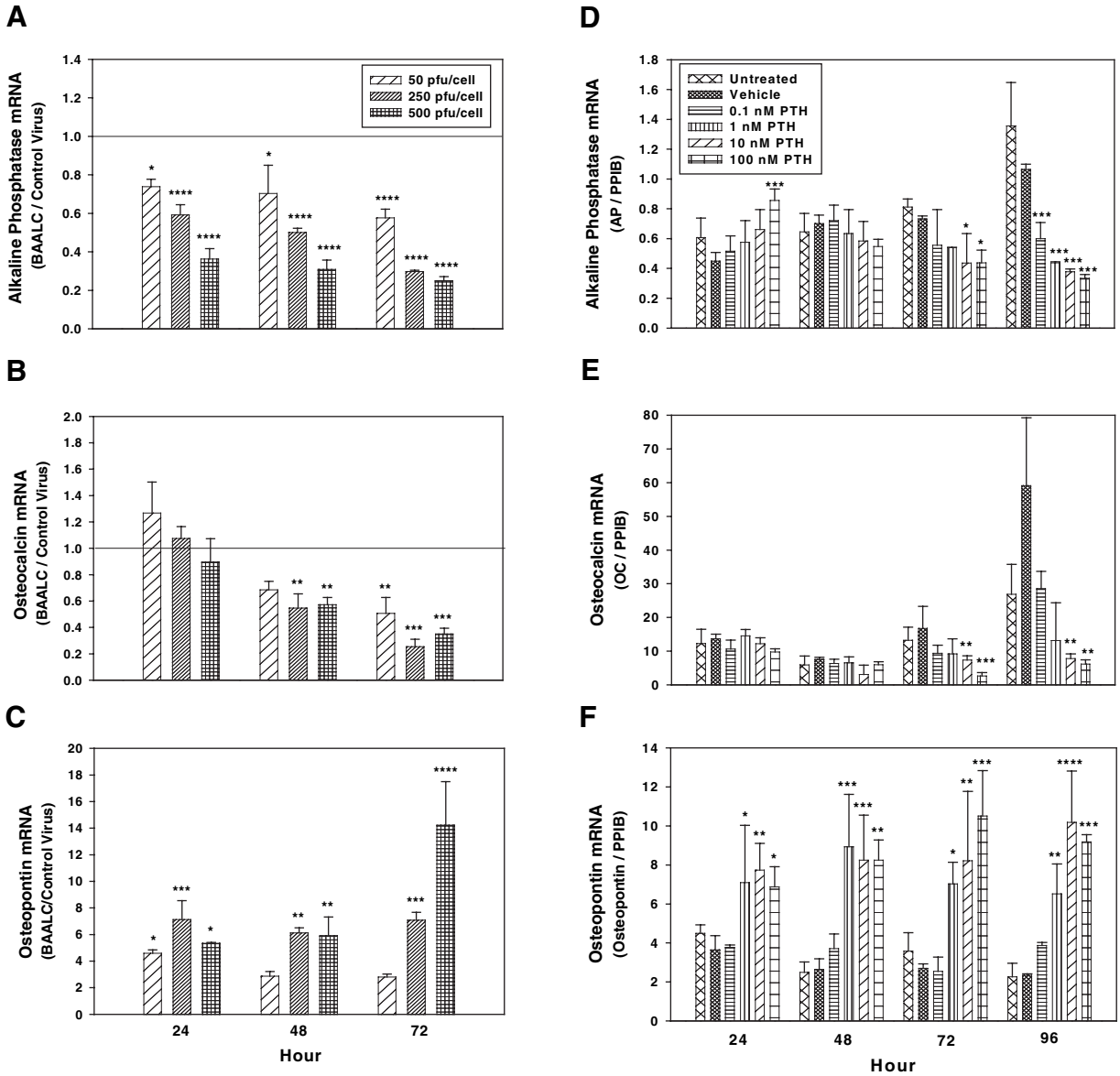


**Fig. 9.** **Panels A–C** show the effects of BAALC overexpression in MC3T3-E1 cells using various amounts of BAALC adenovirus at the indicated times of RNA harvest. The mRNA expression for each of the extracellular matrix genes was normalized to PPIB and the control QBI virus response, where the horizontal bar represents the control virus response. Panel A; alkaline phosphatase, Panel B; osteocalcin, Panel C; osteopontin, $^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.005$, $^{****}P < 0.0001$ different from control virus response (one-way ANOVA). **Panels D–F** show the effects of the indicated concentrations of PTH 1–34 on extracellular matrix gene expression (normalized to PPIB expression) following 24, 48, 72, and 96 h of culture. Panel D; alkaline phosphatase, Panel E; osteocalcin, Panel F; osteopontin, $^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.005$, $^{****}P < 0.0001$ different from vehicle control (one-way ANOVA).

explain our inability to detect BAALC protein expression and regulation by PTH in the osteocytes of the bone sections we analyzed.

Although BAALC was reported as a cytoplasmic protein, it has recently been reported that myristoylation and palmitoylation of BAALC targets the protein to lipid rafts [Wang et al., 2005]. We show in vitro, that there was an increase in BAALC protein associated with the plasma and organelle membrane fraction when UMR-106 cells were treated with PTH. Although these data support the conclusion that BAALC expression is regulated in osteoblasts by PTH (consistent with our in vivo observations), it is unclear why we did not observe differences in BAALC protein expression in the cytoplasmic fraction of cells treated with PTH. Therefore, future experiments directed at

determining whether BAALC cell compartmentalization is regulated may lead to further insights into our findings.

Despite the low endogenous mRNA levels of BAALC in rat tibiae, the full-length form of BAALC appears to be broadly expressed with the highest mRNA levels present both in the adult and fetal brain, suggesting a possible role of BAALC in early brain development and adult brain function. Interestingly, although we show that BAALC mRNA expression was highly regulated by PTH in bone, we found no regulation in other tissues that express significant levels of the PTH receptor including the brain, heart, and kidney [Urena et al., 1993]. The lack of regulation of BAALC by PTH in the brain may be due to the already high expression levels of BAALC in this tissue. It is also possible that due to the short half-life of PTH [Papapoulos et al., 1980; Kent et al., 1985] and the peripheral mode of administration of PTH, sufficient levels of PTH are not crossing the blood–brain barrier. As for the lack of BAALC regulation in the heart and kidney, it is not likely that this is due to the relative expression levels of the PTH receptor in these tissues compared to bone because the kidney, a well-documented PTH target tissue, expresses one of the highest levels of receptor relative to bone and other non-bone tissues [Urena et al., 1993].

Therefore, the lack of BAALC regulation in the non-bone tissues that we analyzed may be due in part to differential expression of cell type specific signaling molecules that mediate PTH action. This conclusion is supported by a recent report demonstrating that expression levels of PTH signaling components (specifically B-Raf splice variants) in various osteoblastic cell lines determined whether PTH increased or decreased proliferation in the given cell line [Fujita et al., 2002]. This may also explain, in addition to any differences in the PTH receptor levels between cell lines, why the magnitude of BAALC regulation by PTH is greater in UMR-106 cells compared to ROS17/2.8 or U2OS cells.

Given the strong correlation between the bone anabolic effects of PTH and the requirement to activate the cAMP pathway by PTH [Rixon et al., 1994; Hilliker et al., 1996; Whitfield et al., 1996; Mohan et al., 2000], we also investigated whether BAALC regulation was dependent on the activation of cAMP/PKA pathway by PTH. Using ROS17/2.8 cells we found that forskolin, an activator of adenylate cyclase, was sufficient to induce BAALC expression while an inhibitor of adenylate cyclase was able to block the induction of BAALC by PTH 1–34. These data suggest that activation of cAMP/PKA is sufficient to induce BAALC expression, which is further supported by the observation that PTH 3–34, which has minimal cAMP/PKA-signaling activity [Cole et al., 1989; Chakravarthy et al., 1990; Fujimori et al., 1992; Armamento-Villareal et al., 1997] but can activate the PKC pathway, was not able to induce BAALC expression. In addition, a peptide PKC inhibitor had little effect on PTH-induced BAALC expression in the ROS 17/2.8 cells. Collectively, these data strongly suggest that activation of the cAMP/PKA pathway by PTH is required to regulate BAALC expression.

Our data suggest that BAALC expression is associated with the PTH anabolic activity in bone. Therefore, we examined whether this increased expression of BAALC is specific to PTH by evaluating the effects of other known bone anabolic agents. We found little to no regulation with 17β-estradiol, dexamethasone, BMP2, and BMP6. It is likely that the lack of regulation we observed by 17β-estradiol and dexamethosone was not due to a lack of the appropriate receptors, as UMR-106 cells have been previously shown to be responsive to 17β-estradiol and dexamethasone [Davis et al., 1994; Gray et al., 1987]. However, we did observe a significant induction of BAALC with $PGE_2$ and 1,25-$(OH)_2$-$D_3$.

1,25-$(OH)_2$-$D_3$ is a hormone that plays an integral role in bone metabolism and often works in conjunction with PTH. Furthermore, similar to PTH, 1,25-$(OH)_2$-$D_3$ promotes osteoblast differentiation and the regulation of BAALC by 1,25-$(OH)_2$-$D_3$ may be one of the many factors involved in mediating 1,25-$(OH)_2$-$D_3$ action in osteoblasts. Although we do not know whether the regulation of BAALC by 1,25-$(OH)_2$-$D_3$ is due to genomic or non-genomic actions, the regulation of BAALC by 1,25-$(OH)_2$-$D_3$ (and $PGE_2$) may be mediated in part through increases in cAMP levels since both $PGE_2$ and 1,25-$(OH)_2$-$D_3$ have been shown to increase intracellular levels of $Ca^{+2}$ and cAMP [Partridge et al., 1981; Farr et al., 1984; Raisz and Martin, 1984; Yamaguchi et al., 1989; Zanello and Norman, 1997, 2004; Falkenstein et al., 2000]. In support of this, we show that a

pharmacological agent, which increases cAMP (e.g., forskolin), will induce BAALC expression.

To address whether BAALC can directly modulate osteoblast function, we showed that BAALC overexpression in MC3T3-E1 osteoblastic cells downregulate AP and osteocalcin and induces osteopontin mRNA expression. Furthermore we show that PTH treatment of these cells causes the same transcriptional responses of these extracellular matrix genes, albeit with a delayed response relative to BAALC adenoviral overexpression. Collectively, these data support the hypothesis that BAALC is playing a role in mediating the action of PTH on bone cell function because BAALC was able to recapitulate the down stream effects of PTH on relevant markers of osteoblast differentiation and function. Even though these in vitro responses of PTH on AP and osteocalcin gene expression described here are in contrast to what has been reported for PTH effects on osteoblast related gene expression in vivo, they are consistent with many in vitro reports of PTH action [Swarthout et al., 2002; Lammi et al., 2004; Qin et al., 2004]. The conflicting responses of PTH on expression of osteoblast related markers observed in vitro versus in vivo are likely due to the fact that there is no well-defined anabolic treatment regimen for PTH in vitro cell systems and therefore it is difficult to make direct comparisons with in vivo results.

We know from related studies in the laboratory, using a rat model of ovariectomy-induced osteopenia, that PTH administration has clear effects on BMD of the tibia as early as 4 days of treatment with a significant increase in BMD evident after 8 days of treatment (data not shown). In this model, the induction of BAALC expression by PTH in the tibia is rapid and precedes the BMD effects of PTH. Collectively, these in vivo observations along with the data showing selective BAALC regulation with an anabolic PTH treatment regimen (not a catabolic treatment regimen) and the in vitro data demonstrating that BAALC regulates AP, osteocalcin, and osteopontin expression, suggest that BAALC expression may play an important function alone or in concert with other factors in mediating the PTH bone anabolic response. However, although this gene and its splice variants have been reported in lymphoblast cells of patients with acute myeloid leukemia [Tanner et al., 2001], and the full-length form of BAALC was recently shown to interact with the $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) [Wang et al., 2005], there is no known function that has been ascribed to this gene based on known protein structural motifs.

In summary, we have identified BAALC as a PTH-induced gene whose regulation by PTH in vivo is associated with PTH-mediated bone formation. We show that BAALC is rapidly regulated by PTH in osteoblasts, and its regulation by PTH is mediated predominantly through cAMP/PKA signaling. Furthermore, we show in vitro that BAALC overexpression regulates markers of osteoblast differentiation similar to the actions of PTH. Collectively, these observations suggest that BAALC is an important mediator of the PTH anabolic action on bone cell function. Based on these findings we believe this gene may serve as a unique therapeutic intervention point for the treatment of osteoporosis.

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