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Intermittent Administration of Parathyroid Hormone (1-34) Stimulates Matrix Metalloproteinase-9 (MMP-9) Expression in Rat Long Bone

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Abstract Intermittent doses of parathyroid hormone (PTH) stimulate bone formation in animals and humans, but the molecular mechanisms underlying this phenomenon are not understood. Bone formation culminates with the expression of type I collagen, osteocalcin, and alkaline phosphatase, but genes that initiate and support the anabolic response are not known. To identify novel PTH-regulated genes in bone during the anabolic response, we used differential display-polymerase chain reaction (DDRT-PCR) to analyze RNA from young male rats injected with either human PTH (1-34) or vehicle control, once daily for 5 days. Total RNA was isolated from the distal femur metaphysis at 1, 6, and 48 h after the final injection and subjected to DDRT-PCR. We identified three PTH-responsive transcripts as matrix metalloproteinase-9 (MMP-9), creatine kinase, and the α1(I) polypeptide chain (COL1A1) of type I collagen. The concomitant upregulation of MMP-9 and COL1A1 during bone formation was particularly intriguing. Further characterization of MMP-9 expression revealed that it was localized to osteoblasts, osteocytes, megakaryocytes, and cells of the bone marrow in the rat distal femur metaphysis. Northern analysis for MMP-9 expression in other tissues indicated that this transcript was present in the kidney and brain. In vitro, PTH regulated the protein synthesis of MMP-9 by osteoblasts of the primary spongiosa. We propose that PTH may promote bone formation by mediating the subtle variation in MMP activities, thus preparing the extracellular matrix for the subsequent bone cell migration and deposition of new osteoid. J. Cell. Biochem. 70:391-401, 1998.

Key words: anabolic; bone; MMP-9; osteoblast; parathyroid hormone

Intermittent doses of parathyroid hormone (PTH) stimulate a net gain in bone mass in normal rats [Tam et al., 1982; Gunness-Hey and Hock, 1984; Hock and Gera, 1992], osteoporotic rats [Hori et al., 1988; Tada et al., 1990], dogs [Podbeseka et al., 1983], and humans [Reeve et al., 1980; Slovic et al., 1981; Hodsman et al., 1997]. Although the mechanisms underlying the PTH-induced anabolic response of the skeleton are not fully understood, this phenomenon is supported in part by an increase in both osteoblast number [Schmidt et al., 1995] and bone-forming surfaces [Hock and Gera, 1992].

The molecular mechanisms that mediate PTH-induced bone formation have been difficult to elucidate primarily because this phenomenon cannot be fully recapitulated in vitro [Dempster et al., 1993]. For example, in vivo, PTH enhances type I collagen expression but in vitro, it attenuates synthesis of this protein (Kream et al., 1986; Partridge et al., 1989; Dempster et al., 1993; Dobnig and Turner, 1995]. The known marker genes for bone formation are few and include the extracellular matrix proteins, osteocalcin, osteopontin, and type I collagen, and the membrane-associated enzyme alkaline phosphatase [Stein et al., 1996]. The expression of these genes typically signals the end of the bone formation process and therefore are of little use by themselves for studying the events that initiate and mediate anabolic bone metabolism.

The PTH-induced anabolic effect on the skeleton likely involves the interaction of a variety

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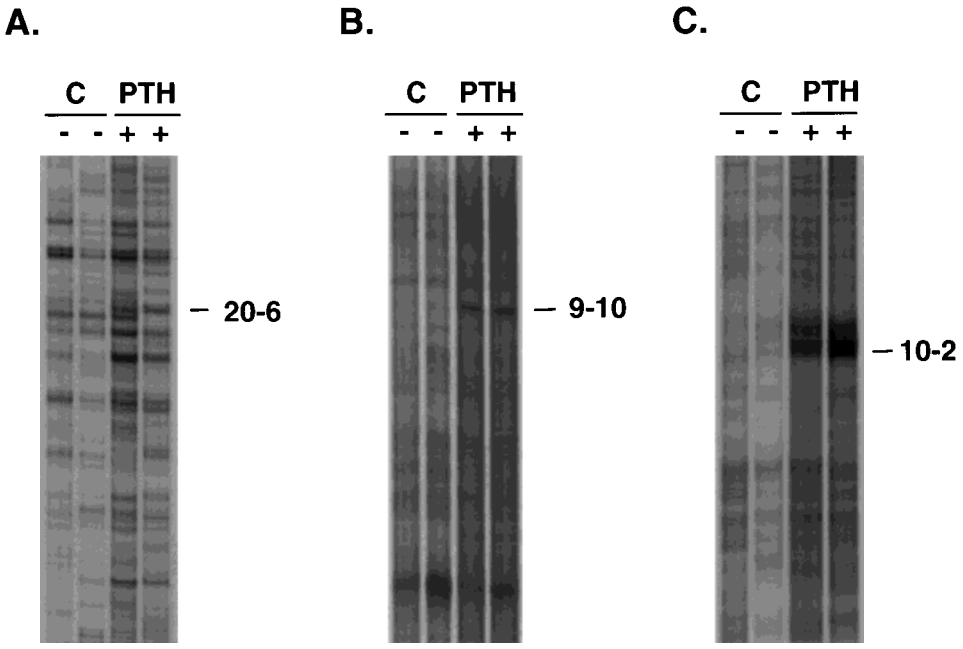


Fig. 1. Gel analysis of differential display-polymerase chain reaction (DDRT-PCR) to analyze RNA from the metaphyseal femurs of young male rats. Total RNA was isolated from vehicle (control) and PTH-treated rats. First-strand cDNA synthesis and PCR fingerprinting reactions were performed as described in Materials and Methods. Four sets of cDNA samples (control vs PTH-treated) were compared. Samples were done in two dilutions (the second lane of the control and PTH samples represent a 1:4 dilution of the first sample lanes). The bands labelled 20-6, 9-10, and 10-2 were of the 24 that exhibited a PTH-induced increase in intensity.

of cell types and numerous proteins in a complex regulatory cascade. Recently, techniques developed to identify changes in the expression of large numbers of genes in cells or tissues have been applied to elucidate the myriad of events that mediate complex physiological processes [Liang and Pardee, 1995; Velculescu et al., 1995]. Differential display reverse transcription-polymerase chain reaction (DDRT-PCR), e.g., has been used to characterize a proliferation-specific protein (PROM-1) expressed in bone cells [Ryoo et al., 1997], to identify type VI collagen as an interleukin-4-responsive gene in cultured human osteoblasts [Ishibashi et al., 1995], and to identify the growth-regulatory protein, stathmin, in human and rat osteoblast-like cells [Kumar and Haugen, 1994]. Therefore, this technique has potential for identifying novel genes that are regulated by PTH during the anabolic response of the skeleton.

Here, we describe the application of DDRT-PCR to identify, sequence, and clone PTH-responsive genes in the primary spongiosa of the rat distal femur. Our results indicated that intermittent administration of PTH upregulated the trabecular osteoblast expression of matrix metalloproteinase-9 (MMP-9 or gelatinase B), along with the α1(I) polypeptide chain (COL1A1) of type I collagen, in the distal femur metaphysis. Additionally, creatine kinase expression was increased with PTH treatment. We propose that the PTH-induced expression of MMP-9 by primary spongiosa osteogenic cells indicates a remodeling of the extracellular matrix and may mediate cell migration and the activation of matrix-bound growth factors.

MATERIALS AND METHODS

Reagents

Synthetic human parathyroid hormone (1-34) [hPTH(1-34)] (Bachem Co., Torrance, CA) was used to stimulate anabolic bone formation in male rats. Ultraspec-II reagent (Biotech Laboratories, Houston, TX), chloroform, isopropanol (Mallinckrodt, Baker, Paris, KY), and RNase-free DNase I (Gibco BRL, Grand Island, NY) were used to isolate total RNA from rat bone and cultured cells. Delta RNA Fingerprinting System (Clontech Laboratories, Palo Alto, CA) was used to obtain the differential displays from both primary spongiosa tissue and cell culture. For fixation, decalcification, and embedding of rat primary spongiosa, we used 10%

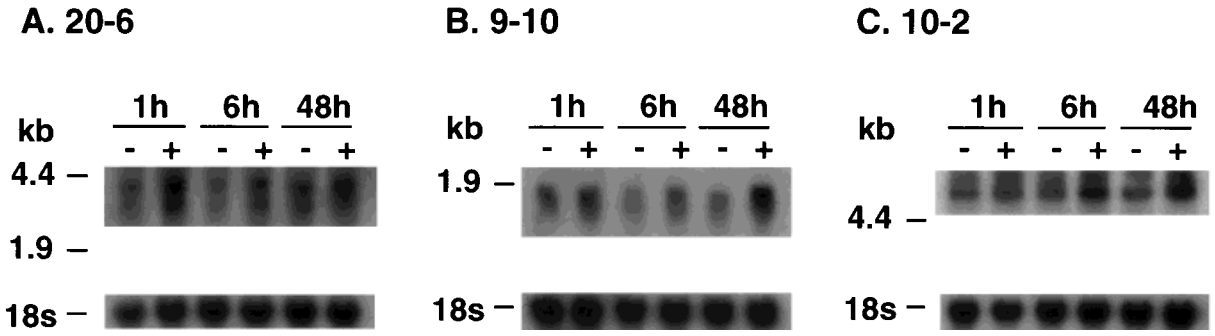


Fig. 2. Northern blot analysis of bands (**A**) 20-6, (**B**) 9-10, and (**C**) 10-2 confirming PTH regulation in bone. These cDNA bands were cut from the sequencing gel **(Fig. 1)**, reamplified by PCR, and labelled with ^{32}P. The PCR products were then used for Northern analysis. For Northern analysis, total RNA (20 µg/lane) from vehicle-treated rats (-) and PTH-treated animals (+) were fractionated on a denatured formamide agarose gel. After gel electrophoresis, RNAs were blotted onto a nitrocellulose membrane and hybridized to the cDNA probes. Intermittent hormone treatment, in vivo, increased the expression of these transcripts at 1, 6, and 48 h after final PTH injection.

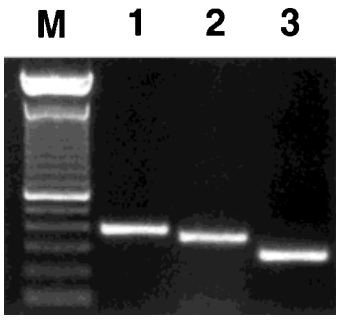


Fig. 3. PCR analysis of affinity captured cDNA fragments **(lane 1)** 20-6, **(lane 2)** 9-10, and **(lane 3)** 10-2. These cDNA probes used for Northern analysis **(Fig. 2)** were stripped from the Northern filter, reamplified by PCR, electrophoresed on a 1% agarose gel, and visualized with ethidium bromide staining.

TABLE I. Cloned Sequences With Significant Homologies to Previously Cloned Sequences in Databases

Clone number	Size of the clone (bp)	Amount of homology (bp/bp)	Homology
20–6	714	333/351	Rat gelatinase B mRNA, bp 969–1320
9–10	716	292/300	Rat creatine kinase gene, bp 3525–3825
10–2	303	111/117	Rat α-1 type I collagen mRNA, segment 3, bp 1–117

normal buffered formalin (NBF) (Fisher Scientific, Pittsburgh, PA), Decalcifier 1®,Surgipath paraffin tissue embedding medium (Surgipath Medical Industries, Grayslake, IL), and Permount™ (Fisher Scientific). The mouse mAb to MMP-9 (Oncogene Research Products, Cambridge, MA) was used for histochemical analysis. For the staining of MMP-9 in rat tissue sections, we used the Vectastain® ABC kit containing biotinylated, affinity-purified horse antimouse IgG secondary antibody (Vector Laboratories). MMP-9 immunopositive cells were detected using the 3,3'-diaminobenzidine (DAB) substrate kit for peroxidase activity (Vector Laboratories). Superfrost-plus slides (Fisher Scientific) were used for histochemical analysis. Rabbit anti-MMP-9 (Chemicon International, Temecula, CA) was used for Western analysis of conditioned media. Goat antirabbit IgG-HRP (Southern Biotechnology Associates, Birmingham, AL) was used as the secondary antibody for the Western analysis and the signal was developed using chemiluminescence (ECL™, Amersham, Buckinghamshire, UK).

Cell Culture

Primary osteoblasts were derived from the trabecular spongiosa of the distal femur metaphysis of the young male rats previously described [Onyia et al., 1995, 1997]. Briefly, muscle and connective tissue were cut away from femurs and the epiphyseal cap was removed to obtain the subjacent 3 mm section of metaphyseal bone. This section of bone was minced and digested with trypsin for 1 hr at 37°C. The released cells were pelleted and resuspended into α-Minimum Essential Medium (α-MEM) containing 100 IU/ml penicillin, 100 µg/ml streptomycin, 25 µg/ml amphotericin, 2 mM L-glutamine (Gibco BRL) and 20% fetal bovine serum (FBS) (Sigma, St.Louis, MO) and seeded into T-150 mm tissue culture flasks ($1x10^7$ cells/

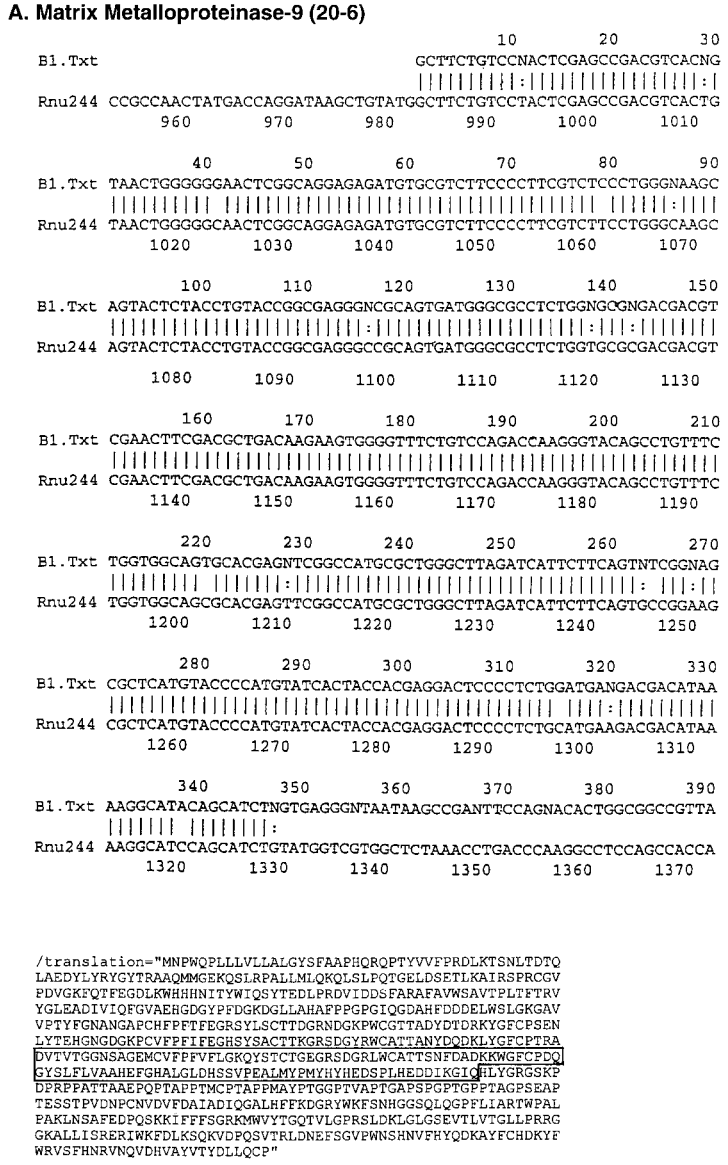


Fig. 4. Identification of cloned sequences using BLAST against the GenBank: (**A**) transcript 20-6 as matrix metalloproteinase (MMP-9/gelatinase B), (**B**) transcript 9-10 as creatine kinase, (**C**) transcript 10-2 as COL1A1.

flask). The media was changed 48 h after seeding. Primary osteoblast cultures were also established from the bone marrow stroma. The femur marrow was flushed, with the culture medium, from the diaphyseal shaft and the exudate passed through a 70 µm nylon, sterile cell strainer. These cells were seeded into culture medium as described above. The first medium change was 6 days postseeding and then every 48 hr. All cells were cultured at 37°C in 5% CO_2 in humidified air.

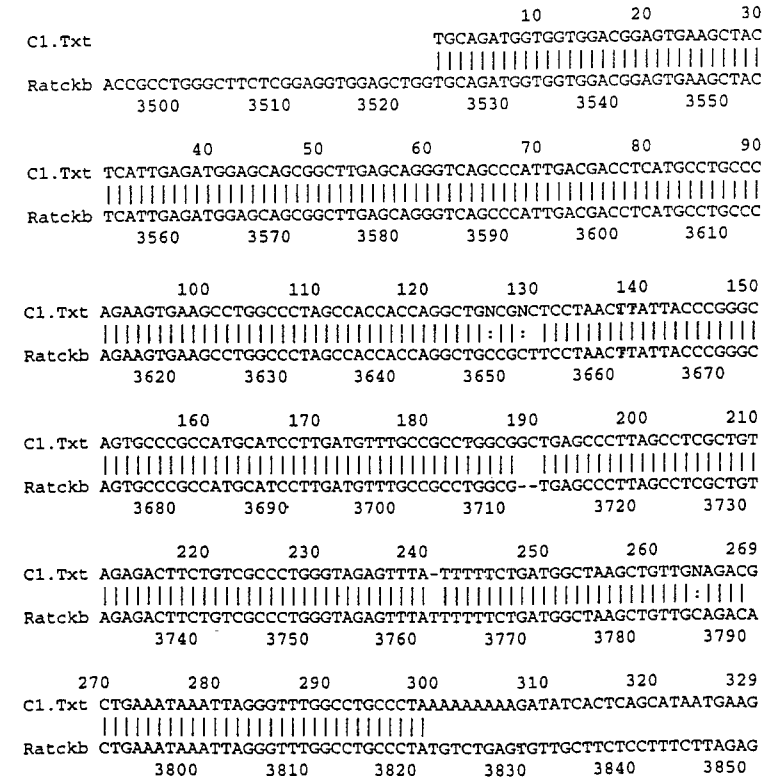
PTH Treatment

In vivo experiments: Young (3-4 wks, 60-70g), male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were given a single s.c. injection of either hPTH (1-34) (40 µg/kg/day) or vehicle (acidified saline, 2% heat inactivated serum) every 24 h for 5 days. Total RNA was isolated from the metaphyseal, distal femurs at 1 h, 6 h, and 48 h after the final injection. In vitro experiments: Cell cultures were treated with hPTH(1-34) [50 nM] or the same volume of vehicle for 24 hr. The conditioned media was collected and processed for Western analysis as described below.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from the tissues and cells using Ultraspec-II reagent, followed

B. Creatine Kinase (9-10)



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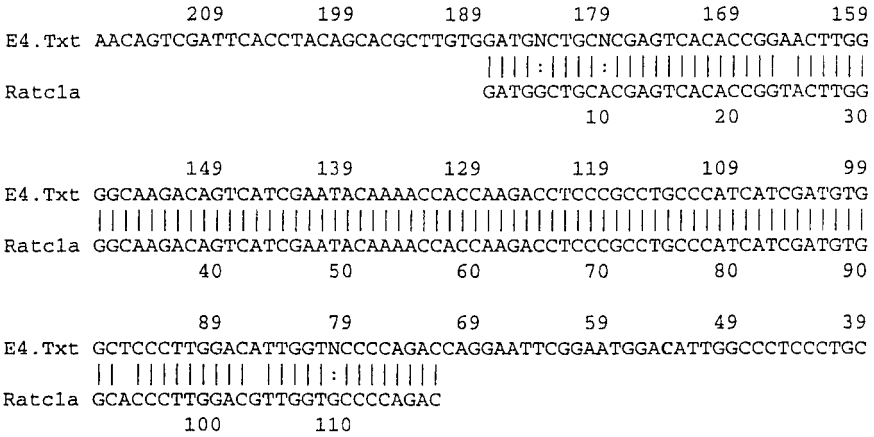
SDEHKTDLNPDNLQGGDDLDPNYVLSSRVRTGRSIRGFCLPPHCSRGERRAIEKLAVE
ALSSLDGDLSGRYYALKSMTEADEQQLIDDHFLFDKPVSPLLLASAMARDWPDARGIW
HNDNKTFLVWINEEDHLRVISMQKGGNMKEVFTRFCTGLTQIETLFKSKNYEFMWNPH
LGYILTCPSNLGTGLRAGVHIKLPHLGKHEKFSEVLKRLRLQKRGTGGVDTAAVGGVF
DVSNADRLGFSEVELVQMVVDGVKLLIEMEQRLEQGQPIDDLMPAQK"

C. Alpha-1 Type I Collagen (10-2)



/translation="GEPGDTGVKGDAGPPGPAGPAGPPGPIGNVGAPGPKGSRGAAGP
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GPAGEKGSPGADGPAGSPGTPGPQGIAGQRGVVGLPGQRGKRGFPGLPGPSGEPGKQG
PSGASGERGPPGPMGPPGLAGPPGESGREGSPGAEGSPGRDGAPGAKGDRGETGPAGP
PGAPGAPGAPGPVGPAGKNGDRGETGPAGPAGPIGPAGARGPAGPQGPRGDKGETGEQ
GDRGIKGHRGFSGLQGPPGSPGSPGEQGPSGASGPAGPRGPPGSAGSPGKDGLNGLPG
PIGPPGPRGRTGDSGPAGPPGPPGPPGPPSGGYDFSFLPQPPQEKSQDGGRYYRA
DDANVVRDRDLEVDTTLKSLSQQIENIRSPEGSRKNPARTCRDLKMCHSDWKSGEYWI
DPNQGCNLDAIKVYCNMETGQTCVFPTQPSVPQKNWYISPNPKEKKHVWFGESMTDGF
QFEYGSEGSDPADVAIQLTFLRLMSTEASQNITYHCKNSVAYMDQQTGNLKKSLLLQG
SNEIELRGEGNSRFTYSTLVDGCTSHTGTWGKTVIEYKTTKTSRLPIIDVAPLDIGAP
DQEFGMDIGPACFV"

Figure 4. (Continued.)

by extraction with chloroform, and precipitation with isopropanol according to the manufacturer's instruction. Subsequently, the RNA was incubated with RNase-free DNase I to remove contaminating chromosomal DNA. After phenol/chloroform extraction and ethanol precipitation, RNA was reversed transcribed using unique 3'-anchored oligo(dT) primers (Clontech) to obtain the first strand cDNA.

Differential Display-Polymerase Chain Reaction

Differential display was performed as described by Liang and Pardee [1992] with some modifications [Welsh et al., 1992; Liang et al., 1993; McClelland et al. 1993]. The mRNA fingerprinting procedure used ten 5'-arbitrary upstream primers and nine downstream oligo(dT) primers (Clontech). The temperature profile for PCR following the reverse transcription of RNA was as follows: one cycle of 94°C/5 min; 40°C/5 min; 68°C/5 min; followed by 30 cycles of 94°C/1 min; 60°C/1 min; 68°C/2 min; followed by a final extension at 68°C/7 min. The labelled PCR products (cDNA), representing the 3' ends of the expressed RNAs, were visualized by autoradiography on a 6% denaturing polyacrylamide sequencing gel. Differentially expressed bands were cut out from the gel and reamplified. The reamplified PCR products were gel purified (Qiagen, Chatsworth, CA) and used as probes for Northern analysis to confirm regulation of each selected transcript.

Northern Blot Analysis

Twenty micrograms of total RNA was separated on a 1% agarose-formaldehyde gel and transferred to a Hybond-N nylon membrane (Amersham). The membrane was prehybridized for 30 min at 65°C in a rapid hybridization buffer (Amersham) before adding denatured cDNA probe (1 x 10^6 cpm/ml). Hybridization was carried out for 2 hr at 65°C. The membrane was washed once in 2X SSC, 0.1% SDS (at room temperature for 20 min) and two times in 0.2X SSC, 0.1% SDS (at 65°C for 30 min) and exposed to X-ray film (Hyperfilm-MP; Amersham) overnight at -80°C.

Affinity Capture of cDNA

A portion of the nylon membrane containing the captured ^{32}P- cDNA probe was cut out using the autoradiogram for band localization [Fengsheng et al., 1994]. The membrane piece was stripped by boiling for 5 min in 100 ml of RNase-free water. An aliquot of the sample was reamplified by PCR using the same primers and conditions as before. The resulting PCR products were gel purified (Qiagen), cloned, and sequenced.

Cloning and Sequencing

Purified cDNAs were cloned into the pCR™ 2.1 TA cloning vector (Invitrogen, San Diego, CA) and transformed into INVaF' cells according to the manufacturer's instructions. For each clone, five positive colonies were picked, amplified in LB broth, and the plasmid isolated using Wizard Plus plasmid preps (Promega, Madison, WI). Clones were sequenced by the DNA sequencing facilities at Eli Lilly Research Laboratories (Indianapolis, IN). All sequences were subjected to BLAST analysis against GenBank, EMBL, and Lilly databases to determine identity.

Immunohistochemistry

Distal femur metaphysis segments were immersed in 10% formalin for 2 h at 25°C, decalcified, and embedded in paraffin. Paraffin sections (5 µm) were cut and floated onto slides. Tissue sections were rehydrated and immersed in 0.3% H_2O_2 in absolute methanol for 20 min to quench any endogenous peroxidase activity. The sections were then blocked in 5% horse serum and incubated for 16 h at 4°C with mouse mAb to MMP-9 (1:10, in DAKO® Antibody Diluent, DAKO, Carpinteria, CA). The secondary biotinylated mouse antibody was applied at concentrations of 2.5 µg/ml PBS for 30 min. Prior to color development, slides were incubated in standard avidin-biotinylated horseradish peroxidase solution (Vector Laboratories) for 30 min. Peroxidase activity was detected by a 2-4 min incubation in DAB substrate solution. Slides were counterstained for 1 min in hematoxylin, processed through a series of ethanol dehydrations, and permanently mounted in Permount™. Controls for nonspecific secondary binding were incubated with normal horse serum instead of primary antibody.

SDS-PAGE and Western Blotting

Conditioned media were collected from primary spongiosa cultures and dialyzed extensively (4-5 days) against distilled water. Equal volumes of each sample were lyophilized and

solubilized in SDS sample buffer containing 15 mg/ml DTT, boiled, and loaded onto a 3-15% gradient SDS-polyacrylamide gel with 3% stacking gels. The electrophoresed proteins were transferred to nitrocellulose (Hoefer, San Francisco, CA) and the membranes blocked for 2 h at 25°C in PBS and 5% milk. Rabbit anti-MMP-9 (1:1,000 in PBS/1% milk, 90 min at 25°C) was used to probe the membranes. Goat antirabbit IgG-HRP was used to develop the signal with chemiluminescence.

RESULTS

Identification of PTH-responsive Genes in Rat Bone

To identify PTH-responsive genes, rats were intermittently injected with hormone or vehicle and RNA was isolated from the distal metaphyseal demur at 1, 6, and 48 hr after the initial injection. The RNA was evaluated by DDRT-PCR using 10 arbitrary primers (25-mer) and nine anchor primers (29-mer) for a total of 90 independent differential display reactions. Each primer combination displayed ~50 cDNA bands yielding 4,500 cDNA transcripts. Hormone treatment altered the expression of gene transcripts as indicated by changes in band intensity (Fig. 1).

The profiles of amplified cDNA obtained with the respective primer combinations were highly reproducible. We choose 24 cDNA bands that were upregulated by PTH (200 bp) for further analysis. These bands were cut from the sequencing gel, reamplified by PCR, and randomly labelled with ^{32}P. The PCR products were then used for Northern analysis. Three of the stronger RNA signals are shown in Figure 2. Intermittent hormone treatment, in vivo, increased the expression of these transcripts at 1, 6, and 48 hr after final PTH injection (Fig. 2). These three cDNA probes were then affinity captured from the Northern filter and reamplified under the same PCR conditions, without adding labelled dNTP, electrophoresed on a 1% agarose gel, and visualized with ethidium bromide staining (Fig. 3).

These PCR products were purified, cloned, and sequenced. Sequence analysis using BLAST and FASTA against the GenBank revealed the identities of the transcripts (Table I and Fig. 4) as MMP-9 (gelatinase B), collagen type I (COL1A1), and creatine kinase.

Tissue and Cellular Distribution of MMP-9

We were particularly intrigued with the upregulation of the matrix metalloproteinase during the anabolic response to PTH; therefore, we evaluated the tissue distribution of MMP-9 expression by immunohistochemistry and Western and Northern analyses. In nonosseous tissues, MMP-9 RNA transcripts were detected in kidney and trace amounts in brain (Fig. 5).

Next, we sought to identify those cells in the metaphyseal primary spongiosa expressing MMP-9 at 5 days after intermittent PTH treatment. Immunohistochemistry indicated that MMP-9 protein was expressed by mature osteoblasts and osteocytes of the primary spongiosa (Fig. 6A). MMP-9 was also expressed by megakaryocytes and other cells of the bone marrow (Fig. 6B). Tissue sections from animals treated with vehicle exhibited a similar distribution and apparent level of expression of this protein. Consistent with the immunohistochemical analysis, Northern analysis indicated that MMP-9 was expressed in cultures of rat primary spongiosa osteoblasts and bone marrow stromal cells (Fig. 7).

We also confirmed the MMP-9 expression by immunoblot analysis of conditioned media. Metaphyseal cells in culture were treated with PTH (50 nM) for 24 h and the conditioned media were subjected to electrophoresis followed by Western analysis. The results indi-

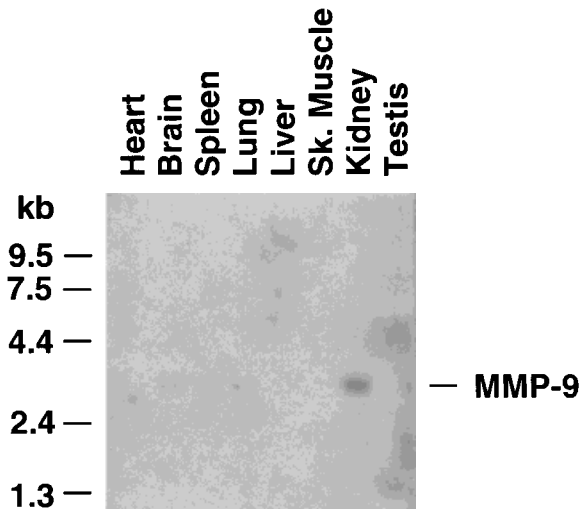

Fig. 5. Tissue distribution of MMP-9 mRNA expression. Multiple Northern Tissue Blot (Clontech) with 2 μg/lane of poly(A^+) RNA from various tissues. Probe was the ^{32}P-labelled cDNA for MMP-9. Expression was observed in kidney and trace amounts were detected in brain.

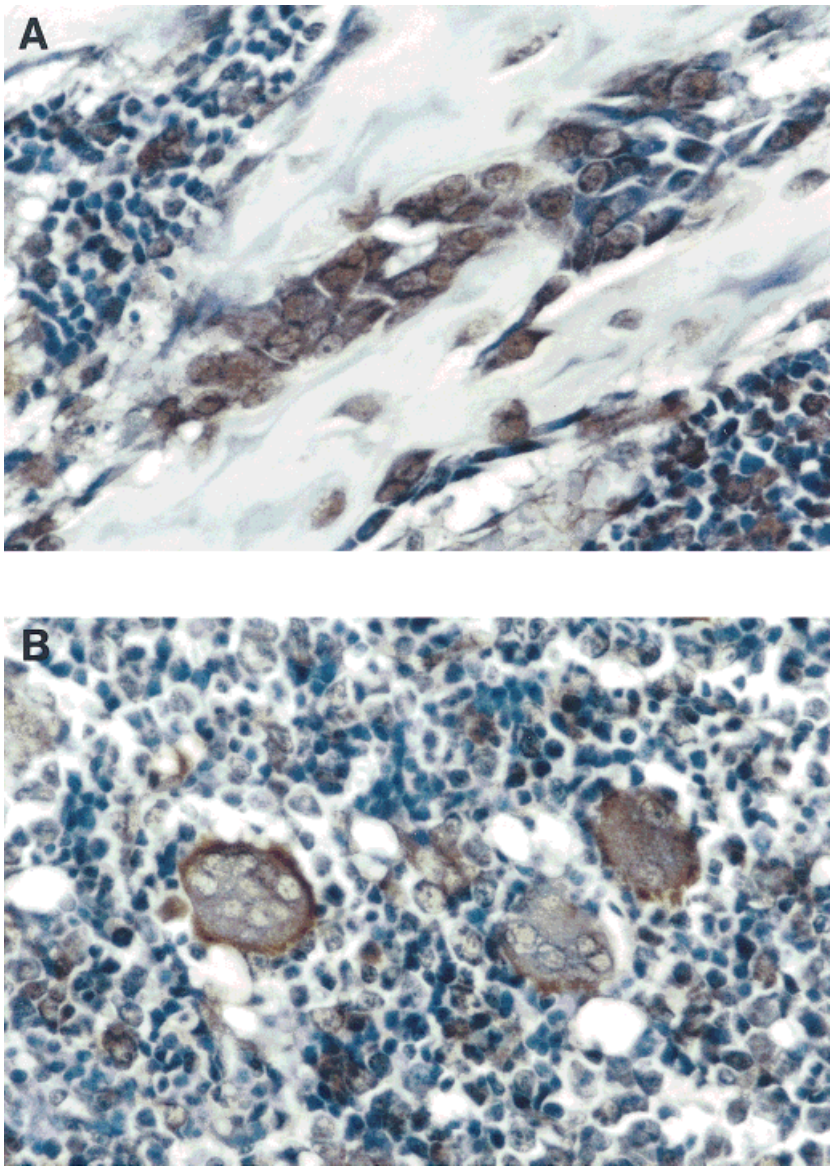

Fig. 6. Immunohistochemical analysis of MMP-9 expression in rat metaphyseal primary spongiosa after 5 days of intermittent PTH treatment. Immunopositive cells stain brown, the sections are counterstained with hematoxylin (blue). **A**. Osteoblasts, osteocytes, and some cells of the bone marrow cavity stain for MMP-9. **B**. Megakaryocytes, in the bone marrow cavity of the primary spongiosa stain for MMP-9. Immunohistochemical analysis of tissue sections from animals treated with vehicle exhibited a similar cellular distribution and level of MMP-9 expression.

cated that a band of M_r=92 kD was present in both control and PTH-treated conditioned media (Fig. 8). Additionally, PTH treatment resulted in a modest increase in the levels of MMP-9 in the conditioned medium of the metaphyseal marrow cells (Fig. 8).

DISCUSSION

The anabolic effects of PTH have been well documented; however, the molecular mechanisms that underlie this phenomenon have not been fully elucidated. Although several PTH-responsive genes have been identified, the critical physiological events that lead to bone formation remain to be established. We have undertaken a systematic approach to evaluate and identify all genes that are likely to be influenced by PTH during bone growth. Using DDRT-PCR, we have demonstrated that several genes were affected by PTH, including COL1A1, creatine kinase, and MMP-9. In osseous tissue, MMP-9 was expressed in osteo-

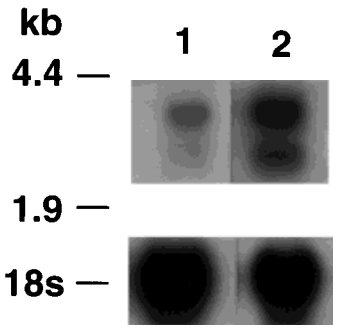

Fig. 7. Northern blot analysis of MMP-9 expression in cultured rat primary spongiosa cells **(lane 1)**, and rat marrow stromal cells **(lane 2)**. Total RNA was harvested from cell cultures at day 9, postseeding, for the primary spongiosa cells and day 14, postseeding, for the stromal cells.

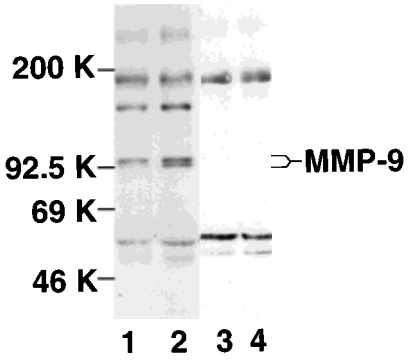

Fig. 8. Western analysis for MMP-9 of conditioned media samples from cultures of rat metaphyseal primary spongiosa osteoblasts. Identical blots were treated with either immune serum **(lanes 1 and 2)** or nonimmune serum **(lanes 3 and 4)**. Conditioned media were from cultures treated with hPTH(1-34) [50 nM, 24 h] **(lanes 2 and 4)** or the same volume of vehicle **(lanes 1 and 3)**.

blasts, osteocytes and cells of the bone marrow cavity.

That intermittent administration of PTH induced COL1A1 and creatine kinase expression in vivo is consistent with the fact that these proteins are known markers for skeletal formation [Dempster et al., 1993; Somjen and Kaye, 1994; Fournier et al., 1996], but the upregulation of MMP-9 raises some interesting questions concerning the role of extracellular matrix remodelling and invasive events in bone growth. PTH may promote bone formation by mediating the subtle variation in MMP activities, thus preparing the extracellular matrix for the subsequent deposition of new matrix. Additionally, osteoblast expression of MMPs may mediate bone cell migration and the activation of matrix-bound growth factors. This is not necessarily incongruous with the putative role of osteoblast MMP expression in PTH-induced bone resorption [Partridge et al., 1987; Delaisse et al., 1988; Hill et al., 1994; Witty et al., 1996; Varghese and Canalis, 1997] but may comprise the difference between anabolic and catabolic doses of this hormone.

The MMPs and their tissue inhibitors (TIMPs) mediate the degradation and remodeling of the extracellular matrix in both pathological and normal physiological processes, ranging from metastasis to regeneration, and including skeletogenesis [Matrisian, 1990; Woessner, 1991; Ennis and Matrisian, 1994; Yang and Bryant, 1994; Guerin and Holland, 1995; La Fleur et al., 1996; Chen and Werb, 1997; Johansson et al., 1997; Stahle-Backdahl et al., 1997]. During human fetal development, collagenase-3 expression was confined to mineralizing skeletal tissue, hypertrophic chondrocytes, and osteoblastic cells involved in ossification beginning at 10 weeks and continuing through gestation (Johansson et al., 1997; Stahle-Backdahl et al., 1997]. No expression of collagenase-3 was detected in other fetal tissues, including skin, lungs, neural tissue, muscle, and liver [Johansson et al., 1997]. In situ hybridization of the murine embryonic mandibular arch revealed that gelatinase A mRNA transcripts were strongly expressed in the perichondrium of Meckel's cartilage and mesenchymal areas at days 13-15 of development [Chin and Werb, 1997]. However, MMP-9, collagenase-3, TIMP-1, and TIMP-2 mRNA were found primarily in the ossifying areas of the mandibles [Chin and Werb, 1997]. A similar expression profile of MMPs was observed in cultured mandibular explants. Interestingly, culture of day 10 mandibular explants with a hydroxamic acid metalloproteinase inhibitor altered the development of the tongue and cartilage, but not bone or teeth [Chin and Werb, 1997].

The expression of MMPs and TIMPs, as mediators of extracellular matrix remodeling, appear critical to many growing or regenerating tissues. Recent evidence suggests that skeletal muscle cells selectively synthesize members of the MMP family that may mediate extracellular matrix remodeling during myogenesis and the regeneration of skeletal muscle [Guerin and Holland, 1995]. The regulation of gelatinase A by NGF is critical to neurite penetration through the extracellular matrix and thus neuronal regeneration [Muir et al., 1994]. The expression of a variety of MMPs and TIMPs ex-

pressed in the rat kidney may mediate the nonpathological turnover of the mesangial glomerular matrix [Martin et al., 1994]. Finally, a 90-kD gelatinase/collagenase was upregulated within hours after limb amputation in the axolotl *Ambystoma mexicanum* [Yang and Bryant, 1994]. This gelatinase exhibited dramatic elevation in activity during the dedifferentiation and blastema stages. It was hypothesized that this 90-kD gelatinolytic activity in limb regeneration was necessary to enable cells to migrate individually and to engage in cell-cell interactions as well as to release sequestered growth factors [Yang and Bryant, 1994].

Further study is required to elucidate the spectrum and temporal sequence of specific MMPs expressed by different osteogenic cells. The relative activities of the various MMPs and their inhibitors (TIMPs) must be determined to understand the extracellular matrix remodeling process, and thus perhaps the subtle difference between PTH-induced bone formation and resorption.

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