[Frontiers in Bioscience 3, d795-803, August 1, 1998]

ROLE OF PTHrP AND PTH-1 RECEPTOR IN ENDOCHONDRAL BONE DEVELOPMENT

Andrew C. Karaplis and Ron A. Deckelbaum

Division of Endocrinology, Department of Medicine, and Lady Davis Institute for Medical Research, McGill University, Montréal, Canada

Received 2/16/98 Accepted 3/23/98

TABLE OF CONTENTS

1. ABSTRACT

Parathyroid hormone-related peptide (PTHrP) has important functions in the control of cellular growth and differentiation. Acting, at least in part, through the PTH-1 receptor, PTHrP profoundly influences chondrocytic and osteogenic cell biology. Studies using knockout and transgenic mouse technology have played a pivotal role in unraveling the physiological role of PTHrP and its receptor in endochondral bone development and adult skeletal homeostasis. Further clarification of these functions will have far-reaching implications in our general understanding of skeletogenesis and the pathophysiology of human skeletal disorders.

2. INTRODUCTION

Parathyroid hormone-related peptide (PTHrP) was initially identified as a tumor-derived, secretory protein with structural similarity to parathyroid hormone (PTH), the major regulator of calcium homeostasis [for review see (1)]. PTH and PTHrP bind to a common G protein-coupled cell surface receptor (PTH/PTHrP or PTH-1 receptor) that recognizes the N-terminal (1-34) region of these peptides (2, 3). Hence, when tumor-derived PTHrP enters the circulation, it activates receptors in classic PTH target organs such as bone and kidney and elicits PTH-like bioactivity. By promoting bone resorption and inhibiting calcium excretion, circulating PTHrP gives rise to the common paraneoplastic syndrome of malignancy-associated humoral hypercalcemia.

The human gene for *PTHrP* has been mapped to the short arm of chromosome 12 (4), whereas the *PTH* gene is assigned to human chromosome 11. An evolutionary relationship between the *PTHrP* and *PTH* genes has been suggested, since human chromosomes 11 and 12 are thought to have arisen by a tetraploidization event of a common ancestral chromosome (5). Considerably more complex than the *PTH* gene, the human *PTHrP* gene comprises eight exons (6) that span more than 15 kb of genomic DNA, utilizes three distinct promoters, two TATA and one GC-rich region, (7-9), and by alternate splicing gives rise to three different isoforms of 139, 141, and 173 amino acids in length. In rodents, only one major form is produced (141 amino acids in the rat, 139 in the mouse due to a two amino acid-deletion), and the existence of multiple promoters has been disputed (7, 10). Human, rat, mouse, and chicken PTHrP share marked amino acid sequence identity throughout the amino terminal and midregion portions of the protein but diverge beyond residue 112. This striking conservation through evolution prompted the suggestion that the protein is indeed of biological value.

Although initially discovered in tumors, PTHrP was subsequently shown to be expressed in a remarkable variety of normal tissues including the fetal and adult skeleton, where acting in concert with its amino terminal PTH-1 receptor, it serves to regulate cellular growth and

differentiation. The present review aims to explore our recent understanding of PTHrP and PTH-1 receptor function in endochondral bone development.

3. PTHRP AND PTH-1 RECEPTOR IN CHONDROCYTE BIOLOGY

3.1 The endochondral skeleton

Endochondral ossification is a complex, multistep process involving the formation of cartilaginous skeleton from aggregated mesenchymal cells and its subsequent replacement by bone. The cellular and molecular events that regulate the highly ordered progression of chondrocytes within the growth plate through the stages of proliferation and differentiation must be under precise spatial and temporal control. Ultimately, it is these events that determine the extent and rate of skeletal growth.

3.2 Chondrocyte differentiation

The generation of mice missing the PTHrP gene provided the first direct evidence of a physiological role for this protein in chondrocyte biology (11). Mice homozygous for the PTHrP-null mutation die in the immediate postnatal period, likely from respiratory failure as a consequence of widespread abnormalities of endochondral bone development. Characterized by diminished proliferation, accelerated differentiation, and premature apoptotic death of chondrocytes, this form of osteochondrodysplasia results in rapid and untimely maturation of the skeleton (11-14).

The critical role of PTHrP as an inhibitor of the chondrocyte differentiation program has been further substantiated by the targeted overexpression of PTHrP in chondrocytes by means of the mouse collagen type II promoter (15). This targeting induces a novel form of chondrodysplasia that mirrors the PTHrP-null phenotype and is characterized by a delay in endochondral ossification so profound that mice are born with cartilaginous endochondral skeletons. However, by 7 weeks of age, this delay in chondrocyte differentiation and ossification has been largely corrected, leaving foreshortened and misshapen but histologically near-normal bones. This ultimate histological healing and the sequence by which it proceeds are reminiscent of that seen in patients with Jansen's metaphyseal chondrodysplasia, a condition arising from constitutive activation of the PTH-1 receptor (16-19). Similar effects on the chondrocyte maturation program have recently been observed in transgenic mice in whom expression of a constitutively active PTH-1 receptor was targeted to the growth plate (20). Therefore, overexpression of PTHrP or constitutive activation of the PTH-1 receptor in the growth plate ultimately result in a similar pattern of abnormalities in endochondral bone formation.

3.3 Chondrocyte apoptosis

The mechanism by which PTHrP inhibits the process of chondrocyte differentiation is just beginning to unravel. Hypertrophic chondrocytes in the growth plate are thought to undergo apoptosis immediately prior to ossification (21) and, therefore, represent the terminal stage of differentiation in the chondrogenic lineage. PTHrP expression might be expected to delay, or even prevent, the progression to terminal differentiation and eventual programmed cell death. Studies using chondrocytic CFK2 cells that overexpress PTHrP demonstrated enhanced cell survival under conditions that promote apoptotic death (22). In addition, quantitative analysis of the growth plate of PTHrP-null mice revealed significantly more apoptotic chondrocytes near the chondro-osseous junction compared to wild-type littermates (14, 23). Thus, PTHrP influences not only chondrocyte proliferation and differentiation, but also programmed cell death.

In several cell types, apoptosis is regulated by the ratio of expression of the cell death inhibitor, Bcl-2, and the cell death inducer Bax. Bcl-2 is expressed in growth plate chondrocytes in a pattern similar to PTHrP (12, 24) with highest levels detected in late proliferative and prehypertrophic chondrocytes (14, 24**).** Both *in vitro* and in transgenic mice, PTHrP overexpression causes a marked increase in Bcl-2 with no detectable change in Bax levels (24). A shift of the Bcl-2/Bax ratio in favor of Bcl-2 delays terminal differentiation, prolongs chondrocyte survival, and leads to the accumulation of cells in their prehypertrophic stage. These observations are deemed very exciting as they place Bcl-2 downstream of PTHrP in the pathway that controls chondrocyte maturation and endochondral skeletal development.

3.4 The hedgehog proteins

A most interesting recent development has been the observation that PTHrP mediates the actions of Indian hedgehog (Ihh) on chondrocyte differentiation. Ihh is a member of a family of proteins, the most notable of which is Sonic hedgehog (Shh), the vertebrate homologue of the *Drosophila* segment polarity gene product, hedgehog (*hh*), which regulates a variety of patterning events during embryonic development. The mouse Hedgehog (Hh) gene family consists of *Sonic* (*Shh*), *Desert* (*Dhh*), and *Indian* (*Ihh*) *hedgehog*, and all encode secreted proteins implicated in cell-cell interactions. Upon secretion, they undergo autocatalytic internal cleavage generating a ~20-kD amino-terminal domain and a ~25-kD carboxyl-terminal domain. While the amino domain possesses all known signaling activity of these proteins, the carboxyl terminal is responsible for the autocatalytic processing (25). In addition to cleavage, this processing causes the covalent attachment of a cholesterol moiety in ester linkage to the carboxylate of the terminal residue of the amino-terminal fragment (26). This modification may set constrains on the diffusion of Hh, by tethering it to the plasma membrane, thereby, spatially restricting the localization of the Hh signal. Local effects, based on a requirement for high concentration of amino-terminal Hh, can be easily envisioned for cells in close proximity to Hh-expressing cells. While long-range signaling also appears to function via diffusion of the amino-terminal fragment, indirect effects have also been reported. Both local- and long-range signaling of Hh proteins on target cells are mediated by a receptor that consists of two subunits, Patched (Ptc), a twelve transmembrane protein which is the binding subunit (27-28), and Smoothened (Smo), a seven transmembrane protein which is the signaling subunit. In the absence of Hh, Ptc associates with Smo and inhibits its activities. In

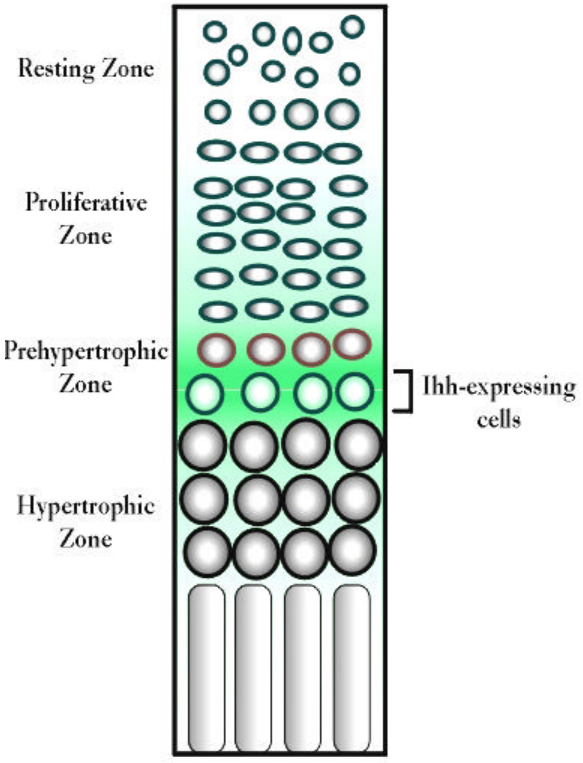

Figure 1. Proposed molecular regulation of cartilage differentiation in the growth plate by Ihh. Prehypertrophic chondrocytes express *Ihh*, and abundant PTHrP, and PTH-1 receptor (not shown). Shading represents *Ihh* signals with the intensity of shading indicating their relative strength. These inductive short- (dark shading) and long-range (lighter shading) signals act in a concentration-dependent manner to determine differential cell fate within the growth plate. The individual cellular response to Ihh signaling may vary in the various zones of the growth plate (proliferative vs hypertrophic), because the interpretation of the gradient differs owing to different states of other gene activities ("on" versus "off").

contrast, binding of Hh to Ptc relieves the Ptc-dependent inhibition of Smo (29). Signaling then ensues and includes downstream components such as the Gli family of transcriptional activators. The three cloned *Gli* genes (*Gli*, *Gli-2* and *Gli-3*) encode a family of DNA-binding zinc finger proteins with related target sequence specificities. Expression of the three mouse genes has been observed in both ectoderm- and mesoderm-derived tissues, suggesting that they play multiple roles during postimplantation development (30).

The importance of the Hh inductive signaling in skeletal patterning has been underscored by a number of observations: (i) the effects of Shh in the differentiation of somites into sclerotome (31) and in the growth and patterning of the developing limb (32, 33), (ii) the skeletal abnormalities arising from targeted disruption in mice (34) and from natural mutations in mice (34, 35) and humans (36) of the *Gli* genes, and (iii) the demonstration that Ihh, produced by growth plate chondrocytes making the transition to the hypertrophic stage of differentiation, attenuates this programmed process (37). Studies have indicated that Ihh action within the vertebrate growth plate is indirect and mediated primarily by the PTHrP signaling pathway (37-38). Ihh acts on cells in the perichondrium, signaling via Ptc and Gli, to induce, directly or indirectly, the release of PTHrP from the periarticular perichondrium. In turn, PTHrP exerts influence upon the growth plate chondrocytes by activating its receptor and thereby attenuating their propensity to differentiate.

While attractive, this paracrine negative feedback loop within the growth plate requires further clarification. At present it remains unclear how the *Hh* signaling pathway functioning on the perichondrium, would lead to increased PTHrP expression by periarticular cells. In other studies, PTHrP and PTH-1 receptor expression was reported in resting, proliferating, and prehypertrophic chondrocytes (12, 23, 24). Moreover, *Ptc*, *Smo*, and *Gli* expression has been observed in chondrocytes within the murine growth plate and in clonal mouse ATDC5 cells undergoing chondrogenesis and cartilage differentiation (39, 40). Such a set up would therefore predict an alternative molecular regulation of cartilage differentiation. We have proposed that Ihh, via direct action on growth plate chondrocytes, induces differential PTHrP expression and hence differential cell fate, by both short and long-range signals, acting as a morphogen in a concentration-dependent fashion (figure 1) (41). Thus, local effects arising from high concentration of amino-terminal Ihh could be envisioned as promoting terminal chondrocyte differentiation independent of PTHrP, while long-range signaling via diffusion of the Ihh amino-terminal fragment would delay the differentiation of proliferating chondrocytes via activation of the PTHrP signaling pathway. Additional evidence substantiating this hypothesis has been provided by recent *in vitro* studies. Thus, *Ihh* mRNA expression parallels that of *Col-X* mRNA in ATDC5 cells undergoing chondrogenesis and subsequent cartilage differentiation (40). Further, ectopic expression of *Hh* in limb bud micromass cultures promotes alkaline phosphatase and *Col-X* expression, characteristics of hypertrophic chondrocytes, in a mechanism independent of the PTHrP paracrine system (42). This apparent dual action of Ihh on the program of chondrocyte maturation will likely require the use of powerful genetic tools for further substantiation in the *in vivo* setting.

3.5 The role of other cytokines

While a number of other signaling molecules produced within the growth plate may alter chondrocyte proliferation and differentiation by their own independent actions, others influence these processes likely by modulating the Ihh/PTHrP pathway. Signaling by bone morphogenetic proteins (BMPs) has been implicated in the development of the vertebrate limb. Recent studies have reported that BMP receptor-IA is specifically expressed in prehypertrophic chondrocytes and modulates the program of chondrocyte maturation (43). Consequently, misexpression of a constitutively active form of BMP receptor-IA delayed chondrocyte differentiation and was associated with decreased *Col-X* and *Ihh* expression, while

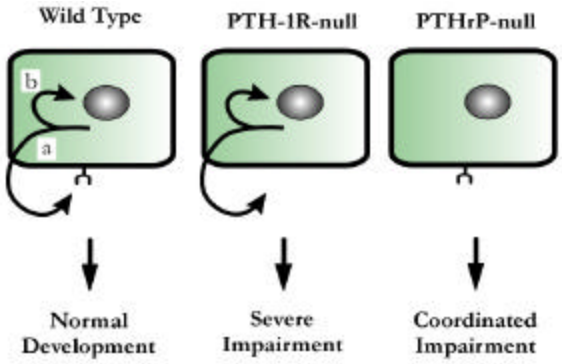

Figure 2. PTHrP signaling in wild type, PTH-1 receptor- and PTHrP-negative cells. In normal cells (left), the biological actions elicited by PTHrP require the coordinated activity between its (a) autocrine/paracrine and (b) intracrine actions. In receptor-deficient cells (center), extracellular signaling is absent, while the nuclear effects proceed unabated. This leads to severely dysregulated cell function and to the more severe phenotype of the receptor-null animals. In contrast, both PTHrP signaling pathways are abolished in ligand-deficient cells (right), leading in a more "coordinated" functional disruption.

PTHrP expression was increased. Analysis of this model suggests that BMP signals are downstream mediators of Ihh function in both a local signaling loop and a longer-range relay system that may involve PTHrP.

In other studies, the overexpression of a truncated type II TGF-beta receptor (a dominant-negative mutant) in skeletal tissue of transgenic mice resulted in degenerative joint disease resembling human osteoarthritis, and growth plate abnormalities characterized by increased *Col-X* and *Ihh* expression (44). Thus, loss of responsiveness to TGF-beta promotes chondrocyte terminal differentiation through a mechanism that likely involves the Ihh/PTHrP pathway.

3.6 Clarifying the role of the PTH-1 receptor

There is abundant evidence that the cloned PTH-1 receptor mediates most PTHrP actions. Finding natural mutations in humans with Jansen's metaphyseal chondrodysplasia has emphasized a critical role for the receptor in skeletal development. In addition, targeted overexpression of constitutively active PTH-1 receptor to the growth plate of transgenic mice delays endochondral bone formation (20). It was, however, the generation of PTH-1 receptor knockout mice that provided unequivocal proof that this receptor mediates the cartilaginous effects of PTHrP (38). The small number of homozygous mice that do survive to the peripartum period exhibit a phenotype similar to that observed in the PTHrP-negative mutants, although they are proportionally smaller. Skeletal alterations are characterized by accelerated differentiation of chondrocytes leading to a severe and lethal form of osteochondrodysplasia.

Of note, however, is the observation that most receptor-negative animals exhibit a more severe phenotype than the PTHrP-null mice do and is characterized by early embryonic lethality (embryonic day E14.5). It was originally speculated that PTHrP synthesized by maternal decidual cells could complement the absence of fetal PTHrP but not that of its receptor. Another possibility may be that circulating PTH can partly compensate for the absence of PTHrP but not for the receptor deficiency. Alternatively, the more severe phenotype may implicate the presence of another member of the PTH/PTHrP ligand family that interacts with the common receptor. The existence of such a protein, however, remains speculative at present.

Perhaps the most intriguing explanation for the early demise of these animals stems from the apparent dual mechanism of action of PTHrP on cellular function (22, 45-47). PTHrP translocates to the nucleolus and inhibits expression of differentiation-associated proteins in chondrocytes (48). It is tempting to speculate that normal embryonic development requires the coordinated activity between the amino-terminal end of the protein acting on the cell surface receptor promoting proliferation and the nucleolar form modulating differentiation (figure 2). In the absence of the amino terminal receptor, the unopposed nucleolar effects of PTHrP would lead to severe imbalance of cellular proliferation/differentiation and hence, to early lethality of the receptor-negative mutants. In contrast, ablation of the ligand would eliminate both receptor- and nucleolar-mediated activities, leading to a more "coordinated" impairment and a less severe phenotype. At present, however, it is reasonable to conclude that the small size and early death of the PTH-1 receptor-null mice remains unexplained.

4. PTHrP AND PTH-1 RECEPTOR IN OSTEOBLAST BIOLOGY

4.1 PTHrP haploinsufficiency and osteopenia

Accumulating evidence indicates that PTHrP also regulates osteogenic cell differentiation and/or function. The evidence can be summarized as follows: First, PTHrP and PTH-1 receptor are expressed in cells of the osteogenic lineage (12, 13, 49-54). Second, in the PTHrP-null mouse, osteoblastic progenitor cells contain inappropriate accumulations of glycogen (12). This finding, also observed in PTHrP-null chondrocytes, is indicative of a defect, metabolic or otherwise, in cells of the osteogenic lineage arising as a consequence of PTHrP deficiency. Third, heterozygous PTHrP-null mice, while phenotypically normal at birth, by three months of age exhibit a form of osteopenia characterized by a marked decrease in trabecular thickness and connectivity (13). Moreover, their bone marrow contains an abnormally high number of adipocytes. Since the same pluripotent stromal cells in the bone marrow compartment can give rise to adipocytes and osteoprogenitor cells (55), the increased number of adipocytes and osteopenia in these mice could be attributed to altered stem cell differentiation as a consequence of PTHrP haploinsufficiency (figure 3). Taken together, these findings suggest that PTHrP is important for the orderly commitment of precursor cells toward the osteogenic lineage and for their subsequent maturation and/or function.

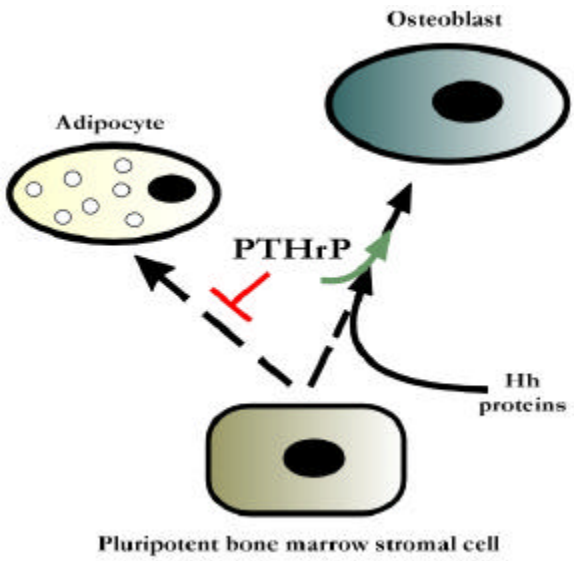

Figure 3. Diagrammatic representation of the undifferentiated mesenchymal stem cell having the potential for differentiation into many cell types including adipocytes and osteoblasts. PTHrP, produced within the skeletal microenvironment, promotes the transition toward the osteogenic lineage while decreased local concentrations in the PTHrP-null heterozygous state would favor the formation of adipocytes. Dashed arrows indicate the unknown number of transitional steps required for these differentiation sequences. Hh proteins likely act upstream of PTHrP in the osteogenic differentiation program.

In these animals, a physiological concentration of PTH is unable to compensate for PTHrP haploinsufficiency. One possible explanation is that a defect in skeletal progenitor cells was caused by PTHrP deficiency *in utero* and was only manifest in the post-natal state. Alternatively, domains of PTHrP not shared with the PTH molecule may subserve unique functions that are important for normal adult skeletal development. PTHrP was shown to translocate to the nucleolus of bone cells *in vitro* and *in situ* and modulate programmed cell death (22). Cell death by apoptosis is necessary for maintenance of homeostatic growth in many adult tissues, including bone. Alterations in this mechanism may also have important implications in the development of the osteopenic state. Further elucidation of the role of PTHrP and its amino-terminal receptor in bone would necessitate their osteoblast-specific disruption in mice. Targeted ablation of *PTHrP* or *PTH-1* receptor gene sequences only in osteoblasts should allow these mice to be viable well beyond the immediate post-natal period, thereby making them amenable for studying the effect of these gene deletions on the adult skeleton.

4.2 Induction of osteogenic differentiation by Hh proteins

Recent studies have shown that members of the Hh family of proteins (Shh and Ihh) can stimulate the osteogenic differentiation of mouse preosteoblastic MC3T3-E1 and fibroblastic C3H10T1/2 cell lines (56, 57). While this stimulatory effect was synergistically enhanced by BMP-2, Hh proteins did not induce *Bmp* gene expression. These cells, however, express *Ptc* indicating that the effects of Hh proteins on the osteoblast differentiation program are direct. What the role of Hh proteins is in modulating the expression of *PTHrP* and its receptor in cells of the osteogenic lineage, remains to be defined.

4.3 PTHrP and bone remodeling

With increasing evidence that PTHrP plays an important role in osteoblast biology, it becomes necessary to understand the regulation of its expression and the mechanisms by which it exerts its effects within the skeletal microenvironment. The effects of PTHrP on bone formation and resorption may be partly mediated through cytokines. *In vitro*, transient treatment with PTHrP stimulates collagen synthesis, an effect mediated by enhancement of local IGF-1 production (58). Osteoblast expression of leukemia inhibitory factor (LIF) mRNA increases following PTHrP administration *in vitro* (59) and *in vivo* (60). The release of LIF likely promotes osteoblast differentiation and may thereby mediate the anabolic actions of PTHrP in bone. Increased expression of interleukin-6 (IL-6) (59, 60) and colony stimulating factor-1 (CSF-1) (61) in osteoblasts has been reported following activation of the PTH-1 receptor. These cytokines in turn influence osteoclast function and/or recruitment, and neutralizing antibodies to the IL-6 receptor (59) and CSF-1 (61) can be blocked their effects.

While a number of factors have been reported to influence PTHrP gene expression in non-skeletal tissues (1), its regulation in bone remains poorly defined. The effect of glucocorticoids on PTHrP gene expression in bone is of interest in view of the severe bone loss associated with their prolonged use. Glucocorticoid administration inhibits PTHrP mRNA expression in human osteogenic cells (62) and increases PTH binding, PTH-stimulated cAMP production and PTH-1 receptor mRNA in an osteoblast cell line (63).

PTHrP may play an important role in the increased bone turnover and bone loss associated with estrogen deficiency. Stimulation of adenylate cyclase activity by PTH and PTHrP in cultured osteoblasts is inhibited by estrogen administration (64, 65). A better understanding of the regulation of PTHrP and its receptor by estrogens is of clinical interest, since increased skeletal responsiveness to the catabolic effects of continuous PTH administration has been observed in postmenopausal women (66).

4.4 Potential therapy for osteoporosis

The anabolic effects of intermittent PTH administration on bone and its therapeutic potential in osteoporosis have been extensively explored [for review see (67)]. With the recognition that PTHrP is the endogenous ligand for the PTH/PTHrP receptor in osteoblasts, its use as an anabolic agent has also been investigated. PTHrP (1-74) was shown to increase bone mass in rats (68). PTHrP (1-36), one of the authentic secretory forms of PTHrP (69), has been reported to have equivalent potency to PTH (1-34) in its actions (70), yet its anabolic effects on bone remain to be defined. PTHrP (1-

34) was less potent than PTH (1-34) in producing an anabolic response in bone (71), although that could be partly attributed to its higher clearance rate (72).

Analogs of PTHrP (1-34) have been developed in an attempt to improve its anabolic efficacy. One such analog, RS-66271, has received much attention because of its pronounced bone anabolic activity *in vivo*. It markedly increased trabecular and cortical bone formation when given intermittently to ovariectomized, osteopenic rats (73). The anabolic benefits of RS-66271 have been confirmed in a non-human primate model of estrogen deprivation osteopenia (74) and in rabbits with glucocorticoid-induced bone loss (75). A rapid increase in size and number of osteoblasts on trabecular surfaces was observed following initiation of treatment (76). The rapidity with which these changes occurred suggests that the lining cells on trabecular surfaces were induced to differentiate into osteoblasts. With cessation of the drug, the osteoblasts reverted to lining cells.

While promising, there are nonetheless some potential concerns arising with the use of PTHrP and its analogs for the treatment of osteoporosis (77). Clearly, more studies using these peptides are needed to verify their safety and efficacy as therapeutic agents.

5. PERSPECTIVE

The perinatal mortality of the knockout mouse severely limits our understanding of PTHrP function in adult tissues. By circumventing the cartilaginous malformations, other abnormalities, skeletal or otherwise, would become evident in the adult PTHrP-null animal. Matings of PTHrP heterozygous mice to transgenic animals overexpressing PTHrP or constitutively active PTH-1 receptor in chondrocytes have been used to generate "rescued" mice that are devoid of PTHrP signaling in all tissues other than cartilage (20, 78). These animals can now be used to investigate other as yet unrecognized abnormalities arising as a consequence of PTHrP deficiency.

PTHrP expression by osteoblasts, which also posses PTH-1 receptor, may have fundamental implications in the regulation of adult skeletal homeostasis. Future studies will undoubtedly provide a better understanding of the role played by PTHrP in bone metabolism under physiological and pathological conditions and effectively target PTHrP and its analogs as potential therapeutic agents in the treatment of osteoporosis.

6. ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council of Canada.

7. REFERENCES

1. W. M. Philbrick, J. J. Wysolmerski, S. Galbraith, E. Holt, J. J. Orloff, K. H. Yang, R. C. Vasavada, E. C. Weir, A. E. Broadus & A. F. Stewart: Defining the roles of parathyroid hormone-related protein in normal physiology. *Physiol Rev* 76, 127-73 (1996)

2. H. Jüppner, A.-B. Abou-Samra, M. Freeman, X.-F. Kong, E. Schipani, J. Richards, L. F. Kolakowski, Jr, J. Hock, J. T. Potts, Jr, H. M. Kronenberg & G. V. Segre: A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. *Science* 254, 1024-6 (1991)

3. A. B. Abou-Samra, H. Jüppner, T. Force, M. W. Freeman, X. F. Kong, E. Schipani, P. Urena, J. Richards, J. V. Bonventre, J. T. Potts, Jr, H. M. Kronenberg & G. V. Segre: 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, 2732-6 (1992)

4. M. Mangin, A. C. Webb, B. E. Dreyer, J. T. Posillico, K. Ikeda, E. C. Weir, A. F. Stewart, N. H. Bander, L. Milstone, D. E. Barton, U. Francke & A. E. Broadus: Identification of a cDNA encoding a parathyroid hormone-like peptide from a humn tumor associated with hypercalcemia of malignancy. *Proc Natl Acad Sci USA* 85, 597- (1988)

5. D. E. Comings: Evidence for ancient tertraploidy and conservation of linkage groups in mammalian chromosomes. *Nature* 238, 455-7 (1972)

6. A. E. Broadus, & A. F. Stewart: Parathyroid hormone-related protein: Structure, processing and physiological actions. In: The Parathyroids. Eds: Bilezikian J. P., Levine M. A., Marcus R.) Raven Press, New York (1994)

7. M. Mangin, K. Ikeda & A. E. Broadus: Structure of the mouse gene encoding parathyroid hormone-related peptide. *Gene* 95, 195-202 (1990)

8. M. Mangin, K. Ikeda, B. E. Dreyer & A. E. Broudus: Identification of an up-stream promoter of the human parathyroid hormone-related peptide gene. *Mol Endocrinology* 4, 851-8 (1990)

9. R. C. Vasavada, J. J. Wysolmerski, A. E. Broadus & W. M. Philbrick: Identification and characterization of a GC-rich promoter of the human parathyroid hormone-related peptide gene. *Mol Endocrinol* 7, 273-82 (1993)

10. A. C. Karaplis, T. Yasuda, G. N. Hendy, D. Goltzman & D. Banville: Gene encoding parathyroid hormone-like peptide: nucleotide sequences of the rat gene and comparison with the human homologue. *Mol Endocrinol* 4, 441-6 (1990)

11. A. C. Karaplis, A. Luz, J. Glowacki, R. T. Bronson, V. L. J. Tybulewicz, H. M. Kronenberg & R. C. Mulligan: Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide (PTHrP) gene. *Genes Dev* 8, 277-289 (1994)

12. N. Amizuka, J. E. Henderson, H. Warshawsky, D. Goltzman & A. C. Karaplis: Parathyroid hormone-related

peptide (PTHrP)-depleted mice show abnormal epiphyseal cartilage development and altered endochondral bone formation. *J Cell Biol* 126, 1611-1623 (1994)

13. N. Amizuka, A. C. Karaplis, J. E. Henderson, H. Warshawsky, M. l. Lipman, Y. Matsuki, S. Ejiri, M. Tanaka, N. Izumi, H. Ozawa & D. Goltzman: Haploinsufficiency of parathyroid hormone-related peptide (PTHrP) results in abnormal postnatal bone development. *Dev Biol* 175, 166-76 (1996)

14. K. Lee, B. Lanske, A. C. Karaplis, J. D. Deeds, H. Kohno, R. A. Nissenson, H. M. Kronenberg & G. V. Segre: Parathyroid hormone-related peptide delays terminal differentiation of chondrocytes during endochondral bone development. *Endocrinology* 137, 5109-18 (1996)

15. E. C. Weir, W. M. Philbrick, M. Amling, L. A. Neff, R. Baron & A. E. Broadus: Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes chondrodysplasia and delayed endochondral bone formation. *Proc Natl Acad Sci USA* 93, 10240-5 (1996)

16. E. Schipani, K. Kruse & H. Juppner: A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* 268, 98-100 (1995)

17. E. Schipani, C. B. Langman, A. M. Parfitt, G. S. Jensen, S. Kikuchi, S. W. Kooh, W. G. Cole & H. Jüppner: Constitutively activated receptors for parathyroid hormone-related peptide in Jansens metaphyseal chondrodysplasia. *N Engl J Med* 335, 708-14 (1996)

18. K. Kruse: Calcium metabolism in the Jansen type of metaphyseal dysplasia. *Eur J Pediatr* 152, 912-5 (1993)

19. J. W. Spranger, L. O. Langer & H. R. Wiedemann: Bone dysplasia: an atlas of constitutional disorders of skeletal development. Philadelphia: Saunders, (1974)

20. E. Schipani, B. Lanske, J. Hunzelman, A. Luz, C. S. Kovacs, K. Lee, A. Pirro, H. M. Kronenberg & H. Juppner: Targeted expression of constitutively active receptors for parathyroid hormone and parathyroid hormone-related peptide delays endochondral bone formation and rescues mice that lack parathyroid hormone-related peptide. *Proc Natl Acad Sci USA* 94, 13689-94 (1997)

21. C. E. Farnum & N. J. Wilsman: Condensation of hypertrophic chondrocytes at the chondro-osseous junction of growth plate cartilage in Yucatan swine:relationship to long bone growth. *Am J Anat* 186, 346-58 (1989)

22. J. E. Henderson, N. Amikuza, H. Warshawsky, D. Biasotto, B. M. K. Lanske, D. Goltzman & A. C. Karaplis: Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. *Mol Cell Biol* 15, 4064-75 (1995)

23. N. Amizuka, J. E. Henderson, K. Hoshi, H. Warshawsky, H. Ozawa, D. Goltzman & A. C. Karaplis: Programmed cell death of chondrocytes and aberrant chondrogenesis in mice homozygous for parathyroid hormone-related peptide gene deletion. *Endocrinology* 137, 5055-67 (1996)

24. M. Amling, L. Neff, S. Tanaka, D. Inoue, K. Kuida, E. Weir, W. M. Philbrick, A. E. Broadus & R. Baron: Bcl-2 lies downstream of parathyroid hormone-related peptide in a signaling pathway that regulates chondrocyte maturation during skeletal development. *J Cell Biol* 136, 205-13 (1997)

25. J. A. Porter, S. C. Ekker, W. J. Park, D. P. v. Kessler, K. E. Young, C. H. Chen, Y. Ma, A. S. Woods, R. J. Cotter, E. V. Koonin & P. A. Beachy: Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. *Cell* 86, 21-34 (1996)

26. J. A. Porter, K. E. Young & P. A. Beachy: Cholesterol modification of hedgehog signaling proteins in animal development. *Science* 274, 255-9 (1996)

27. V. Marigo, R. A. Davey, Y. Zuo, J. M. Cunningham & C. J. Tabin: Biochemical evidence that Patched is the Hedgehog receptor. *Nature* 384, 176-9 (1996)

28. D. M. Stone, M. Hynes, M. Armanini, T. A. Swanson, Q. Gu, R. L. Johnson, M. P. Scott, D. Pennica, A. Goddard, H. Phillips, M. Noll, J. E. Hooper, F. d. Sauvage & A. Rosenthal: The tumour-suppressor gene *patched* encodes a candidate receptor for Sonic hedgehog. *Nature* 384, 129-34 (1996)

29. R. Nusse: Patching up Hedgehog. *Nature* 384, 119-20 (1996)

30. C.-C. Hui, D. Slusarski, K. A. Platt, R. Holmgren & A. L. Joyner: Expression of three mouse homologs of the *Drosophila* segment polarity gene cubitus interruptus, *Gli*, *Gli-2*, and *Gli-3*, in ectoderm-and mesoderm-derived tissues suggests multiple roles during postimplantation development. *Dev Biol* 162, 402-13 (1994)

31. R. L. Johnson, E. Laufer, R. D. Riddle & C. Tabin: Ectopic expression of Sonic hedgehog alters dorsal-ventral patterning of somites. *Cell* 79, 1165-73 (1994)

32. M. J. Cohn, & C. Tickle: Limbs: a model for pattern formation within the vertebrate body plan. *Trends Genet* 12, 253-7 (1996)

33. C. Chiang, Y. Litingtung, E. Lee, K. E. Young, J. L. Corden, H. Westphal & P. A. Beachy: Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* 383, 407-13 (1996)

34. R. Mo, A. M. Freer, D. L. Zinyk, M. A. Crackower, J. Michaud, H. H. Q. Heng, K. W. Chik, X.-M. Shi, L.-C. Tsui, S. H. Cheng, A. L. Joyner & C.-C. Hui: Specific and redundant functions of *Gli2* and *Gli3* zinc finger genes in skeletal patterning and development. *Development* 124, 113-23 (1997)

35. C.-C. Hui & A. L. Joyner: A mouse model of Greig cephalopolysyndactyly syndrome: the *extra-toes*^{J} mutation

contains an intragenic deletion of the *Gli3* gene. *Nature Genet* 3, 241-6 (1993)

36. A. Vortkamp, M. Gessler & K.-H. Grzeschik: GLI3 zinc-finger gene interrupted by translocations in Greig syndrome families. *Nature* 352, 539-40 (1991)

37. A. Vortkamp, K. Lee, B. Lanske, G. V. Segre, H. M. Kronenberg & C. J. Tabin: Regulation of rate of cartilage differentiation by indian hedgehog and PTH-related protein. *Science* 273, 613-22 (1996)

38. B. Lanske, A. C. Karaplis, K. Lee, A. Luz, A. Vortkamp, A. Pirro, M. Karperien, L. H. K. 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, 663-6 (1996)

39. J. Helms, M. Iwasai & A. Lee: Expression of hedgehog, BMP and GLI genes during cartilage and bone development. *Annual Meeting of the Orthopaedic Research Society* (Abstract), 34 (1997)

40. H. Akiyama, C. Shigeno, Y. Hiraki, C. Shukunami, H. Kohno, M. Akagi, J. Konishi & T. Nakamura: Cloning of a mouse smoothened cDNA and expression patterns of hedgehog signalling molecules during chondrogenesis and cartilage differentiation in clonal mouse EC cells, ATDC5. *Biochem Biophys Res Commun* 235, 142-7 (1997)

41. A. C. Karaplis & L. Vautour: Parathyroid hormone-related peptide and the parathyroid hormone/parathyroid hormone-related peptide receptor in skeletal development. *Curr Opin Nephrol Hypertension* 6, 308-13 (1997)

42. N. S. Stott & C.-M. Chuong: Dual action of sonic hedgehog on chondrocyte hypertrophy: retrovirus mediated ectopic sonic hedgehog expression in limb bud micromass culture induces novel cartilage nodules that are positive for alkaline phosphatase and type X collagen. *J Cell Sci* 110, 2691-701 (1997)

43. H. Zou, R. Wieser, J. Massague & L. Niswander: Distinct roles of type I bone morphogenetic protein receptors in the formation and differentiation of cartilage. *Genes Dev* 11, 2191-203 (1997)

44. R. Serra, M. Johnson, E. H. Filvaroff, J. Laborde, D. M. Sheehan, R. Derynck & H. L. Moses: Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol* 139, 541-52 (1997)

45. T. Massfelder, P. Dann, T. L. Wu, R. Vasavada, J. J. Helwig & A. F. Stewart: Opposing mitogenic and anti-mitogenic actions of parathyroid hormone-related protein in vascular smooth muscle cells: a critical role for nuclear targeting. *Proc Natl Acad Sci USA* 94, 13630-5 (1997)

46. M. H. C. Lam, C. M. House, K. I. Mitchellhill, B. Sarcevic, A. Cures, R. Ramsey, B. E. Kemp, T. J. Martin, J. M. Moseley & M. T. Gillespie: Cellular localization of PTHrP is regulated by phosphorylation at Thr85 by cyclin dependent kinases p34cdc2 and p33cdk2. *J Bone Miner Res* 12 Sup. 1, S210 (1997)

47. M. H. Lam, S. L. Olsen, W. A. Rankin, P. W. Ho, T. J. Martin, M. T. Gillespie & J. M. Moseley: PTHrP and cell division: expression and localization of PTHrP in a keratinocyte cell line (HaCaT) during the cell cycle. *J Cell Physiol* 173, 433-46 (1997)

48. J. E. Henderson, B. He, D. Goltzman & A. C. Karaplis: Constitutive expression of parathyroid hormone-related peptide (PTHrP) stimulates growth and inhibits differentiation of CFK2 chondrocytes. *J Cell Physiol* 169, 33-41 (1996)

49. K. Lee, A. Karaplis, A. T. Bond & G. V. Segre: Expression of PTHrP and PTH/PTHrP receptor transcripts is not spatially or temporally correlated in rat fetal development. *14th Annual Meet. Am. Soc. Bone Mineral Research*, Minneapolis, MN, 1992

50. K. Lee, J. D. Deeds, S. Chiba, M. Un-No, A. T. Bond & G. V. Segre: Parathyroid hormone induces sequential c-fos expression in bone cells in vivo: In situ localization of its receptor and c-fos messenger ribonucleic acids. *Endocrinology* 134, 441-50 (1994)

51. B. Fermor & T. M. Skerry: PTH/PTHrP receptor expression on osteoblasts and osteocytes but not resorbing bone surfaces in growing rats. *J Bone Miner Res* 10, 1935-43 (1995)

52. S. Celic, P. J. Chilco, J. D. Zajac, T. J. Martin & D. M. Findley: A type 1 collagen substrate increases PTH/PTHrP receptor mRNA expression and suppresses PTHrP mRNA expression in UMR106-06 osteoblast-like cells. *J Endocrinol* 150, 299-308 (1996)

53. N. Suda, M. T. Gillespie, K. Traianedes, H. Zhou, P. W. M. Ho, D. K. Hards, E. H. Allan, T. J. Martin & J. M. Moseley: Expression of parathyroid hormone-related protein in cells of osteoblast lineage. *J Cell Physiol* 166, 94-104 (1996)

54. L. K. McCauley, A. J. Koh, C. A. Beecher, Y. Cui, T. J. Rosol & R. T. Franceschi: PTH/PTHrP receptor is temporally regulated during osteoblast differentiation and is associated with collagen synthesis. *J Cell Biochem* 61, 638-47 (1996)

55. M. Owen & A. J. Friedenstein: Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp* 136, 42-60 (1988)

56. N. Kinto, M. Iwamoto, M. Enomoto-Iwamoto, S. Noji, H. Ohuchi, H. Yoshioka, H. Kataoka, Y. Wada, G. Yuhao, H. E. Takahashi, S. Yoshiki & A. Yamaguchi: Fibroblasts expressing sonic hedgehog induce osteoblast differentiation and ectopic bone formation. *FEBS Letters* 404, 319-23 (1997)

57. T. Nakamura, T. Aikawa, M. Iwamoto-Enomoto, M. Iwamoto, Y. Higuchi, P. Maurizio, N. Kinto, A. Yamaguchi, S. Noji, K. Kurisu & T. Matsuta: Induction of osteogenic

differentiation by hedgehog proteins. *Biochem Biophys Res Commun* 237, 465-9 (1997)

58. E. Canalis, T. L. McCarthy & M. Centrella: Differential effects of continuous and transient treatment with parathyroid hormone related peptide (PTHrP) on bone collagen synthesis. *Endocrinology* 126, 1806-12 (1990)

59. E. M. Greenfield, S. M. Shaw, S. A. Gornick & M. A. Banks: Adenyl cyclase and interleukin-6 are downstream effectors of parathyroid hormone resulting in stimulation of bone resorption. *J Clin Invest* 96, 1238-44 (1995)

60. J. H. Pollock, M. J. Blaha, S. A. Lavish, S. Stevenson & E. M. Greenfield: *In vivo* demonstration that parathyroid hormone and parathyroid hormone-related protein stimulate expression by osteoblasts of interleukin-6 and leukemia inhibitory factor. *J Bone Miner Res* 11, 754-9 (1996)

61. E. C. Weir, C. W. G. M. Lowik, I. Paliwal & K. L. Insogna: Colony stimulating factor-1 plays a role in osteoclast formation and function in bone resortion induced by parathyroid hormone and parathyroid hormone-related peptide. *J Bone Miner Res* 11, 1474-81 (1996)

62. C. A. Walsh, M. A. Birch, W. D. Fraser, R. Laton, J. Dorgan, S. Walsh, D. Sansom, J. N. Beresford & J. A. Gallagher: Expression and secretion of parathyroid hormone-related protein by human bone-derived cells in vitro: effects of glucocorticoids. *J Bone Miner Res* 10, 17-25 (1995)

63. P. Urena, A. Iida-Klein, X.-F. Kong, H. Juppner, H. M. Kronenberg, A. Abou-Samra & G. V. Segre: Regulation of parathyroid (PTH)/PTH-related peptide receptor messenger ribonucleic acid by glucocorticoids and PTH in ROS 17/2.8 and OK Cells. *Endocrinology* 134, 451-6 (1994)

64. M. Ernst, J. K. Heath & G. A. Rodan: Estradiol effects on proliferation, messenger ribonucleic aci for collagen and insulin-like growth factor-I, and parathyroid hormone-stimulated adenylate cyclase activity in osteoblastic cells from calvariae and long bones. *Endocrinology* 125, 825-33 (1989)

65. S. Fukayama & A. H. Tashjian: Direct modulation by estradiol of the response of human bone cells (SaOS-2) to human parathyroid hormone (PTH) and PTH-related protein. *Endocrinology* 124, 397-401 (1989)

66. C. Joborn, S. Ljunghall, K. Larsson, E. Lindh, T. Naessen, L. Wide, G. Akerstrom & J. Rastad: Skeletal responsiveness to parathyroid hormone in healthy females: Relationship to menopause and estrogen replacement. *Clin Endocrinol* 34, 335-9 (1991)

67. J. Reeve: PTH: a future role in the management of osteoporosis? *J Bone Miner Res* 11, 440-5 (1996)

68. E. C. Weir, G. Terwilliger, L. Sartori & K. L. Insogna: Synthetic parathyroid hormone-like protein (1-74) is anabolic for bone *In vivo*. *Calcif Tissue Int* 51, 30-4 (1992)

69. J. J. Orloff, D. Reddy, A. E. d. Papp, K. H. Yang, N. E. Soifer & A. F. Stewart: Parathyroid hormone-related protein as a prohormone: posttranslational processing and receptor interactions. *Endocr Rev* 15, 40-60 (1994)

70. M. Evenhart-Caye, S. E. Inzucchi, J. Guinness-Henry, M. A. Mitnick & A. F. Stewart: Parathyroid hormone (PTH)-related protein(1-36) is equipotent to PTH(1-34) in humans. *J Clin Endocrinol Metab* 81, 199-208 (1996)

71. J. M. Hock, J. Fonseca, M. Gunness-Hey, B. E. Kemp & T. J. Martin: Comparison of the anabolic effects of synthetic parathyroid hormone-related protein (PTHrP) 1-34 and PTH 1-34 on bone in rats. *Endocrinology* 125, 2022-7 (1989)

72. L. J. Fraher, K. Klein, R. Marier, D. Freeman, G. N. Hendy, D. Goltzman & A. B. Hodsman: Comparison of the pharmacokinetics of parenteral parathyroid hormone-(1-34) [PTH-(1-34)] and PTH-related peptide-(1-34) in healthy young humans. *J Clin Endocrinol Metab* 80, 60-4 (1995)

73. B. H. Vickery, Z. Avnur, Y. Cheng, S. Chiou, D. Leaffer, J. P. Caulfield, D. B. KImmel, T. Ho & J. L. Krstenansky: RS-66271, a C-terminally substituted analog of human parathyroid hormone-related protein (1-34), increases trabecular and cortical bone in ovariectomized, osteopenic rats. *J Bone Miner Res* 11, 1943-51 (1996)

74. Z. Avnur, G. I. McRae, D. Tallentire & B. H. Vickery: RS66271 rapidly increases bone mineral density at spine, hip and forearm in ovariectomized cynomolgus macaques. *J Bone Miner Res* 10 S1, S196 (1995)

75. B. H. Vickery, K. Bergstrom, A. DeRosier, L. Katigbak & R. V. Waters: RS66271 very rapidly reverses bone loss induced by prednisone in rabbits. *J Bone Miner Res* 10 S1, S257 (1995)

76. D. Leaffer, M. Sweeney, L. A. Kellerman, Z. Avnur, J. L. Krstenansky, B. H. Vickery & J. P. Caulfield: Modulation of osteogenic cell ultrastructure by RS-23581, an analog of human parathyroid hormone (PTH)-related peptide-(1-34), and bovine PTH-(1-34). *Endocrinology* 136, 3624-31 (1995)

77. A. F. Stewart: PTHrP(1-36) as a skeletal anabolic agent for the treatment of osteoporosis. *Bone* 19, 303-6 (1996)

78. J. J. Wysolmerski, W. M. Philbrick, M. E. Dunbar, B. Lanske, H. M. Kronenberg, A. C. Karaplis & A. E. Broadus: Rescue of the parathyroid hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development. *Development* 125, 1285-94 (1998)

Key words: Review, PTHrP signaling, Endochondral ossification, Skeletal dysplasia, Chondrocytes, Osteoblasts

---

Send correspondence to: Andrew C. Karaplis, M.D., Ph.D., Division of Endocrinology, Sir Mortimer B. Davis-Jewish General Hospital and Lady Davis Institute for Medical Research, 3755 Côte Ste. Catherine Road, Montréal, Québec, Canada H3T 1E2, Tel:(514)-340-8222x4907, Fax: (514)-340-7573, E-mail: akarapli@ldi.jgh.mcgill.ca