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# Parathyroid Hormone-Related Protein: Biochemistry and Molecular Biology

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**ABSTRACT:** This article critically reviews the current state of knowledge regarding the recently identified and cloned novel hormone parathyroid hormone-related protein (PTHrP). PTHrP is produced by tumors associated with the syndrome of humoral hypercalcemia of malignancy giving rise to the parathyroid hormone (PTH)-like symptoms characteristic of the syndrome.

Areas that will be reviewed include identification, purification and cloning, localization, actions, and significance of PTHrP in cancers and normal physiology. The structure and regulation of the PTHrP gene that may be ancestrally related to the PTH gene will also be discussed.

Studies *in vivo* and *in vitro* with synthetic and recombinant PTHrP sequences and antibodies developed against them have established that the PTH-like actions of PTHrP are mediated via the N-terminal sequences, which show some limited sequence homology with PTH.

Evidence for PTH and non-PTH-like actions of PTHrP in normal physiology, which implicate a role for PTHrP in fetal and neonatal development, is also presented.

**KEY WORDS:** PTHrP, hypercalcemia, cancer, development, calcium.

## I. INTRODUCTION

The term, *humoral hypercalcemia of malignancy* (HMM) is used to describe patients with certain cancers in whom the blood calcium level is elevated in the absence of metastatic deposits in bone. The syndrome occurs most commonly with squamous cell carcinomata, especially of the lung, but also with a variety of other tumors, especially renal cortical carcinoma and several cancers of other than squamous cell origin.[1-3] A humoral mechanism of hypercalcemia has also been recognized relatively recently in patients with breast cancer,[4] and, furthermore, in the retrovirus (HTLV-1)-associated adult T-cell leukemia, the mechanism of hypercalcemia appears to be the same as that in patients with the HHM syndrome and solid tumor.[5]

It has been recognized since the 1930s that the high plasma calcium and low plasma phosphorus levels occurring in many patients with cancers, especially of the lung or kidney, resemble the biochemical changes of primary hyperparathyroidism. Because of this it was suggested by Albright[6] that malignancy-associated hypercalcemia might be due to production by the cancers of parathyroid hormone (PTH). This concept of "ectopic" PTH production by cancers gained favor and was thought to explain hypercalcemia in cancer, especially when bone metastases were not detected.[7]

Eventually it became clear, however, that the tumor product most likely responsible for the hypercalcemia was not PTH itself but some factor that exerted physiological actions very similar to those of PTH, most likely by interacting with the PTH receptor.[8-10] In 1987 this cancer protein was isolated, cloned, and expressed, revealing the ex-

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istence of a PTH-related protein (PTHrP) that fulfilled these predictions made on the basis of previous clinical research.[3,11]

## II. THE EXISTENCE OF A PTH-LIKE FACTOR IN CANCERS

Clinical studies of the early 1980s showed convincingly the striking similarities between the biochemical features of the HHM syndrome and those of primary hyperparathyroidism.[12-14] Since the immunoreactive PTH levels in those patients were suppressed, it became more evident that the responsible agent must be different, but capable of reproducing the actions of PTH. By this time, biological assays for PTH-like activity had been developed that were very sensitive and specific, robust, and suited to the assay of the large numbers of samples that would be required in purification work.[8-10]

These assays, depending on the ability of PTH to activate adenylate cyclase, either in kidney or bone cell targets, were used in the work over the next few years, which resulted in the isolation of parathyroid hormone-related protein (PTHrP). Extracts of tumors from HHM patients containing no immunoassayable PTH were shown to be capable of activating adenylate cyclase in kidney membranes and increasing cyclic AMP production in osteoblast-like bone cells.[9,10] This adenylate cyclase-stimulating activity could be shown in PTH target tissues only and was inhibited by peptide antagonists of PTH. However, no inhibition of this activity resulted from preincubation with an antibody to PTH, which was capable of completely obliterating the PTH response in these systems. A very sensitive cytochemical assay for PTH was also used to show that some tumor mRNA injected into *Xenopus* oocytes produced PTH-like activity inhibited by PTH antagonists but not by anti-PTH antisera.[15] Furthermore, this same sensitive PTH assay was employed to find increased circulating biological activity in patients with HHM in the presence of low or undectable immunoreactive PTH.[16] All of this was strong evidence that HHM cancers produced a substance chemically distinct from PTH, but that acts upon PTH target cells through, or in close association with, the PTH receptor. The production of such activity was also shown in certain animal tumors associated with humoral hypercalcemia,[10,17] in cell cultures established from these tumors, and in a cultured cell line established from a renal cortical carcinoma removed originally from a hypercalcemic patient.[8]

## III. ISOLATION OF PTHrP

We found that a human lung cancer cell line (BEN) that we had been studying for several years produced substantial amounts of this PTH-like adenylate-cyclase-stimulating activity. The BEN cell line had been established originally from a patient with hypercalcemia and squamous cell carcinoma of the lung.[18] PTH-like biological activity could be detected in dilutions of BEN-conditioned medium, and activity was unimpaired by prior incubation with a goat antiserum against human PTH(1-34), which completely blocked the activity of human PTH(1-34) itself. On the other hand, specific peptide antagonists of PTH were able to inhibit the activity from BEN cell medium.[11]

Purification of the active components from BEN cell medium was achieved by processing large batches of conditioned medium through cation exchange chromatography and several reverse-phased high-pressure liquid chromatography steps, with monitoring of purification at all stages by the use of the sensitive biological assay measuring the cyclic AMP response in parathyroid hormone-responsive UMR 106.01 osteogenic sarcoma cells. A protein of molecular weight 17 to 18,000 Da was purified in this way.[19] Amino-terminal sequence analysis of purified material pointed to significant N-terminal homology with PTH, with 8 of the first 13 residues identical with those in PTH, and the specific biological activity of this protein in the PTH assay was about 6 times greater than that of PTH itself.[19] An apparently similar protein was purified from a breast cancer by Burtis et al.[20] but no specific biological activity or sequence data were shown. The earlier publication of Rabbani et al.[21] reported quite low specific activity proteins purified from a rat Leydig cell tumor and a human squamous cell carcinoma from a patient with HHM. Amino acid analysis of these proteins sug-

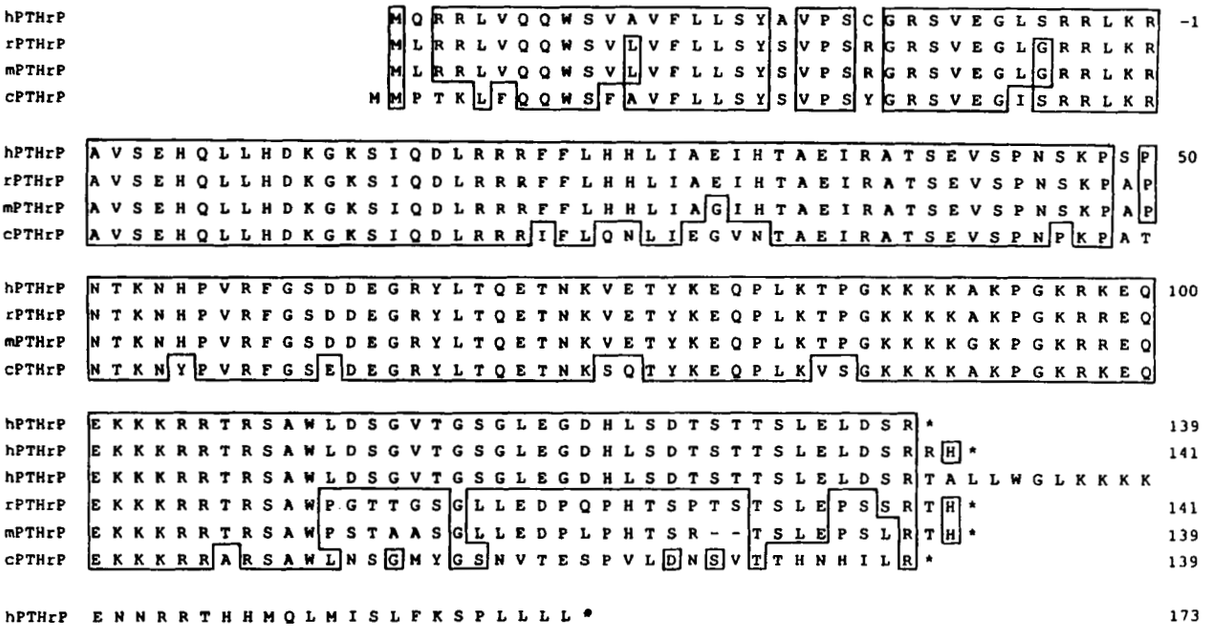


**FIGURE 1.** Amino-acid sequence comparison of human (h), rat (r), mouse (m), and chicken (c) PTHrPs. Residues of identity with the human species are boxed, and dashes have been introduced to maximize the homologies. The differing C-terminal regions of human PTHrP (i.e., from residue 139), resulting from alternate splicing, are indicated.

gested the presence of an unrelated protein contaminated by a small amount of PTH-like activity.

Subsequent purification work of BEN-cell-conditioned medium yielded amino-acid sequence up to residue 50, using a combination of amino-terminal sequencing and the sequencing of an overlapping tryptic peptide, as well as a sequence from a tryptic peptide comprising residues 68 to 79.[22] The amino acid sequence data were confirmed when the total sequence was deduced from the nucleotide sequence of the isolated cDNA.[22] Subsequently two other groups isolated proteins with identical N-terminal sequences from a breast tumor and from cultured renal carcinoma cells.[23,24]

The striking homology with PTH about the amino-terminal region is not maintained in the remainder of the molecule, which therefore represents a previously unrecognized hormone. It seemed most likely that PTH and PTHrP were related in evolution, having arisen from a common ancestral gene, and diverging through gene duplication. For this reason the temporary name, *parathyroid hormone-related protein,* has been applied. The PTH-like portion of the molecule is confined to the amino-terminal region.

## IV. CLONING OF PTHrP AND PREDICTED STRUCTURE

Knowledge of the first 24 amino acids of the sequence was used to prepare synthetic oligonucleotides probes, in order to isolate cDNA clones from a cDNA library in λgt10, prepared from the BEN cells. Two 72-mer oligonucleotides were synthesized, one based on mammalian frequency tables and the other using codons from PTH for the positions of amino acid match. Full-length cDNA clones were isolated and found to encode a prepropeptide of 36 amino acids and a mature protein of 141 amino acids, containing no cysteines, no methionines, and no potential $NH_2$-linked glycosylation sites (Figure 1).[22] The striking homology with PTH about the N-terminus was obvious, but the remainder of the sequence was unique. There is little homology in the leader sequence with PTH, but the overall structure of the leader sequence suggests that it may function in a manner analogous to the prepro-sequence of PTH.[25] A cleavage site present six or eight residues before the $NH_2$-terminus provides for formation of a short basic, prosequence like that found in PTH, which would then be cleaved off

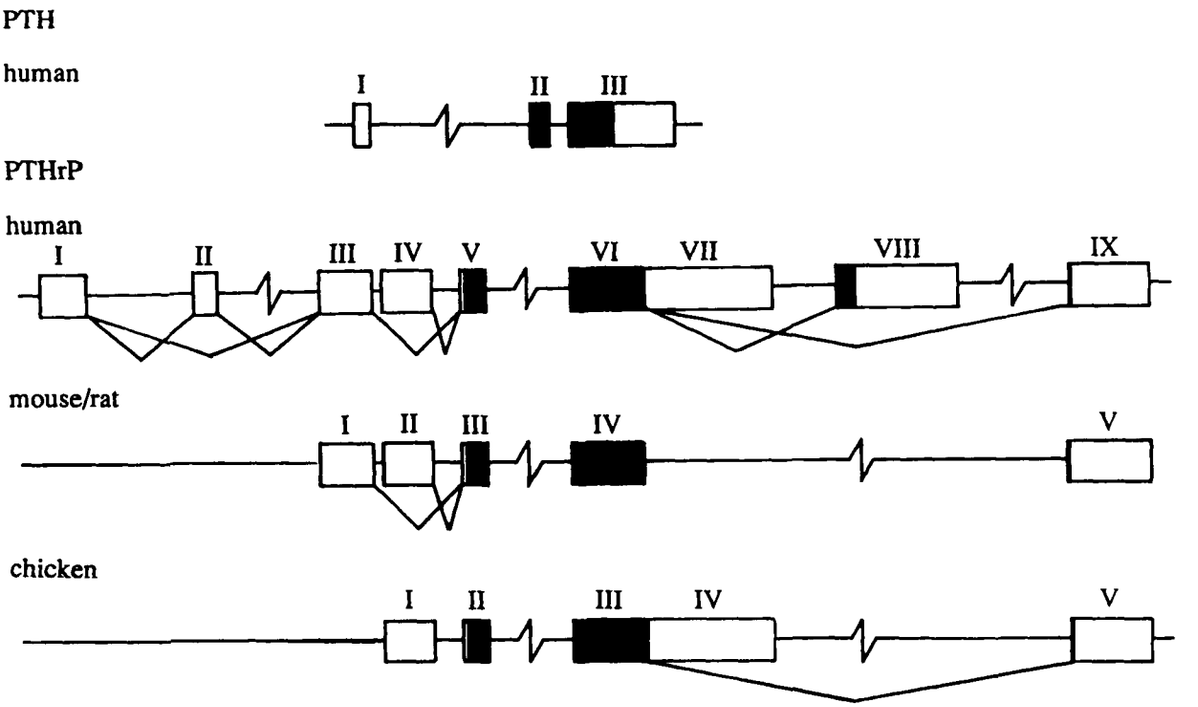


**FIGURE 2.** Comparison of the genomic organization of the human *PTH* and *PTHrP* genes from mouse/rat and chicken. The coding regions and untranslated sequences are indicated by the closed and open boxes, respectively. The human *PTHrP* gene has been aligned below the human *PTH* gene at the common intron/exon boundary 5′ to their respective coding regions, while the mouse/rat and chicken structures are aligned over areas of sequence homology within the exon regions of the human gene sequence. Potential alternate splicing events of the human, mouse, rat, and chicken genes are indicated below.

to give the mature protein. A long stretch of basic amino acids, which includes two potential amidation sites, is located between residues 86 and 108, providing potential cleavage points for post-translational processing. Chicken, rat, and mouse PTHrPs have now also been cloned,[26-28] and there is remarkable conservation of the primary sequence of the predicted proteins up to residue 111, after which there is considerable divergence. Examination of the nucleic acid sequences suggest that this divergence, in part, results from deletions and reading-frame shifts.

The cDNA for PTHrP was subsequently cloned independently by Mangin et al.[29] from a renal cortical carcinoma. Thiede et al.[30] used the published cDNA sequence to clone PTHrP cDNA, which is also from a renal cortical carcinoma cell culture, as starting material. The combined studies of the isolated PTHrP cDNAs by Thiede et al.,[30] Mangin et al.,[29] and Suva et al.,[23] and studies of the structure of the gene by Mangin et al.,[31,32] Suva et al.,[33] Yasuda et al.,[34] and Karaplis et al.,[35] reveal that PTHrP species of 139, 141, and 173 amino acids can be produced and that multiple mRNA species with variable 5′ and 3′ untranslated regions result from alternate splicing of the single-copy gene (Figure 2). Studies in various tumors and tissues have revealed, in many cases, multiple PTHrP mRNA and protein species.[22,33,36-38]

Proof that the isolated PTHrP cDNA reflected a functional protein was obtained by expressing biologically active PTHrP. A vector was constructed so that the full-length protein, including its prepro sequence, would be synthesized, by cytomegalovirus promoter-directed synthesis. The secretion and generation of an active molecule requires cleavage of this prepro sequence. With a similar vector constructed for expression of PTH as a positive control, transfection of these plasmids into COS-7 monkey kidney cells yielded PTH-like bioactivity in cul-

**TABLE 1**
**Related Genes Located on Chromosomes 11 and 12**

| Chromosome 11 | | Chromosome 12 | |
|---|---|---|---|
| **Location** | **Gene** | **Location** | **Gene** |
| q13 | Glutathione S transferase | q13-q14 | Glutathione S transferase 3-like 1 |
| p15.4 | Parathyroid hormone | p11.2-p12.1 | Parathyroid hormone-related protein |
| p14.1-p15 | Lactate dehydrogenase A<br>Lactate dehydrogenase C | p12.2-p12.1 | Lactate dehydrogenase B |
| p15.5 | Tyrosine hydroxylase | q22-q24.2 | Phenylalanine hydroxylase |
| p15.5 | Harvey ras sarcoma 1 | p12.1 | Kirsten ras sarcoma 2 |
| q13 | Murine mammary tumor virus integration site (v-int-2) | q12-q13 | Murine mammary tumor virus integration site (v-int-1) |
| p15.5 | Insulin-like growth factor | q22-24.2 | Insulin-like growth factor 1 |
| p15.5 | Insulin | | |
| p11-q12 | Coagulation factor II (prothrombin) | p12 | Coagulation factor VII (von Willebrand factor) |
| q12-q13.1 | Complement component inhibitor 1 | p13 | Complement component 1s |

ture media, as measured by biological assay.[22]

## V. *PTHrP* GENE ORGANIZATION AND REGULATION

The human *PTHrP* gene has been located on chromosome 12,[29,33] whereas that for PTH is located on chromosome 11.[39] These two chromosomes are considered to be related in evolution, and there are several examples of evolutionarily related proteins whose genes are located on chromosome 12, and chromosome 11 (Table 1).

The genomic structure for the human *PTHrP* gene has been studied predominantly by three laboratories,[28,29,31-34] and reveals a single-copy gene comprising nine exons (Figure 2). The *PTHrP* gene is thus far more complex than the *PTH* gene, which has only three exons. However, they share a common exonic region encoding the prepro sequence. The rat and mouse *PTHrP* gene structures have also been determined[27,28] and are much simpler than the human gene, comprising four, or potentially five exons, equivalent to coding exons, V, VI, and IX, and the untranslated exons III and IV of the human gene. The structure of the chicken gene has also been elucidated[40] and indicates, unlike the rat and mouse genes, that alternate splicing of the primary mRNA transcript occurs at the 3′ end, specifying two different protein species (Figure 2). The conservation of exons equivalent to IV, V, VI, and IX of human *PTHrP* among the three other species of genes examined would imply that these constitute the minimum gene structure. Furthermore, the promoter region 5′ to human exon IV appears to be the major promoter for *PTHrP* gene transcription, since this region is conserved among the human, mouse, rat, and chicken genes. In support of this notion, our regulation studies have identified that agents that affect *PTHrP* gene transcription act primarily through this promoter sequence.

Exon VI of *PTHrP* comprises the majority of the coding region for the mature protein, up to residue 139, where a splice donor site is located. Read-through of exon VI into exon VII results in the introduction of a termination signal producing a protein product of 139 amino acids in length, while participation of exons VII and IX, via alternate splicing, results in protein products of 173 and 141 amino acids, respectively[27,31] (Figures 1 and 2). Thus, alternate 3′ splicing results in proteins with variable C termini, the biological significance of which is unknown at present. It has been suggested that 3′ untranslated sequences of some genes may play a role in development or in tissue-specific gene expression. The presence of the motif AUUUA, which has been implicated in the rapid turnover of many cytokines,[41] and some protooncogene mRNA in each of the 3′ untranslated sequences (i.e., those

specified by exons VII, VIII, and IX), is in agreement with the short half-life of PTHrP mRNA, which we have determined to be less than 2 h, and may be indicative that PTHrP has a role in differentiation or is expressed in a tissue-specific manner, such as other mRNA species that contain this motif.[42,43]

In addition to the variable 3′ exonic regions, multiple promoters contribute to the production of different mRNA species. We had obtained an indication of this in the course of cloning the PTHrP cDNA, in which we noted that one of the six clones that we isolated (pBRF52)[22] contained a divergent 5′ untranslated region, suggesting the involvement of alternatively spliced mRNAs. Subsequent analysis of the 5′ region of the gene[32,33] confirmed the presence of multiple promoter sites. 5′ to exons I and IV are canonical TATA consensus sequences, while 5′ to exon III is a GC-rich region, containing a Sp-1 and two AP-2 binding sites; elements known to act as promoters in the absence of RNA II-dependent polymerase, as has been reported for insulin-like growth factor II and platelet-derived growth factor receptor.[44,45] This arrangement suggests that tissue-specific expression of the gene may occur as a function of the use of differing promoter regions. In agreement with this hypothesis, differential use of the 5′ exons has been reported between esophageal, renal, and squamous cell lines.[27,32,33] For example, the data so far indicate that renal cell lines utilize promoter sequences 5′ to exon I and exon IV, while squamous cell lines utilize a GC promoter region within exon III, as well as the TATA sequences 5′ to exons I and IV. The extent of exon III is, at present, undefined. This exon has been determined to be 240 nucleotides in length by RNase protection experiments using RNA from a renal carcinoma cell line.[32] However, by S1 and primer extension analyses, this exon in esophageal cells is apparently 827 nucleotides, while in two squamous cell lines this exon is 113 nucleotides in length. This apparent difference in size may result either from the use of different transcription factors (e.g., AP-2, Sp1, or RNA II dependent polymerase) in these cell lines or the extension of RNA present in the intronic sequences of unprocessed PTHrP transcripts. The existence and use of alternate promoters for the *PTHrP* gene points to complex regulation, such as has been described for the α-amylase gene,[42] with high potential for tissue-specific expression. The multiple promoters and very long upstream region of the human *PTHrP* gene is suggestive of novel mechanisms of regulation of *PTHrP* gene expression. The longer 5′ untranslated regions generated by the use of exons, I, II, and III, contain a number of AUG sequences, each of which is followed by in-frame stop codons. None of these upstream AUGs is preceeded by the Kozak[46] consensus for the initiation of transcription. The precise role of these AUGs or secondary loop structures in mRNA translation is unclear, but it is thought that they could exert some translational control over gene expression. Upstream AUGs have been shown to be involved in the control of translation in both yeast GCN4[47] and mouse bc12 genes.[48]

The presence in the *PTHrP* gene of features characteristic of high-turnover cytokines and the short half-life of PTHrP mRNA provides the means for sensitive and complex regulation of PTHrP production. The *PTHrP* gene contains consensus regulatory motifs for cyclic AMP, 1,25-dihydroxy vitamin $D_3$ ($1,25(OH)_2D_3$), and glucocorticoids.[49-53] Dexamethasone and $1,25(OH)_2D_3$ both decrease the expression of PTHrP mRNA in cancer cells,[51,52] and transfection experiments indicate that these agents decrease gene transcription. In the BEN cells, in which calcitonin increases cyclic AMP production, calcitonin treatment led to increased PTHrP mRNA, probably by a transcriptional effect.[54] Epidermal growth factor (EGF) increases PTHrP production by an osteosarcoma cell line[55] and PTHrP mRNA production in human keratinocytes,[56] and transforming growth factor β (TGFβ) also increases PTHrP mRNA and protein in both keratinocytes and squamous skin cancer cells.[57] The effect of TGFβ also appears to be a transcriptional one, and this may be a particularly important finding, given the fact that TGFβ and PTHrP appear to be coproduced in a number of tissues during development,[58,59] and also that TGFβ is commonly produced by tumors. It is possible that TGFβ is an important paracrine stimulator of PTHrP production. Investigation of potential regulation of PTHrP production by other tumor-derived cytokines will be important in defining the etiology of HHM. It is also possible

that such interactions may have physiological relevance with respect to tumor maintenance.

Thus the detailed regulation of the *PTHrP* gene by these and other agents will be important to determine. Regulation by calcium ions will be of particular interest. PTH secretion by the parathyroids is regulated by a reduction in serum calcium, which can stimulate PTH release in the short term and regulate transcription in the longer term.[60,61] The production of PTHrP-like biological activity by a rat parathyroid cell line can be influenced by calcium,[62] although very little change could be detected at the mRNA level.[63] It has yet to be demonstrated whether calcium levels can influence PTHrP production by tumors. If, as will be discussed later, PTHrP is the important calcium-regulating hormone of the fetal parathyroid glands, it will be important to know whether, as is the case with PTH, calcium ion changes can influence PTHrP transcription.

## VI. STRUCTURE POTENCY STUDIES OF PTHrP

As is the case with PTH itself, the PTH-like biological activity of PTHrP is contained within the first 34 amino acids. PTHrP (1-34) is at least equipotent with PTH in promoting cyclic AMP formation in osteoblasts, and in some assays it is more potent.[64] Furthermore, PTHrP (1-141), (1-108), (1-84), and PTHrP (1-34) are all equipotent on a molar basis in promoting cAMP formation and plasminogen activator activity.[65] Truncation of the peptide to PTHrP (1-29) reduces the activity to 10% of PTHrP(1-34) and shorter peptides are essentially inactive.[64] All of these structure-activity relationships observed with recombinant and synthetic peptides are also retained in studies of the effects on bone resorption by isolated osteoclasts, mediated indirectly by actions of PTHrP through osteoblasts.[66] Thus the structure/activity relationships are very similar to those of PTH,[67] and it has been shown that the nonhomologous regions of PTHrP(1-34) (i.e., residues 15 to 24) contribute to receptor binding in much the same way as their PTH counterparts.[68] Furthermore, PTHrP peptides with N-terminal deletions retain a greater ability to compete for PTH receptor binding than do their PTH equivalents.[69]

The tertiary structure of PTHrP has been studied by Barden and Kemp,[70] and reveals that the 1-34 end of the molecule has a surprisingly compact structure, with an alpha helix between residues 4 and 14 (Figure 3). There is a cluster of hydrophobic residues — Ile-15, Leu-18, Phe-22, Leu-24, Leu-27, Ile-28, and Ile-31. Residues 30 to 34 and the N-terminal residues project out from the main body of the structure. Removal of residues 30 to 34 destabilizes the end of the helix, reducing binding to the PTH receptor. Removal of residues 26 to 29 destroys the second turn between residues 17 and 27, and may account for the loss of binding. Competition binding studies show that PTH(1-34) and PTHrP(1-34) bind with the same relative potencies as those observed in other bioassays and that each ligand competes for the alternative ligand.[71] Cross-linking of either ligand to PTH-responsive cells results in the identification of potential receptor components of identical mobility on polyacrylamide gels.[72,73] There is thus no longer any doubt that the PTH-like actions of PTHrP are mediated via the 1-34 sequence of PTHrP binding to the PTH receptor and that the amino-terminal "PTH-like" portion of PTHrP can essentially reproduce all the actions of PTH, as will be discussed in the following section.

## VII. PTH-LIKE ACTIONS OF PTHrP

PTHrP can reproduce all the features of the syndrome of HHM by its actions via PTH receptors on bone and kidney. Furthermore, antibodies raised against synthetic PTHrP (1-34) can reduce the hypercalcemia and bone abnormalities associated with HHM in animal models of HHM derived by the transplantation of human and animal tumors into nude mice.[74]

PTHrP peptides are active in promoting bone resorption *in vitro*[64,75,76] and *in vivo*,[77-79] and like PTH itself, they do so by acting first on cells of the osteoblast lineage, with subsequent activation of osteoblasts.[66] As with PTH, cyclic AMP appears to be major mediator of the actions of PTHrP on its target cells, and effects of PTHrP on intracellular calcium[80] alkaline phosphatase and plasminogen activator have also been shown.[81]

The actions of PTHrP on the kidney have been studied in the isolated perfused rat kid-

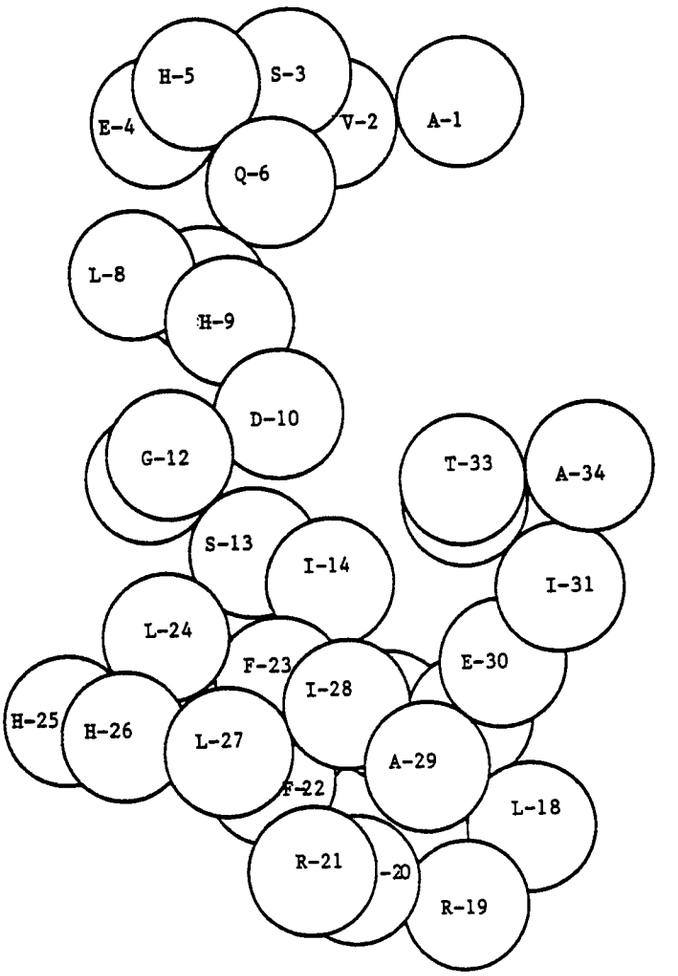


**FIGURE 3.** The tertiary structure of human PTHrP (1-34).[138]

ney,[82,83] in the rat *in vivo*,[77-79,84,84] and in the ovine fetus *in utero*.[86] All these studies show clearly that both PTHrP and PTH promote cyclic AMP and phosphorus excretion and reduce calcium excretion. The study by Ellis et al.,[7] in the perfused rat kidney, showed that prolonged infusion with PTHrP (1-141) resulted in reduced bicarbonate excretion, a response that differed from that with PTH or with PTHrP (1-34). It is possible that this observation might explain the mild alkalosis that often accompanies the HHM syndrome, whereas primary hyperparathyroidism is often associated with mild hyperchloremic acidosis.

As is the case with PTH, PTHrP promotes 1-α-hydroxylation of vitamin D,[75,76] although there are some clinical observations of low plasma levels of $1,25(OH)_2D_3$ in HHM patients that are discrepant with this,[87,88] and for which there is no obvious explanation at present.

Other actions of PTHrP that reflect those of PTH include the ability to relax vascular and other smooth muscle.[89-91] This response is one that will be most interesting to investigate further, because it may reflect more a physiological function of PTHrP, rather than of PTH, and is consistent with PTHrP production and local action on smooth muscles at various sites.[92]

## VIII. UNIQUE ACTIONS OF PTHrP

In addition to the PTH-like actions of PTHrP, there is increasing evidence for other biological activities within the PTHrP molecule, not shared with PTH, and giving rise to the concept that PTHrP is a polypeptide precursor of a number of

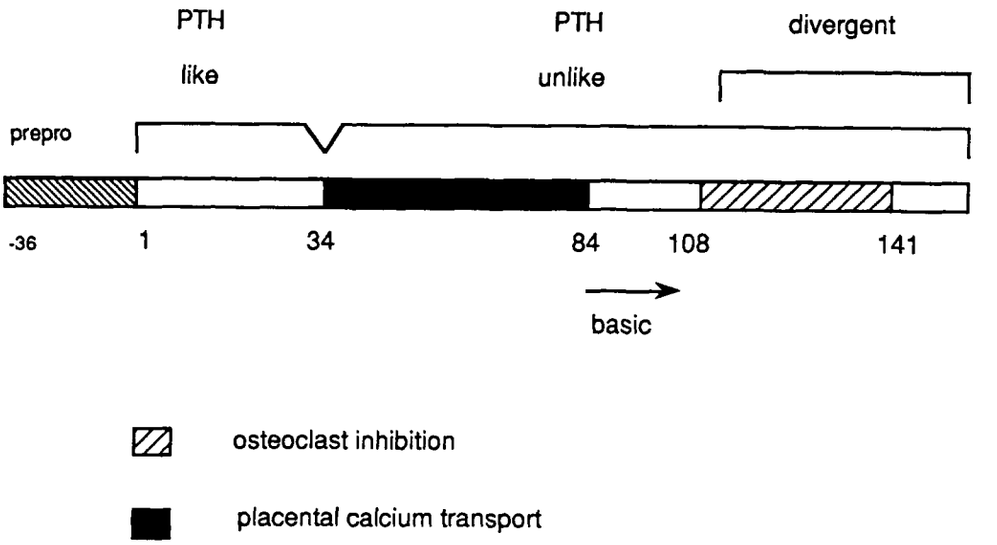


**FIGURE 4.** Organization of prepro-PTHrP indicating the location of the different biological actions.



biological activities, analogous with proopiomelanocortin (Figure 4). The first of these "non-PTH" effects is the action of PTHrP to promote calcium transport across the placenta.

Both placenta and fetal parathyroids contain PTHrP-like material that has been identified both immunohistochemically and by bioassay.[93,94] Extracts from both of these tissues, as well as purified and recombinant PTHrP, can stimulate calcium transport across the placenta in sheep,[93] providing the calcium required for skeletal development and maintaining the fetus hypercalcemic relative to the mother.[95] It seems likely that the placenta is the early source of PTHrP, since it is present in larger amounts in placenta from early gestation and since later the fetal parathyroids take over as the major source of PTHrP. The mechanisms involved remain to be elucidated.

Parathyroidectomy of the fetus abolishes the calcium gradient between mother and fetus, but neither PTH nor $1,25(OH)_2D_3$ (or other vitamin D metabolites) has any effect on placental calcium transport, implying that the effects are mediated by regions of PTHrP that are not homologous with PTH and that a unique receptor is involved.[95] Recent evidence of Care et al.[96] suggest that the placental calcium transport activity lies within the 1-84 sequence distal to the first 34 amino acids. The placental model is potentially important and may provide the means to study the unique actions of PTHrP on calcium transport, separate from the actions that mimic those of PTH. The fetal parathyroid production of PTHrP has been established by carrying out PTHrP assays on extracts of fetal parathyroids of several species,[97] by immunohistochemistry on fetal lamb and human parathyroid tissue, and by Western blotting of extracts.[98]

The third biological activity within the PTHrP molecule that is potentially very important is a surprising one. The carboxy-terminal region of PTHrP (107-139) is an extremely potent inhibitor of bone resorption, an observation made first in experiments in which this peptide and analogues acted directly on isolated osteoclasts to inhibit their activity.[99] It is possible that carboxy-terminal PTHrP acts as a paracrine regulator of bone resorption. Evidence has been obtained for the production by resorbing fetal bones of PTH-like activity, which appears to be amino-terminal PTHrP,[100] and furthermore, immunohistological evidence for PTHrP production in fetal bones has been obtained.[92] The finding that carboxy-terminal PTHrP exists free in the circulation in some patients with cancer[101,102] also raises the possibility that it might, in some circumstances, have a humoral role. The receptor and mode of action of the C-terminal fragment remains to be elucidated.

Figure 4 illustrates the organization of PTHrP,

summarizing the location of these several biological activities. There are many potential processing sites within PTHrP, and it will be important to determine the specific mechanisms and sites of postsynthetic processing that can give rise to these constituent activities.

## IX. PTHrP IN CANCER

Localization studies of PTHrP using immunohistochemistry and Northern blotting in tumors from patients with a variety of cancers have indicated that PTHrP may be more commonly involved in the cause of hypercalcemia associated with malignancy than has been previously appreciated. The more recent development of useful radioimmunoassays has also confirmed the presence of PTHrP in the circulation of patients with HHM.

Immunohistochemical analyses of tumor sections using specific antibodies predominantly raised to PTHrP 1-34, but also to unique mid 50-69 and C-terminal 106-141 epitopes, have shown that PTHrP is present in the cells of all squamous cell carcinomata investigated,[11,103] indicating the potential of these tumors to produce HHM, even though in many cases secretion of PTHrP into the circulation might be insignificant and patients remain normocalcemic. Positive staining has also been demonstrated in other tumors associated with HHM, particularly renal carcinomata, and the presence of PTHrP mRNA has been demonstrated in these two tumor types most commonly associated with HHM.[36,102-107] The involvement of a humoral factor in the etiology of hypercalcemia associated with breast cancer has been indicated previously in studies by Percival[108] and Isales,[4] but the traditional view has been that the hypercalcemia in breast cancer patients results predominantly from the action of metastases in bone.[1] The immunohistochemical localization of PTHrP in 60% of unselected breast cancers[109] has indicated a need to investigate PTHrP levels in breast cancer patients and the hypothesis that PTHrP may have a role in the establishment of metastases in bone. Studies in our laboratory have indicated that circulating levels of PTHrP are raised in 60% of patients with breast cancer and hypercalcemia.[110]

Another cancer frequently associated with hypercalcemia is the group of lymphoid tumors, including those from HTLV-1-infected patients. It had long been thought that in these cases hypercalcemia resulted from the action of bone-resorbing cytokines released by circulating cancer cells,[111] however, many patients with HTLV-1 hypercalcemia exhibit the clinical and biochemical features associated with the HHM syndrome. Evidence has now been obtained for the presence of PTHrP immunoreactivity in 40% of a small group of patients with HTLV-1-positive tumors,[112] and PTHrP mRNA has been identified in HTLV-1-infected lymphoid cells.[5,113] In a recent study of 13 ATL patients, all showed expression of the *PTHrP* gene.[5] The implication of a *tax* gene product that is the HTLV-1 transactivating factor in PTHrP production in these cells has also been reported,[5] and it was found that the *tax* gene product caused transactivation of the *PTHrP* gene promoter linked to the CAT reporter gene in transfection assays.

It is of particular interest to note the high rate of detection of PTHrP and PTHrP mRNA in parathyroid adenomata and secondary hyperplastic glands, but not in primary hyperplasia.[104,105] We have identified PTHrP in fetal parathyroids in sheep and human embryos, originally in the sheep by identification of PTH-like bioactivity that was not PTH, and more recently by immunohistology.[94]

The presence of PTHrP in parathyroid adenomata may represent the reexpression of the evolutionary and fetal origins of PTHrP. The mechanisms involved in the expression of PTHrP in chronic renal failure are not clear, but it is presumed that low serum calcium is the stimulus for the hyperplasia, the implication being that PTHrP may normally be present in parathyroids at very low levels and may be subject to the same feedback mechanisms as PTH. Immunoreactive PTHrP levels measured using N- and C-terminal (109-138) radioimmunoassays were not raised in patients with primary hyperparathyroidism,[101,110,114,115] except in one study that is very difficult to interpret because of the low sensitivity and apparently high normal values it reports.[116] However Burtis et al.,[101] reported the presence of elevated PTHrP (109-138) but not (1-74) levels in patients with chronic renal failure. This finding

was interpreted to be due to the inability of the kidney to clear the C-terminal peptides, which are likely to result from normal proteolytic cleavage. By inference PTHrP may circulate in normal subjects, although it is not detectable with current assays.

## X. PTHrP IN NORMAL PHYSIOLOGY

At the present time there is no indication of a role for PTHrP as a circulating hormone in normal mammals or humans after birth. From the preliminary radioimmunoassay data available at the time of writing, it is clear that circulating levels in normal humans are very low. No assay can convincingly and repeatedly assay PTHrP in normal subjects, with detection limits in assays of the order of 0.2 to 2 p*M*.[101,110,116] PTHrP levels are readily detected with these assays in 80 to 100% of patients with the HHM syndrome.

On the other hand, there are increasing suggestions that PTHrP may have a role in the fetus and in early development. Recently, maternal milk has also been shown to contain very high concentrations of PTHrP, of the order of 100 ng/ml.[117] High levels have been detected in milk from a variety of mammalian species, including the marsupial, which is born with a very immature uncalcified skeleton. It is not yet known if maternal milk PTHrP is important for the neonate, but it is of interest to note that suckling and prolactin induce PTHrP mRNA in mammary tissue.[118,119] Recent evidence also indicates measurable levels of PTHrP in the circulation of lactating mothers at a time when large amounts of PTHrP are found in milk.[120] At this time it is not clear whether circulating PTHrP in lactating women simply represents spillover from the large amounts produced by the breast or if PTHrP may also serve some endocrine role in the mother, for example, in the mobilization of calcium from the maternal skeleton. It is possible that milk PTHrP can contribute, for example, to intestinal cell differentiation or alternatively, and more likely, to the transport of calcium into milk, consistent with the correlation between milk PTHrP and calcium levels noted in cows.[121]

We have discussed earlier the data suggesting that PTHrP is a hormone of the fetal parathyroid gland, to be replaced around the time of parturition by PTH. The existence of a fetal factor with properties resembling PTH has long been suspected. If PTHrP is the predominant secretory product of the parathyroid gland of the mammalian fetus, its physiological role is similar to that of PTH itself, i.e., to raise the extracellular fluid calcium by whatever means are available to it. In the case of the postnatal animal, that is by promoting bone resorption, calcium retention by the kidney, and by indirectly promoting calcium absorption. In the fetus, the means available is the active placental calcium pump, transporting calcium from mother to fetus and restricting calcium excretion into amniotic fluid.

Using fetal sheep as a model, we have obtained evidence for the placental calcium transport effect of PTHrP[93,96] and have identified this as an action of some part of the PTHrP molecule that is not "PTH-like". More recently, also in studies in the fetal lamb we have demonstrated that both PTH and PTHrP cause a rapid reduction of calcium excretion by the fetal kidney.[86] The calcium-conserving effect of PTHrP by such a renal action seems, therefore, to be one that is found within the "PTH-like" portion of the molecule.

From the evolutionary point of view, such an adaptation to achieve a physiological purpose might indicate that PTH is the latecomer in evolution and that we might expect to find the PTH-related gene expressed in lower species, producing a protein that will contribute to maintenance of the extracellular fluid calcium in those species by the means available to them. In postnatal life in mammals, on the other hand, PTHrP may have no significant function as a circulating hormone, except in certain diseases, notably the cancers associated with HHM and perhaps also parathyroid diseases. The molecule responsible for hormonal regulation of calcium homeostasis in the postnatal mammals is PTH, having evolved to carry out a set of functions through a single class of receptors. It should be expected that a second receptor will be found for PTHrP.

PTHrP has been detected in normal animals in fetal epithelia, developing fetal bone undergoing osteogenesis, fetal parathyroids, placenta, lactating mammary tissue, skeletal and smooth muscle, normal stomach, brain, pancreas, heart,

lung, spleen, kidneys, testis, ovary, uterus, and normal skin.[28,92,106,107,122-124] In the nonpregnant adult, there is no location yet identified that could constitute a major source of circulating PTHrP, consistent with the view that PTHrP has a predominantly local role in normal physiology in the adult.

We have already mentioned evidence for potential local role of PTHrP in smooth-muscle relaxation. The presence of PTHrP in smooth muscle and skeletal muscle in the fetus may also imply local effects involving calcium metabolism. It is particularly interesting, in this respect, that a recent study reports increased levels of PTHrP mRNA in rat uterine smooth muscle just prior to parturition,[124] and the same authors have also shown a correlation between PTHrP mRNA in the vascular bed of the shell gland that correlates with the movement of eggs down the oviduct in birds.[90] PTHrP mRNA has been identified by *in situ* hybridization in mammalian central nervous system, and brain extracts have been shown to stimulate adenylate cyclase in parathyroid-hormone-responsive cells.[122] While its expression in normal brain may be the basis of its expression in neuroendocrine tumors, there is as yet no evidence for a functional role for PTHrP in the brain. An effect of PTH on calcium transport independent of cAMP in rat brain synaptosomes has been reported,[125] however, and PTH mRNA has recently been detected in the brain, although its distribution appears not to be as widespread as that for PTHrP.[126]

Consistent with the presence of PTHrP squamous cell cancers is its presence in normal adult skin, which has been demonstrated both immunohistochemically[11,103,105] and by mRNA hybridization.[36] The possibility that PTHrP is predominantly an epithelial cell product is supported by the demonstration of PTHrP in epithelia from many locations in the human fetus. It is possible that PTHrP may have an important role in epithelial differentiation, a property that would be relevant when expressed in cancers and that also has relevance to the constant renewal processes in normal skin. The fact that PTHrP is located in the keratinocyte layer of normal skin is certainly of interest, but it may be that only extremely small amounts reach the circulation from that source, where it may function as a locally active cytokine. The primary physiological role for PTHrP as a paracrine effector may be the local regulation of calcium transport, which itself may be important in epithelial differentiation,[127,128] and this hypothesis is supported by the finding that PTHrP is present in nonlactating hypertrophic breast tissue and released into the circulation.[130] No potential major source of PTHrP that might contribute to significant circulating levels in the adult has yet been identified, and the physiological significance of the PTHrP and its mRNA detected in a variety of normal tissues remains to be elucidated.

## XI. IMMUNOLOGY OF PTHrP

The existence of this protein helps to explain the HHM syndrome in cancers and opens up areas for study on the physiological functions of PTHrP. The structural similarity to PTH within the amino-terminal region is sufficient to explain the PTH-like effects of PTHrP. Despite this similarity in primary sequence, antibodies against PTH or PTHrP are usually very selective, and antibodies can readily be obtained, which, for example, recognize PTHrP readily and PTH not at all. Use of this is being made in radioimmunoassays being carried out in clinical studies[109,110] in our immunohistology studies and in the use of anti-PTHrP antibodies in the treatment of the HHM syndrome.

When the immunogenicity of the PTHrP and of human PTH (1-34) are predicted based on the assumptions of Welling et al.,[130] the difference between the two is noted, particularly in the area of greatest sequence homology.[11] The first 10 amino acids of PTH are notoriously poorly immunoreactive, in contrast to the N-terminal sequence of PTHrP, which is highly antigenic. We have used antisera against PTHrP amino-terminal fragments in immunohistochemical studies of tumors, in which cross-reaction with human PTH remains insignificant, even under high concentration conditions.[103,104] Antisera were chosen for use in immunohistochemistry on the basis of their specificity in radioimmunoassay, their ability to neutralize PTHrP biological activity without effect on PTH, and their low background in immunoperoxidase studies. Thus, the immunolog-

ical difference between these two molecules is substantial, despite the structural, and indeed functional, similarities. Care must be taken in relying on this, however, and lack of cross-reactivity under radioimmunoassay conditions does not exclude significant cross-reaction at the high antibody concentrations used in immunohistochemistry. Before the discovery of PTHrP and recognition of its similarity to PTH, there was a long literature on the detection of "PTH" by immunohistochemistry in tumors. Palmieri et al.[131] used an immunofluorescence method with a guinea-pig antiserum against partially PTH to show fluorescence in the cytoplasm in six of seven cancers from patients with HHM. Similarly, immunoreactivity ascribed to PTH was detected in a rhabdoid Wilms tumor[132] using an uncharacterized anti-PTH antiserum, and in squamous cell cancers from four patients with HHM,[133] employing an antiserum against PTH (1-84). In all these cases the findings can be explained by significant cross-reaction of the anti-PTH against PTHrP under the conditions used. It seems likely on the basis of present evidence that PTHrP production may be an almost invariable finding in squamous cell cancers. The ectopic production of PTH probably occurs very rarely, and there have been only two very carefully documented cases of PTH synthesized by human cancers reported within the last few years.[134,135]

Another major use of antisera against PTHrP is in the therapy of malignancy-associated hypercalcemia. We studied the effect of anti-PTHrP (1-34) antiserum and affinity-purified anti-PTHrP (1-16) antiserum on serum calcium and cAMP excretion in two animal modes of humoral hypercalcemia of malignancy. These consisted of two tumors from patients with the HHM syndrome, one a squamous cell carcinoma of the lung, the other of the larynx, and maintained as transplanted tumors in athymic mice.[136] In each case, the mice bearing the tumors develop hypercalcemia, hypophosphatemia, and elevated urinary cAMP. With each antiserum and in both tumor modes, intravenous injection of antiserum resulted in significant lowering of serum calcium and urinary cAMP in the tumor-bearing mice. The effects were first seen 3 h after injection and lasted for at least 48 h.[73] The fact that serum calcium levels did not return completely to normal in all animals suggests either that more antibody was necessary or that other factors (e.g., cytokines, TGFα, and TGFβ) might contribute to the development of hypercalcemia.[137] The 24- to 48-h duration of the hypocalcemic effect is consistent with the half-life of IgG in the mouse. Using the same experimental model, antibody treatment was shown to be as effective as tumor resection in correcting the bone abnormalities occurring in these tumor-bearing animals. The data suggest that PTHrP secreted by the tumors is at least responsible, to a major extent, for the hypercalcemia. It also raises the interesting possibility that monoclonal antibodies against PTHrP could be applied to the emergency treatment of hypercalcemia in the HHM syndrome.

## XII. CONCLUDING COMMENTS

The PTHrP molecule is parent to three demonstrated bioactivities involved in calcium regulation, of which the PTH-like action is the most characterized. In addition to the endocrine effects of PTHrP, a growing body of largely indirect evidence suggests a local role for PTHrP in epithelial growth and/or differentiation, which may also relate to local calcium regulation. Although the placental transport and osteoclast inhibitory actions can be clearly demonstrated with synthetic and recombinant PTHrP preparations, there is still much to understand about the significance of the non-PTH-like actions of PTHrP in normal physiology and the molecular synthetic and processing events that may generate them as either endocrine or autocrine/paracrine regulators.

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## REFERENCES


1. **Mundy, G. R. and Martin, T. J.,** The hypercalcemia of malignancy: pathogenesis and treatment, *Metabolism*, 31, 1247, 1982.
2. **Martin, T. J. and Mundy, G. R.,** Hypercalcemia of malignancy, in *Clinical Endocrinology of Calcium Metabolism*, Martin, T. J. and Raisz, L. G., Eds., Marcel Dekker, New York, 1987, 171.
3. **Martin, T. J.,** Humoral hypercalcemia of malignancy, *Bone Mineral*, 4, 83, 1988.
4. **Isales, C., Carcangiu, M. L., and Stewart, A. F.,** Hypercalcemia in breast cancer: reassessment of the mechanism, *Am. J. Med.*, 82, 1143, 1987.
5. **Watanabe, T., Yamaguchi, K., Takatsuki, K., Osame, M., and Yoshida, M.,** Constitutive expression of parathyroid hormone-related protein gene in human T cell leukemia virus type 1 (HTLV-1) carriers and adult T cell leukemia patients that can be transactivated by HTLV-1 tax gene, *J. Exp. Med.*, 172, 759, 1990.
6. **Albright, F.,** Case records of the Massachusetts General Hospital (case 27401), *N. Engl. J. Med.*, 225, 789, 1941.
7. **Lafferty, F. W.,** Pseudohyperparathyroidism, *Medicine*, 45, 247, 1966.
8. **Strewler, G. J., Williams, R. D., and Nissenson, R. A.,** Human renal carcinoma cells produce hypercalcemia in the nude mouse and a novel protein recognized by parathyroid hormone receptors, *J. Clin. Invest.*, 71, 769, 1983.
9. **Stewart, A. F., Insogna, K. L., Goltzman, D., and Broadus, A. E.,** Identification of adenylate cyclase-stimulating activity and cytochemical glucose-6-phosphate dehydrogenase stimulating activity in extracts of tumors from patients with humoral hypercalcemia of malignancy, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 1454, 1983.
10. **Rodan, S. B., Insogna, K. L., Vignery, A. M.-C., Stewart, A. F., Broadus, A. E., D'Souza, S., Bertolini, D. R., Mundy, G. R., and Rodan, G. A.,** Factors associated with humoral hypercalcemia of malignancy stimulate adenylate cyclase in osteoblastic cells, *J. Clin. Invest.*, 72, 1511, 1983.
11. **Martin, T. J., Allan, E. H., Caple, I. W., Care, A. D., Danks, J. A., Diefenbach-Jagger, H., Ebeling, P. R., Gillespie, M. T., Hammonds, R. G., Heath, J. A., Hudson, P. J., Kemp, B. E., Kubota, M., Kukreja, S. C., Moseley, J. M., Ng, K. W., Raisz, L. G., Rodda, C. P., Simmons, H. A., Suva, L.J., Wettenhall, R. E. H., and Wood, W. I.,** Parathyroid hormone-related protein: isolation, molecular cloning and mechanism of action, *Rec. Progr. Hormone Res.*, 45, 467, 1989.
12. **Kukreja, S. C., Shermerdiak, W. P., Lad, T. E., and Johnson, P. A.,** Elevated nephrogenous cyclic AMP with normal serum parathyroid hormone levels in patients with lung cancer, *J. Clin. Endocrinol. Metab.*, 51, 167, 1980.
13. **Stewart, A. F., Horst, R., Deftos, L. J., Cadman, E. C., Lang, R., and Broadus, A. E.,** Biochemical evaluation of patients with cancer-associated hypercalcemia. Evidence for humoral and non-humoral groups, *N. Engl. J. Med.*, 303, 1377, 1980.
14. **Rude, R. K., Sharp, C. F. Jr., Fredericks, R. S., Oldham, S. B., Elbaum, N., Link, J., Irvin, L., and Singer, F. R.,** Urinary and nephrogenous adenosine 3'5'-monophosphate in the hypercalcemia of malignancy, *J. Clin. Invest.*, 52, 765, 1981.
15. **Broadus, A. E., Goltzman, D., Webb, A. C., and Kronenberg, H. M.,** Messenger ribonucleic acid from tumors associated with humoral hypercalcemia of malignancy directs the synthesis of a secretory parathyroid hormone-like peptide, *Endocrinology*, 117, 1661, 1985.
16. **Goltzman, D., Stewart, A. F., and Broadus, A. E.,** Malignancy-associated hypercalcemia evaluation with a cytochemical bioassay for parathyroid hormone, *J. Clin. Endocrinol. Metab.*, 53, 899, 1981.
17. **Rosol, T. J., Capen, C. C., and Brooks, C. L.,** Bone and kidney adenylate cyclase-stimulating activity produced by a hypercalcemia canine adenocarcinoma line (CAC-8) maintained in nude mice, *Cancer Res.*, 47, 690, 1987.
18. **Ellison, M., Woodhouse, D., Hillyard, C. J., Dowsett, M., Coombes, R. C., and Gilby, E. D.,** Immunoreactive calcitonin production by human lung cancer cells in culture, *Br. J. Cancer*, 32, 373, 1975.
19. **Moseley, J. M., Kubota, M., Diefenbach-Jagger, H., Wettenhall, R. E. H., Kemp, B. E., Suva, L. J., Rodda, C. P., Ebeling, P. R., Hudson, P. J., Zajac, J. D., and Martin, T. J.,** Parathyroid hormone-related protein purified from a human lung cancer cell line, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 5048, 1987.
20. **Burtis, W. J., Wu, J., Bunch, C. M., Wysolmerski, J. J., Insogna, K. I., Weir, E. C., Broadus, A. E., and Stewart, A. F.,** Identification of a novel 17,000-dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy, *J. Biol. Chem.*, 262, 7151, 1987.
21. **Rabbani, S. A., Mitchell, J., Roy, D. R., Kremer, R., Bennett, H. P. J., and Goltzman, D.,** Purification of peptides with parathyroid hormone-like bioactivity from human and rat malignancies associated with humoral hypercalcemia, *Endocrinology*, 118, 1200, 1986.
22. **Suva, L. J., Winslow, G. A., Wettenhall, R. E. H., Kemp, B. E., Hudson, P. J., Diefenbach-Jagger, H., Moseley, J. M., Rodda, C. P., Martin, T. J., and Wood, W. I.,** A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression, *Science*, 237, 893, 1987.
23. **Stewart, A. F., Wu, T., Goumas, D., Burtis, W. J., and Broadus, A. E.,** N-terminal amino-acid se-

quence of two novel tumor-derived adenylate cyclase-stimulating proteins: identification of parathyroid hormone-like and unlike domains, *Biochem. Biophys. Res. Commun.*, 146, 672, 1987.

24. **Strewler, G. J., Stern, P. H., Jacobs, J. W., Eveloff, J., Klein, R. F., Leung, S. C., Rosenblatt, M., and Nissenson, R. A.,** Parathyroid hormone-like protein from human renal carcinoma cells. Structural and functional homology with parathyroid hormone, *J. Clin. Invest.*, 80, 1803, 1987.
25. **Habener, J. F., Rosenblatt, M., Dee, P. C., and Potts, J. T. Jr.,** Cellular processing of pre-proparathyroid hormone involves rapid hydrolysis of leader sequences, *J. Biol. Chem.*, 254, 10596, 1979.
26. **Thiede, M. A. and Rutledge, S. J.,** Nucleotide sequence of a parathyroid hormone-related protein expressed by the 10 day chicken embryo, *Nucleic Acids Res.*, 18, 3062, 1990.
27. **Yasuda, T., Banville, D., Rabbani, S. A., Hendy, G., and Goltzman, D.,** Rat parathyroid hormone-like peptide: comparison with the human homologue and expression in malignant and normal tissue, *Mol. Endocrinol.*, 3, 519, 1989.
28. **Mangin, M., Ikeda, K., and Broadus, A. E.,** Structure of the mouse parathyroid hormone-related peptide gene, *Gene*, 95, 195, 1991.
29. **Mangin, M., Webb, A. C., Dreyer, B. E., Posillico, J. T., Ikeda, K., Weir, E. C., Stewart, A. F., Bander, N. H., Milstone, L., Barton, D. E., Francke, U., and Broadus, A. E.,** Identification of a cDNA encoding a parathyroid hormone-like peptide from a human tumor associated with humoral hypercalcemia of malignancy, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 597, 1988.
30. **Thiede, M. A., Strewler, R. A., Nissenson, R. A., Rosenblatt, M., and Rodan, G. A.,** Human renal carcinoma expresses two messages encoding a parathyroid hormone like peptide: evidence for the alternate splicing of a single copy gene, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 4605, 1988.
31. **Mangin, M., Ikeda, K., Dreyer, B. E., and Broadus, A. E.,** Isolation and characterization of the human parathyroid hormone-like peptide gene, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 2408, 1989.
32. **Mangin, M., Ikeda, K., Dreyer, B., and Broadus, A. E.,** Identification of an upstream promoter of the human parathyroid hormone-related peptide gene, *Mol. Endocrinol.*, 4, 851, 1990.
33. **Suva, L. J., Mather, K. A., Gillespie, M. T., Webb, G. C., Ng, K. W., Winslow, G. A., Wood, W. I., Martin, T. J., and Hudson, P. J.,** Structure of the 5′ flanking region of the gene encoding human parathyroid-hormone-related protein (PTHrP), *Gene*, 77, 95, 1989.
34. **Yasuda, T., Banville, D., Hendy, G. N., and Goltzman, D.,** Characterization of the human parathyroid hormone-like peptide gene, *J. Biol. Chem.*, 264, 7720, 1989.
35. **Karaplis, A. C., Yasuda, T., Hendy, G. N., Goltzman, D., and Banville, D.,** Gene-encoding parathyroid hormone-like peptide: nucleotide sequence of the rat gene and comparison with the human homolog, *Mol. Endocrinol.*, 4, 441, 1990.
36. **Ikeda, K., Mangin, M., Dreyer, B. E., Webb, A. C., Posillico, J. T., Stewart, A. F., Bander, N. H., Weir, E. C., Insogna, K. L., and Broadus, A. E.,** Identification of transcripts encoding a parathyroid hromone-like peptide in messenger RNAs from a variety of human and animal tumors associated with humoral hypercalcemia of malignancy, *J. Clin. Invest.*, 81, 2010, 1988.
37. **Mangin, M., Ikeda, K., Dreyer, B. E., Milstone, L., and Broadus, A. E.,** Two distinct tumor-derived parathyroid hormone-like peptides result from alternative ribonucleic acid processing, *Mol. Endocrinol.*, 2, 1049, 1988.
38. **Grill, V., Diefenbach-Jagger, H., Ebeling, P. R., Glatz, J. A., Moseley, J. M., Hammonds, G. R., Wood, W. I., and Martin, T. J.,** Characterization of parathyroid hormone-related protein with region specific antisera, *J. Bone Min. Res.*, 3 (Suppl 1), A779, 1989.
39. **Mannens, M., Slater, R. M., Heyting, C., Guerts van Kessel, A., Goedde-Salz, B., Frants, R. R., van-Ommen, G. J. B., and Pearson, P. L.,** Regional localization of DNA probed on the short arm of chromosome 11 using anirdia-Wilms' tumor associated deletions, *Hum. Genet.*, 75, 180, 1987.
40. **Thiede, M. A.,** personal communication 1990.
41. **Shaw, G. and Karmen, R.,** A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates mRNA degradation, *Cell*, 46, 659, 1986.
42. **Young, R. A., Hägenbuchle, O., and Schibler, V.,** A single mouse α-amylase gene specifies two different tissue-specific mRNAs, *Cell*, 23, 451, 1981.
43. **Yaffe, D., Nudel, U., Mayer, Y., and Neuman, S.,** Highly conserved sequences in the 3′ untranslated regions of mRNAs coding for honologous proteins in distantly related species, *Nucleic Acids Res.*, 13, 372, 1985.
44. **de Pagler-Holshiuzen, P., Jansen, M., van-Schaik, F. M. A., van der Kammen, R., Oosterwijk, C., van der Brande, J. L., and Sussenbach, J. S.,** The human insulin-like growth factor II gene contains two development-specific promoters, *FEBS Lett.*, 214, 259, 1987.
45. **Vu, T. H., Martin, G. R., Lee, P., Mark, D., Wang, A., and Williams, L. T.,** Developmentally regulated use of alternative promoters creates a novel platelet-derived growth factor receptor transcript in mouse teratocarcinoma and embryonic stem cells, *Mol. Cell Biol.*, 9, 4563, 1989.
46. **Kozak, M.,** Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs, *Nucleic Acids Res.*, 12, 857, 1984.

47. **Mueller, P. P. and Hinnebusch, A. G.,** Multiple upstream AUG codons mediate translational control of GCN4, *Cell,* 45, 201, 1986.
48. **Negrini, M., Silini, E., Zozak, C., Tsujimoto, Y., and Croce, C. M.,** Molecular analysis of mbcl-2: structure and expression of the murine gene homologous to the human gene involved in follicular lymphoma, *Cell,* 49, 455, 1987.
49. **Ikeda, K., Lu, C., Weir, E. C., Mangin, M., and Broadus, A. E.,** Regulation of parathyroid hormone-related peptide gene expression by cycloheximide, *J. Biol. Chem.*, 285, 5398, 1990.
50. **Deftos, L. J., Gazdar, A. F., Ikeda, K., and Broadus, A. E.,** The parathyroid hormone-related protein associated with malignancy is secreted by neuroendocrine tumors, *Mol. Endocrinol.*, 3, 503, 1989.
51. **Ikeda, K., Lu, C., Weir, E. C., Mangin, M., and Broadus, A. E.,** Transcriptional regulation of the parathyroid hormone-related peptide gene by glucocorticoids and vitamin D in a human C-cell line, *J. Biol. Chem.*, 264, 15743, 1989.
52. **Lu, C., Ikeda, K., Deftos, L. J., Gazdar, A. F., Mangin, M., and Broadus, A. E.,** Glucocorticoid regulation of parathyroid hormone-related peptide gene transcription in a human neuroendocrine cell line, *Mol. Endocrinol.*, 3, 2034, 1989.
53. **Minghetti, P. P., Gibbs, P. E. M., and Norman, A. W.,** Computer analysis of 1,25-dihydroxyvitamin $D_3$ receptor regulated promoters: identification of a candidate $D_3$-response element, *Biochem. Biophys. Res. Commun.*, 2, 869, 1989.
54. **Rizzoli, R., Sappino, A. P., Aubert, M. L., and Bonjour, J.-P.,** Effect of cAMP-stimulators on parathyroid hormone-related protein (PTHrP) release by a cultured lung squamous cell carcinoma (BEN cells). Abstracts of the Third International Workshop on Cells and Cytokines in Bone and Cartilage, *Calcif. Tiss. Int.*, 46 (Suppl. 2), 1990,
55. **Rodan, S. B., Wesolowski, G., Ianacone, J., Thiede, M. A., and Rodan, G. A.,** Production of parathyroid hormone-like peptide in a human osteosarcoma cell line: stimulation by phorbol esters and epidermal growth factor, *J. Endocrinol.*, 122, 219, 1988.
56. **Ernst, M., Rodan, G. A., and Thiede, M. A.,** Rapid induction of parathyroid hormone-like peptide (PTH-LP) in keratinocytes: implication of PTH-LP in the proliferative response to epidermal growth factor, *J. Bone Min. Res.*, 4 (Suppl. 1), A309, 1989.
57. **Kiriyama, T., Glatz, J. A., Ho, P., Fukumoto, S., Moseley, J. M., Gillespie, M. T., and Martin, T. J.,** Regulation of parathyroid hormone-related protein production by transforming growth factor-beta in COLO 16 squamous carcinoma cells, in press.
58. **Lehnart, S. A. and Akhurst, R. J.,** Embryonic expression of TGF beta type-1 RNA suggests both paracrine and autocrine mechanisms of action, *Development,* 104, 263, 1988.
59. **Senior, P. V., Heath, D. A., and Beck, F.,** Expression of parathyroid hormone-related protein in the rat before birth: demonstration by hybridization histochemistry, *J. Mol. Endocrinol.*, 6, 1, 1991.
60. **Farrow, S. M., Karmali, R., Gleed, J. H., Hendy, G. N., and O'Riordan, J. L. H.,** Regulation of preproparathyroid hormone messenger RNA and hormone synthesis in human parathyroid adenomata, *J. Endocrinol.*, 117, 133, 1988.
61. **Russell, J., Lettieri, D., and Sherwood, L. M.,** Direct regulation by calcium of cytoplasmic messenger ribonucleic acid coding for pre-proparathyroid hormone in isolated bovine parathyroid cells, *J. Clin. Invest.*, 72, 1851, 1983.
62. **Zajac, J. D., Callaghan, J., Eldridge, C., Diefenbach-Jagger, H., Suva, L. J., Hudson, P., Moseley, J. M., Michelangeli, V. P., and Pasquini, G.,** Production of parathyroid hormone-related protein by a rat parathyroid cell line, *Mol. Cell. Endocrinol.*, 67, 107, 1989.
63. **Ikeda, K., Weir, E. C., Sakaguchi, K., Burtis, W., Zimering, M., Mangin, M., Dreyer, B., Brandi, M. L., Aurbach, G. D., and Broadus, A. E.,** Clonal rat parathyroid cell line expresses a parathyroid hormone-related peptide but not parathyroid hormone itself, *Biochem. Biophys. Res. Commun.*, 162, 108, 1989.
64. **Kemp, B. E., Moseley, J. M., Rodda, C. P., Ebeling, P. R., Wettenhall, R. E. H., Stapleton, D., Diefenbach-Jagger, H., Ure F., Michelangeli, V. P., Simmons, H., Raisz, L. G., and Martin, T. J.,** Parathyroid hormone-related protein of malignancy: active synthetic fragments, *Science,* 238, 1568, 1987.
65. **Hammonds, R. G., McKay, P., Winslow, G. A., Diefenbach-Jagger, H., Grill, V., Glatz, J. A., Rodda, C. P., Moseley, J. M., Wood, W. I., and Martin, T. J.,** Purification and characterization of recombinant human parathyroid hormone-related protein, *J. Biol. Chem.*, 264, 14806, 1989.
66. **Evely, R. S., Bonommo, A., Schneider, H.-G., Moseley, J. M., Gallagher, J., and Martin, T. J.,** The action of parathyroid hormone-related protein (PTHrP) on bone resorption by isolated osteoclasts, *J. Bone Min. Res.*, 6, 85, 1991.
67. **Tregear, G. W., Van Reitschotten, J., Greene, E., Keutman, H. T., Niall, H. D., Reit, B., Parsons, J. A., and Potts, J. T., Jr.,** Bovine parathyroid hormone: minimum chain length of synthetic peptide required for biological activity, *Endocrinology,* 93, 1349, 1973.
68. **Sadi, A., Samra, A., Uneno, S., Jueppner, H., Keutmann, H., Potts, J. T., Jr., Segre, G. V., and Nussbaum, S. R.,** Non-homologous sequences of parathyroid hormone related peptide bind to a common receptor in ROS 17/2.8 cells, *Endocrinology,* 125, 2215, 1989.
69. **McKee, R. L., Goldman, M. E., Caulfield, M. P., De Haven, P. A., Levy, T. J., Nutt, R. F., and**

**Rosenblatt, M.,** The 7-34 fragment of human hypercalcemia factor in a partial agonist/antagonist for parathyroid stimulated cAMP production, *Endocrinology*, 122, 3008, 1988.

70. **Barden, J. A. and Kemp, B. E.,** NMR study of a 34-residue N-terminal fragment of the parathyroid hormone related protein secreted during humoral hypercalcemia of malignancy, *Eur. J. Biochem.*, 184, 379, 1989.
71. **Nissenson, R. A., Diep, D., and Strewler, G. J.,** Synthetic peptides comprising the amino-terminal sequence of a parathyroid hormone-like protein from human malignancies, *J. Biol. Chem.*, 263, 12866, 1989.
72. **Jueppner, H., Abou-Samra, A. B., Uneno, S., Gu, W. X., Potts, J. T., and Segre, G. V.,** The parathyroid hormone-like peptide associated with humoral hypercalcemia of malignancy and parathyroid hormone bind to the same receptor on the plasma membrane of ROS 17/2.8 cells, *J. Biol. Chem.*, 263, 8557, 1988.
73. **Shigeno, C., Hiraki, Y., Westerberg, D. P., Potts, J. T., Jr., and Segre, G. V.,** Photoaffinity labeling of parathyroid hormone receptors in clonal rat osteosarcoma cells, *J. Biol. Chem.*, 263, 3864, 1988.
74. **Kukreja, S. C., Schavin, D. H., Wimbuscus, S., Ebeling, P. R., Danks, J. A., Rodda, C. P., Wood, W. I., and Martin, T. J.,** Antibodies to parathyroid hormone-related protein lower serum calcium in athymic mouse models of malignancy associated hypercalcemia due to human tumors, *J. Clin. Invest.*, 82, 1798, 1988.
75. **Raisz, L., Simmons, H. A., Vargas, S. J., Kemp, B. E., and Martin, T. J.,** Comparison of the effects of amino-terminal synthetic parathyroid hormone related peptide (PTHrP) of malignancy and parathyroid hormone on resorption of cultured fetal rat long bones, *Calcif. Tiss. Int.*, 46, 233, 1990.
76. **Thorikay, M., Kramer, S., Reynolds, F. H., Sorvillo, J. M., Doescher, L., Wu, T., Morris, C. A., Burtis, W. J., Insogna, K. L., Valenzuela, D. M., and Stewart, A. F.,** Synthesis of a gene encoding parathyroid hormone-like protein (1-141). Purification and biological characterization of the expressed protein, *Endocrinology*, 124, 111, 1989.
77. **Horiuchi, N., Caulfield, M. P., Fisher, J. E., Goldman, M. E., McKee, R. L., Reagan, J. E., Levy, J. J., Nutt, R. F., Rodan, S. B., Scholfield, T. L., Clemens, T. L., and Rosenblatt, M.,** Similarity of synthetic peptide from human tumor to parathyroid hormone *in vivo* and *in vitro*, *Science*, 238, 1566, 1987.
78. **Yates, A. J. P., Gutierrez, G. E., Smoleus, P., Travis, P. S., Katz, M. S., Aufdemorte, T. B., Boyce, B. F., Hymer, T. K., Poser, J. W., and Mundy, G. R.,** Effects of a synthetic peptide of a parathyroid hormone-related protein on calcium homeostasis, renal tubular calcium reabsorption and bone metabolism *in vivo* and *in vitro* in rodents, *J. Clin. Invest.*, 81, 932, 1988.
79. **Stewart, A. F., Mangin, M., Wu, T., Goumas, D., Insogna, K. L., Burtis, W. J., and Broadus, A. E.,** Synthetic human parathyroid hormone-like protein stimulates bone resorption and causes hypercalcemia in rats, *J. Clin. Invest.*, 81, 596, 1988.
80. **Civitelli, A., Martin, T. J., Hruska, K. A., and Avioli, L. V.,** Parathyroid hormone-related peptide (PTHrP) transiently increases intracellular calcium in osteoblastic-like cells, *Endocrinology*, 125, 1204, 1990.
81. **Rodan, S. B., Noda, M., Wesolowski, G., Rosenblatt, M., and Rodan, G. A.,** Comparison of postreceptor effects of 1-34 human hypercalcemic factor and 1-34 human parathyroid hormone in rat osteosarcoma cells, *J. Clin. Invest.*, 81, 924, 1988.
82. **Ebeling, P. R., Adam, W. R., Moseley, J. M., and Martin, T. J.,** Actions of parathyroid hormone-related protein on the isolated rat kidney, *J. Endocrinol.*, 12, 45, 1988.
83. **Ellis, A. G., Adam, W. R., and Martin, T. J.,** Comparison of the effects of parathyroid hormone (PTH) and recombinant PTH-related protein on bicarbonate excretion by the isolated, perfused rat kidney, *J. Endocrinol.*, 126, 403, 1990.
84. **Zhou, H., Leaver, D. D., Moseley, J. M., Kemp, B. E., Ebeling, P. R., and Martin, T. J.,** Actions of parathyroid hormone-related protein on the rat kidney *in vivo*, *J. Endocrinol.*, 122, 227, 1989.
85. **Rizzoli, R., Caverzasio, J., Chapuy, M. C., Martin, T. J., and Bonjour, J.-P.,** Role of bone and kidney in parathyroid hormone-related peptide-induced hypercalcemia in rats, *J. Bone Min. Res.*, 4, 759, 1989.
86. **McIsaac, R. J., Heath, J. A., Rodda, C. P., Moseley, J. M., Care, A. D., Martin, T. J., and Caple, I. W.,** Role of fetal parathyroid hormone-related protein in the hormonal regulation of placental calcium, magnesium and inorganic phosphate transport, in *Reproduction Fertility and Development*, 3, 417, 1991.
87. **Stewart, A. F., Vignery, A., Silvergate, A., Ravin, N. D., Li Volsi, V., Broadus, A. E. and Baron, R.,** Quantitative bone histomorphometry in humoral hypercalcemia of malignancy: uncoupling of bone cell activity, *J. Clin. Endocrinol. Metab.*, 55, 219, 1981.
88. **Yamamoto, I., Kitamura, N., Aoki, J., Kawamura, J., Dokoh, S., Morita, R., and Torizuka, K.,** Circulating 1,25-dihydroxyvitamin D concentrations in patients with renal cell carcinoma-associated hypercalcemia are rarely suppressed, *J. Clin. Endocrinol. Metab.*, 64, 175, 1987.
89. **Mok, L. L. S., Ajiwe, E., Martin, T. J., Thompson, J. C., and Cooper, C. W.,** Parathyroid hormone-related protein relaxes smooth muscle and shows cross-desensitization with PTH, *J. Bone Min. Res.*, 4, 433, 1989.

90. **Thiede, M. A., Harm, S. C., McKee, R. L., Grasser, W., and Leach, R. M.,** Parathyroid hormone-related peptide is a paracrine/autocrine vasorelaxant, in press.
91. **Winquist, R. J., Baskin, E. P., and Vlasuk, G. P.,** Synthetic tumor-derived human hypercalcemic factor exhibits PTH-like vasorelaxation in renal arteries, *Biochem. Biophys. Acta*, 149, 227, 1987.
92. **Moseley, J. M., Hayman, J. A., Danks, J. A., Alcorn, D., Grill, V., Southby, J., and Horton, M. A.,** Immunohistochemical detection of parathyroid hormone-related protein (PTHrP) in human fetal epithelia, *J. Clin. Endocrinol. Metab.*, 1991, in press.
93. **Rodda, C. P., Kubota, M., Heath, J. A., Ebeling, P. R., Moseley, J. M., Care, A. D., Caple, I. W., and Martin, T. J.,** Evidence for a novel parathyroid hormone-related protein in fetal lamb parathyroid glands and sheep placenta: comparisons with a similar protein implicated in humoral hypercalcemia of malignancy, *J. Endocrinol.*, 117, 261, 1988.
94. **Loveridge, N., Caple, I. W., Rodda, C. P., Martin, T. J., and Care, A. D.,** Further evidence for a parathyroid hormone-related protein in fetal parathyroid glands of sheep, *Q. J. Exp. Physiol.*, 74, 781, 1988.
95. **Care, A. D., Caple, I. W., and Pickard, D. W.,** The roles of the parathyroid and thyroid glands on calcium homeostasis in the ovine fetus, in *The Physiological Development of the Fetus and Newborn*, Jones, C. T. and Nathaniels, P., Eds., Academic Press, New York, 1988,135.
96. **Care, A. D., Abbas, S. K., Pickard, D. W., Barri, M., Drinkhill, M., Findlay, J. B. C., White, I. R., and Caple, I. W.,** Stimulation of ovine placental transport of calcium and magnesium by mid-molecule fragments of human parathyroid hormone-related protein, *Exp. Physiol.*, 75, 605, 1990.
97. **Abbas, S. K., Pickard, D. W., Illingworth, D., Storer, J., Purdie, D. W., Moniz, C., Dixit, M., Caple, I. W., Ebeling, P. R., Rodda, C. P., Martin, T. J., and Care, A. D.,** Measurement of parathyroid hormone-related protein in extracts of fetal parathyroid glands and placental membranes, *J. Endocrinol.*, 124, 139, 1990.
98. **McIsaac, R. J., Caple, I. W., Danks, J. A., Diefenbach-Jagger, H., Grill, V., Moseley, J. M., Southby, J., and Martin, T. J.,** Ontogeny of parathyroid hormone-related protein in the ovine parathyroid gland, *Endocrinology*, in press.
99. **Fenton, A. J., Kemp, B. E., Kent, G. N., Moseley, J. M., Zheng, M. H., Rowe, D. J., Britto, J. M., Martin, T. J., and Nicholson, G. C.,** Multiple hormone activities contained in the parathyroid hormone-related protein molecule: potent inhibition of bone resorption by carboxy-terminal fragment, *Endocrinoloy*, in press.
100. **Bergmann, P., Nijs-De Wolf, N., Pepersack, T., and Corvilain, J.,** Release of parathyroid hormone-like peptides by fetal rat long bones in culture, *J. Bone Min. Res.*, 5, 741, 1990.
101. **Burtis, W. J., Brady, T. G., Orloff, J. J., Ersbak, J. B., Warrell, R. P., Olson, B. R., Wu, T. L., Mitnick, M. E., Broadus, A. E., and Stewart, A. E.,** Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer, *N. Engl. J. Med.*, 322, 1106, 1990.
102. **Grill, V.,** personal communication, 1990.
103. **Danks, J. A., Ebeling, P. R., Hayman, J., Chou, S. T., Moseley, J. M., Dunlop, J., Kemp, B. E., and Martin, T. J.,** Parathyroid hormone-related protein of cancer: immunohistochemical localization in cancers and in normal skin, *J. Bone Min. Res.*, 4, 273, 1989.
104. **Danks, J. A., Ebeling, P. R., Hayman, J. A., Diefenbach-Jagger, H., Collier, F., Grill, V., Southby, J., Moseley, J. M., Chou, S. T., and Martin, T. J.,** Immunohistochemical localization of parathyroid hormone-related protein in parathyroid adenoma and hyperplasia, *J. Pathol.*, 16, 27, 1990.
105. **Hayman, J. A., Danks, J. A., Ebeling, P. R., Moseley, J. M., Kemp, B. E., and Martin, T. J.,** Expression of parathyroid hormone-related protein in normal skin and in tumors of skin and skin appendages, *J. Pathol.*, 158, 203, 1989.
106. **Drucker, D. J., Asa, S. L., Henderson, J., and Goltzman, D.,** The parathyroid hormone-like peptide gene is expressed in the normal and neoplastic human endocrine pancreas, *Mol. Endocrinol.*, 3, 1589, 1990.
107. **Ikeda, K., Weir, E., Mangin, M., Dannies, P. S., Kinder, B., Deftos, L. J., Brown, E., and Broadus, A. E.,** Expression of messenger ribonucleic acids encoding a parathyroid hormone-like peptide in normal human and animal tissues with abnormal expression in human parathyroid adenomas, *Mol. Endocrinol.*, 2, 1230, 1988.
108. **Percival, R. C., Yates, A. J. P., Gray, R. E. S., Galloway, J., Rogers, K., Neal, F. E., and Kanis, J. A.,** Mechanisms of malignant hypercalcemia in carcinoma of the breast, *Br. Med. J.*, 2, 7766, 1985.
109. **Southby, J., Kissin, M. W., Danks, J. A., Hayman, J. A., Moseley, J. M., Henderson, M. A., Bennet, R. C., and Martin, T. J.,** Immunohistochemical localization of parathyroid hormone-related protein in human breast cancer, *Cancer Res.*, 50, 7710, 1990.
110. **Grill, V., Ho, P., Moseley, J. M., Johanson, N., Lee, S., Body, J., Kukreja, S., and Martin, T. J.,** Parathyroid hormone-related protein: elevated levels both in humoral hypercalcemia of malignancy and in hypercalcemia complicating metastatic breast cancer, in press.
111. **Mundy, G. R., Ibbotson, K. J., D'Souza, S. M., Simpson, E. L., Jacobs, J. W., and Martin, T. J.,** The hypercalcemia of cancer, *N. Engl. J. Med.*, 310, 1718, 1984.
112. **Horton, M. A., Danks, J. A., and Moseley, J. M.,** PTHrP expression in HTLV-1 positive human T-cell lymphoma: implications for mechanism of hypercal-

cemia, *J. Bone Min. Res.*, 4 (Suppl. 1), A800, 1989.
113. **Motokura, T., Fukumoto, S., Takahashi, S., Watanabe, T., Matsumoto, T., Igarishi, T., and Ogata, E.,** Expression of parathyroid hormone-related protein in a human T cell lymphotrophic virus type I-infected T cell line, *Biochem. Biophys. Res. Commun.*, 154, 1182, 1988.
114. **Budayr, A. A., Nissenson, R. A., Klein, R. F., Pun, K. K., Clark, O. H., Diep, D., Arnaud, C. D., and Strewler, G. J.,** Increased serum levels of a parathyroid hormone-like protein in malignancy-associated hypercalcemia, *Ann. Intern. Med.*, 111, 807, 1989.
115. **Ratcliffe, W. A., Norbury, S., Heath, D. A., and Ratcliffe, J. G.,** Development and validation of an immunoradiometric assay for parathyroid hormone-related protein in unextracted plasma, *J. Endocrinol.*, 1991, in press.
116. **Henderson, J. E., Shustik, C., Kremer, R., Rabbani, S. A., Hendy, G. N., and Goltzman, D.,** Circulating concentrations of parathyroid hormone-like peptide in malignancy and hyperparathyroidism, *J. Bone Min. Res.*, 5, 105, 1990.
117. **Budayr, A. A., Halloran, B. P., King, J. C., Diep, D., Nissenson, R. A., and Strewler, G. J.,** High levels of a parathyroid hormone-like protein in milk, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 7183, 1989.
118. **Thiede, M. A. and Rodan, G. A.,** Expression of a calcium-mobilizing parathyroid hormone-like peptide in lactating mammary tissues, *Science*, 242, 278, 1988.
119. **Thiede, M. A.,** The mRNA encoding a parathyroid hormone-like protein is produced in mammary tissue in response to elevation in serum prolactin, *Mol. Endocrinol.*, 3, 1443, 1989.
120. **Grill, V., Ho, P., Hillary, J., and Moseley, J. M.,** unpublished observation, 1991.
121. **Law, F. M. K., Moate, P. J., Leaver, D. D., Diefenbach-Jagger, H., Grill, V., Ho, P. W. M., and Martin, T. J.,** Parathyroid hormone-related protein in milk and its correlation with bovine milk calcium, *J. Endocrinol.*, 128, 21, 1991.
122. **Weir, E. C., Brines, M. L., Ikeda, K., Burtis, W. J., Broadus, A. E., and Robbins, R. J.,** Parathyroid hormone-related peptide gene is expressed in the mammalian central nervous system, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 108, 1990.
123. **Chan, S. D. H., Strewler, G. J., King, K. L., and Nissenson, R. A.,** Expression of a parathyroid hormone-like protein and its receptor during differentiation of embryonal carcinoma cells, *Mol. Endocrinol.*, 4, 638, 1990.
124. **Thiede, M. A., Daifotis, A. G., Weir, E. C., Brines, M. L., Burtis, W. J., Ikeda, K., Dreyer, B. E., Garfield, R. E. and Broadus, A. E.,** Intrauterine occupancy controls expression of the parathyroid hormone-related peptide gene in preterm rat myometrium, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 6969, 1990.
125. **Fraser, C. L., Sarnacki, P., and Badayr, A.,** Evidence that PTH-mediated calcium transport in rat brain synaptosomes is independent of cAMP, *J. Clin. Invest.*, 81, 982, 1988.
126. **Fraser, R. A., Kronenberg, H. M., Pang, P. K. T., and Harvey, S.,** Parathyroid hormone messenger ribonucleic acid in the rat hypothalamus, *Endocrinology*, 127, 2517, 1990.
127. **Yuspa, S. H., Kilkenny, A. E., Steinart, P. M., and Roop, D. R.,** Expression of murine epidermal differentiation molecules is tightly regulated by restricted extracellular calcium concentrations in vitro, *J. Cell Biol.*, 109, 1207, 1989.
128. **McGrath, C. M. and Soule, H. D.,** Calcium regulation in normal human mammary epithelial cell growth in culture, *In Vitro*, 20, 652, 1984.
129. **Lepre, F., Grill, V., Danks, J. A., Ho, P., Law, F. M., Moseley, J. M., and Martin, T. J.,** Hypercalcemia in pregnancy and lactation due to parathyroid hormone-related protein production, *Bone Mineral*, 10, S317, 1990.
130. **Welling, G. W., Wejser, W. J., van der Zee, R., and Welling-Webster, S.,** Production of sequential antigenic regions in proteins, *FEBS Lett.*, 188, 215, 1985.
131. **Palmieri, G. M. A., Nordquist, R. E., and Omenn, G. S.,** Immunochemical localization of parathyroid hormone in cancer tissue from patients with ectopic hyperparathyroidism, *J. Clin. Invest.*, 53, 1726, 1974.
132. **Mayes, L. C., Kasselberg, A. G., Rolott, J. S., and Lukens, J. N.,** Hypercalcemia associated with immunoreactive parathyroid hormone in a malignant rhabdoid tumor of the kidney (rhabdoid Wilm's tumor), *Cancer*, 54, 882, 1984.
133. **Ilardi, C. F. and Faro, J. C.,** Localization of parathyroid hormone-like substance in squamous cell carcinoma, *Arch. Path. Lab. Med.*, 109, 752, 1975.
134. **Yamamoto, K., Yamasaki, R., Sakai, H., Tezuka, U., Takahashi, M., Iizuka, M., Sekiya, T., and Saito, S.,** Ectopic production of parathyroid hormone by a small cell lung cancer in a patient with hypercalcemia, *J. Clin. Endocrinol. Metab.*, 68, 976, 1989.
135. **Nussbaum, S. R., Gaz, R. D., and Arnold, A.,** Hypercalcemia and ectopic secretion of parathyroid hormone by an ovarian carcinoma with rearrangement of the gene for parathyroid hormone, *N. Engl. J. Med.*, 323, 1324, 1990.
136. **Abrahamson, E. C., Kukla, L. J., Shevrin, D. H., Lad, T. E., McGuire, W. P., and Kukreja, S. C. A.,** Model for malignancy-associated humoral hypercalcemia, *Calcif. Tiss. Int.*, 36, 563, 1984.
137. **Mundy, G. R., Ibbotson, K. J., D'Souza, S. M.,** Tumor products and the hypercalcemia of malignancy, *J. Clin. Invest.*, 76, 391, 1985.
138. **Barden, J. and Kemp, B. E. K.,** unpublished, 1990.