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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, 5/10, C07K 14/72, 16/28, A61K 38/17 // C07K 14/635

A1

(11) International Publication Number: WO 00/32771

(43) International Publication Date: 8 June 2000 (08.06.00)

(21) International Application Number: PCT/US99/11883

(22) International Filing Date: 28 May 1999 (28.05.99)

(30) Priority Data:
60/110,467 30 November 1998 (30.11.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*
*With a statement concerning non-prejudicial disclosure or exception to lack of novelty.*

(54) Title: PTH1R AND PTH3R RECEPTORS, METHODS AND USES THEREOF

(57) Abstract

The present invention relates to novel parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP) receptors (PTH1R and PTH3R) isolated from zebrafish. The receptors of the present invention share homology with previously identified parathyroid hormone (PTH)/parathyroid related protein (PTHrP) receptors. Isolated nucleic acid molecules are provided encoding the zebrafish PTH1R and PTH3R receptors. PTH1R and PTH3R receptor polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of PTH1R and PTH3R receptor activity and to diagnostic and therapeutic methods.

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# PTH1R and PTH3R Receptors, Methods and Uses Thereof

## *Background of the Invention*

### *Statement as to Rights to Inventions Made Under Federally-Sponsored Research and Development*

Part of the work performed during development of this invention utilized U.S. Government funds. The U.S. Government has certain rights in this invention.

### *Field of the Invention*

The present invention is related to the fields of molecular biology, developmental biology, physiology, neurobiology and medicine. The invention provides polynucleotides encoding the zebrafish PTH1R receptor and polynucleotides for a novel zebrafish PTH3R receptor, as well as vectors and cells containing said polynucleotides. The invention further provides polypeptides for the PTH1R and PTH3R receptors. The polynucleotides and polypeptides of the invention are useful for the identification of agonist and antagonists of PTH1R or PTH3R receptor function and as reagents in the treatment of diseases or disorders associated with PTH1R or PTH3R function.

### *Related Art*

The parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor (PTH1R) mediates in mammals and frogs the actions of PTH and PTHrP. Both peptides, most likely evolved through a gene duplication event from a common ancestral gene, and have retained limited homology within the amino-terminal region. Because of their structural conservation, PTH and PTHrP bind with similar affinity to the PTH1R, and activate this common receptor with similar or indistinguishable efficacy. Due to this unusual ligand-specificity, the PTH1R mediates the endocrine actions of PTH, the most important peptide regulator of calcium homeostasis in mammals, and the autocrine/paracrine actions of PTHrP, which is important for normal chondrocyte proliferation and differentiation (Karaplis; Lanske), and most likely for other, still incompletely defined functions including pancreas, skin, and breast development, as well as tooth eruption (Wysolmerski, Philbrick, Rupi and Stewart, Stewart, Braodus).

In addition to the PTH1R, a PTH type-2 receptor (PTH2R) has been isolated from mammals and teleosts (Usdin; Rubin) which preferentially mediates the actions of PTH, and an incompletely characterized hypothalamic peptide (Usdin). Its biological importance remains uncertain, as does the importance of additional receptors for which there is growing biological and pharmacological evidence. For example, receptors with specificity for amino-terminal PTH and PTHrP have been described for keratinocytes, squamous carcinoma cell lines, and central nervous system cells (Orloff *et al.*, 1995 and 1996; Fukayama, 1997), and there is evidence for a PTHrP-selective receptor in the mammalian supraoptic nucleus (Yamamoto *et al.*, 1997 and 1998). In addition, the midregional portion of PTHrP stimulates an increase in intracellular free calcium in some cell lines and increases placental calcium transport (Kovacs *et al.*, 1996; Wu *et al.*,; Orloff *et al.*,), and the carboxy-terminal portion of PTH binds to a distinct receptor on clonal cell lines (Inomata, 1995; Takasu 1998).

Research on the parathyroid hormone (PTH) has been extensive. Formulations of PTH and compounds with PTH activity have been described (*see* US Patent Nos. 5,496,801; 5,814,603; 5,208041;), and methods to produce the same are also known (*see* US Patent Nos. 5,616,560 and 5,010,010). In addition, analogs and inhibitors of PTH may be found in the prior art (see US Patent Nos. 4,423,037; 5,693616; 5,695,955 and 5,798,225).

Thus, the invention furthers the art by focusing on PTH receptors and providing reagents and methods that are distinct and separate from PTH.

## *Summary of the Invention*

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel PTH1R receptor having the amino acid sequence shown in Figure 1 (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number ____ on ____. The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of PTH1R polypeptides or peptides by recombinant techniques. The invention further provides an isolated PTH1R polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel PTH3R receptor having the amino acid sequence shown in Figure 4 (SEQ ID NO:5) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number ______ on _____. The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of PTH3R polypeptides or peptides by recombinant techniques. The invention further provides an isolated PTH3R polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by the PTH1R or PTH3R receptor, which involves contacting cells which express the PTH1R or PTH3R receptor with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a screening assay for agonists and antagonists is provided which involves determining the effect a candidate compound has on PTH or PTHrP binding to the PTH1R or PTH3R receptor. In particular, the method involves contacting the PTH1R or PTH3R receptor with a PTH or PTHrP polypeptide and a candidate compound and determining whether PTH or PTHrP polypeptide binding to the PTH1R or PTH3R receptor is increased or decreased due to the presence of the candidate compound.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of PTH1R or PTH3R activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated PTH1R or PTH3R polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of PTH1R or PTH3R receptor activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an PTH1R or PTH3R antagonist.

The invention further provides a diagnostic method useful during diagnosis or prognosis of diseases and disorders associated with PTH1R or PTH3R receptor expression or function.

## *Brief Description of the Figures*

FIG. 1. A) Schematic representation of exons M6/7 and M7 (A), and of the cDNA encoding the PTH1R or the PTH3R (B). Vertical boxes depict the predicted location of the membrane-spanning helices; recognition sites for restriction enzymes are shown by //. For and Rev arrows indicate the

approximate location of primers used for RT-PCR, 5′ RACE, and 3′ RACE for zPTH1R and zPTH3R. C) The nucleotide sequence of the PTH1R receptor cDNA. D) The nucleotide sequence of the PTH3R receptor cDNA.
⇐, degenerate primers; ←, zPTH1R-specific; ⬅, zPTH3R-specific; ς, potential sites for N-linked glycosylation.

Figure 2 A) The amino acid sequence of the PTH1R receptor; B) The amino acid sequence of the PTH3R receptor.

Figure 3 Alignment of the amino acid sequences of the zPTH1R, zPTH2R, and zPTH3R. The sequences were aligned using the GAP and pileup algorithms of the GCG package (Genetics Computer Group, Wisconsin). Conserved consensus sites for potential N-glycosylation are identified by #; Gaps were introduced to maximize sequence homology. The seventeen residues which are lacking in one splice variant of the characterized zPTH2R (Rubin *et al.*, in press) are boxed; the residues which are predicted to comprise the signal peptide are outlined with a stippled box; and those residues which are likely to be PTH3R-specific are boxed.

Fig. 4A-D. COS-7 cells transiently expressing the zPTH1R or the zPTH3R were evaluated for competitive inhibition of radioligand binding (A,B) and agonist-stimulated cAMP production (C,D). Binding studies (as described in Materials and Methods) used either $^{125}$I-[Nle$^{8,21}$,Tyr$^{34}$]rPTH-(1-34)amide (Panel A) or $^{125}$I-[Tyr$^{36}$]hPTHrP-(1-36)amide (Panel B) as radioligand and varying amounts of unlabeled peptide. Data are expressed as % of maximal binding. Cyclic AMP accumulation is expressed as % of maximal for the zPTH1R (C) or the zPTH3R (D); basal cAMP accumulation was 3-4 pmol/well for either receptor; maximal accumulation of 106.2 pmol/well for the zPTH1R, and 285.4 pmol/well for the zPTH3R. ■□, PTH; ●O, PTHrP; filled symbols represent zPTH1R, open symbols represent zPTH3R. All data represent the mean ± SEM of at least three independent transfections.

Fig. 5. Accumulation of total IP in COS-7 cells transiently expressing the zPTH1R, or the zPTH3R or the hPTH1R (schipani). Hydrolysis of total IPs was assessed as described (Akiko) in the absence or presence of PTHrP ($10^{-6}$ M) or PTH ($10^{-6}$ M). Data are expressed as fold above basal and represent the mean ± SEM of at two independent experiments.

Fig. 6. A phylogenetic analysis, as described in Materials and Methods, indicated that the single most parsimonious tree had a length of 1549 steps, and a consistency index excluding uninformative characters of 0.863. The bootstrap confidence intervals are shown next to the branch points and indicate the percentage of trials which support a given branch in 100 branch-and-bound iterations.

## *Detailed Description of the Preferred Embodiments*

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel PTH1R polypeptide having the amino acid sequence shown in Figure 2A (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The PTH1R protein of the present invention shares sequence homology with previously identified PTH1R and PTH2R sequences. The nucleotide sequence shown in Figure 1C (SEQ ID NO:1) was obtained by sequencing a cDNA clone (zPTH1R), which was deposited on ____ at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number ____. The cDNA was inserted between the EcoRI and SphI site of plasmid pcDNAI/Amp (Invitrogen).

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel PTH3R polypeptide having the amino acid sequence shown in Figure 2B (SEQ ID NO:4), which was determined by sequencing a cloned cDNA. The PTH3R protein of the present invention shares sequence homology with the PTH1R protein sequence of the invention and other PTH1R and PTH2R protein sequences previously. The nucleotide sequence shown in Figure 1C (SEQ ID NO:3) was obtained by sequencing a cDNA clone(zPTH3R), which was deposited on ____ at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number ______. The cDNA was inserted between the BamHI and NotI site of plasmid pcDNAI/Amp (Invitrogen).

*Nucleic Acid Molecules*

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined by manual sequencing, and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by manual sequencing are typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in Figures 1C or 1D, a nucleic acid molecule of the present invention encoding a PTH1R or PTH3R polypeptide, respectively, may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figure 1C (SEQ ID NO:1) was discovered using oligonucleotide primers in a polymerase chain reaction (PCR) with total RNA isolated from adult zebrafish. The determined nucleotide sequence of the PTH1R cDNA of Figure 1C (SEQ ID NO:1) contains an open reading frame encoding a protein of about 536 amino acid residues, with a predicted leader sequence of about 24 amino acid residues, and a deduced molecular weight of about 61.4 kDa for the non-glycosylated form. The amino acid sequence of the predicted mature PTH1R receptor is shown in Figure 2A from amino acid residue about 25 to residue about 536. The PTH1R protein shown in Figure 2A (SEQ ID NO:2) is about 76 % identical to human PTH1R sequence and about 68 % identical to human PTH2R sequence.

Also illustrative of the invention is the nucleic acid molecule described in Figure 1D (SEQ ID NO:3) was discovered in a zebrafish cDNA library (Clontech). The determined nucleotide sequence of the Figure 1D (SEQ ID NO:3) contains an open reading frame encoding a protein of about 523 amino acid residues, with a predicted leader sequence of about 21 amino acid residues, and a deduced molecular weight of about 59.2 kDa. The PTH3R protein shown in Figure 2B (SEQ ID NO:4) is about 68% and 57% similar to human PTH1R and human PTH2R, respectively.

As indicated, the present invention also provides the mature form(s) of the PTH1R and PTH3R receptors of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature PTH1R polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. ____ and as shown in Figure 2A (SEQ ID NO:2). The present invention also provides a nucleotide sequence encoding the mature PTH3R polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. _____ and as shown in Figure 2B (SEQ ID NO:4). By the mature PTH1R protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit ____ is meant the mature form(s) of the PTH1R receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the zebrafish DNA sequence of the clone contained in the vector in the deposited host. By the mature PTH3R protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit _____ is meant the mature form(s) of the PTH3R receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the zebrafish DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature PTH1R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____ may or may not differ from the predicted "mature"PTH1R protein shown in Figure 2A (amino acids from about 25 to about 536) depending on the accuracy of the predicted cleavage. The mature PTH3R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____ may or may not differ from the predicted "mature" PTH3R protein shown in Figure 2B (amino acids from about 22 to about 523) depending on the accuracy of the predicted cleavage site.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the methods of McGeoch (*Virus Res. 3*:271-286 (1985)) and von Heinje (*Nucleic Acids Res. 14*:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein. A computational method may be found in the computer program "PSORT" (K. Nakai and M. Kanehisa, Genomics 14:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated.

In the present case, the predicted amino acid sequence of the complete PTH1R and PTH3R polypeptides of the present invention were analyzed for structural properties by comparison to the rat PTH1R sequence. This analysis provided predicted a cleavage site between amino acids 24 and 25 in Figure 2A (SEQ ID NO:2) and a cleavage site between amino acids 21 and 22 in Figure 2B (SEQ ID NO:4). Thus, the leader sequence for the PTH1R receptor protein is predicted to consist of amino acid residues 1-24 in Figure 2A (amino acids 1 to 24 in SEQ ID NO:2) , while the predicted mature PTH1R protein consists of residues 25-536 (amino acids 25 to 536 in SEQ ID NO:2). The leader sequence for the PTH3R receptor protein is predicted to consist of amino acid residues 1-21 in Figure 2B (amino acids 1-21 in SEQ ID NO:4), while the predicted mature PTH3R protein consists of residues 22-523 (amino acids 22 to 523 in SEQ ID NO:4).

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

As one of ordinary skill would appreciate, however, due to the possibilities of sequencing errors, the PTH1R receptor polypeptide encoded by the deposited cDNA comprises about 536 amino acids, but may be anywhere in the range of 510-561 amino acids; and the leader sequence of this protein is about 24 amino acids, but may be anywhere in the range of about 10 to about 30 amino acids. As one of ordinary skill would also appreciate, however, due to the possibilities of sequencing errors, the PTH3R receptor polypeptide encoded by the deposited cDNA comprises about 523 amino acids, but may be anywhere in the range of 500-550 amino acids; and the leader sequence of this protein is about 24 amino acids, but may be anywhere in the range of about 15 to about 35 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in Figure 1C (SEQ

ID NO:1) or Figure 1D (SEQ ID NO:3); DNA molecules comprising the coding sequence for the PTH1R receptor shown in Figure 2A (SEQ ID NO:2) or the PTH3R receptor shown Figure 2B (SEQ ID NO:4); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the PTH1R or the PTH3R receptor. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the PTH1R polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. ____ on ____. Another aspect, the invention provides isolated nucleic acid molecules encoding the PTH3R polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. ____ on ____. Preferably, these nucleic acid molecules will encode the mature polypeptides encoded by the above-described deposited cDNA clones. In a further embodiment, nucleic acid molecules are provided encoding the PTH1R or the PTH3R polypeptide or the PTH1R or the PTH3R polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or the nucleotide sequence of the PTH1R cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the PTH1R gene in human tissue, for instance, by Northern blot analysis. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:3 or the nucleotide sequence of the cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNAs or the nucleotide sequence shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments of about 50-1550 nt in length, and more preferably at fragments least about 600 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNAs or as shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNAs or the nucleotide sequence as shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3).

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the PTH1R receptor extracellular domain (predicted to constitute amino acid residues from about 25 to about 147 in Figure 2A (or amino acid residues from about 25 to about 147 in SEQ ID NO:2)); a polypeptide comprising the PTH1R receptor transmembrane domain (predicted to constitute amino acid residues from about 148 to about 416 in Figure 2A (or amino acid residues from about 148 to about 416 in SEQ ID NO:2)); and a polypeptide comprising the PTH1R receptor extracellular domain with all or part of the transmembrane domain deleted. As above with the leader sequence, the amino acid residues constituting the PTH1R receptor extracellular and transmembrane domains have been predicted. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding: a polypeptide comprising the PTH3R receptor extracellular domain (predicted to constitute amino acid residues from about 22 to about 145 in Figure 2B (or amino acid residues from about 22 to about 145 in SEQ ID NO:4)); a polypeptide comprising the PTH3R receptor transmembrane domain (predicted to constitute amino acid residues from about 146 to about 402 in Figure 2B (or amino acid residues from about 146 to about 402 in SEQ ID NO:4)); and a polypeptide comprising the PTH3R receptor extracellular domain with all or part of the transmembrane domain deleted. As above with the leader sequence, the amino acid residues constituting the PTH3R receptor extracellular and transmembrane domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the PTH1R or PTH3R receptor protein. As one skilled in the art would know, a nucleic acid sequence may be used to predict the polypeptide sequence encoded therein. Such information may then be used to predict antigenic determinants in the polypeptide that may be related to the corresponding polynucleotide regions encoding the antigenic determinants identified by the analysis. Methods for predicting the antigenic determinants of a polypeptide are well known in the art.

Methods for determining other such epitope-bearing portions of the PTH1R or the PTH3R protein are described in detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC

Deposit Nos.______ or ______. By "stringent hybridization conditions" is intended overnight incubation at 42 °C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 °C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNAs or the nucleotide sequence as shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the PTH1R receptor cDNA shown in Figure 1C (SEQ ID NO:1) or the 3′ terminal poly(A) tract of the PTH3R receptor cDNA shown in Figure 1D (SEQ ID NO:3)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a PTH1R or PTH3R polypeptide may include, but are not limited to those encoding the amino acid sequence of the mature polypeptides, by themselves; the coding sequence for the mature polypeptides and additional sequences, such as those encoding the amino acid leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*: 767 (1984). As discussed below, other such fusion proteins include the PTH1R receptor fused to Fc at the – or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the PTH1R or PTH3R receptor. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the PTH1R or PTH3R receptor or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length PTH1R polypeptide having the complete amino acid sequence in SEQ ID NO:2, including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature PTH1R receptor (full-length polypeptide with the leader removed) having the amino acid sequence at positions from about 25 to about 536 in SEQ ID NO:2; (d) a nucleotide sequence encoding the full-length PTH1R polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No.____; (e) a nucleotide sequence encoding the mature PTH1R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883; (f) a nucleotide sequence encoding the PTH1R receptor extracellular domain; (g) a nucleotide sequence encoding the PTH1R receptor transmembrane domain; (h) a nucleotide sequence encoding the PTH1R receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h).

Embodiments of the invention also include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length PTH3R polypeptide having the complete amino acid sequence in Figure 2B (SEQ ID NO:4), including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:5, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature PTH3R receptor (full-length polypeptide with the leader removed) having the amino acid sequence at positions from about 22 to about 523 in SEQ ID NO:4; (d) a nucleotide sequence encoding the full-length PTH3R polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. _____; (e) a nucleotide sequence encoding the mature PTH3R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____; (f) a nucleotide sequence encoding the PTH3R receptor extracellular domain; (g) a nucleotide sequence encoding the PTH3R receptor transmembrane domain; (h) a nucleotide sequence encoding the PTH3R receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a PTH1R or PTH3R polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the PTH1R or PTH3R receptor. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1C or 1D or to the nucleotides sequence of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having PTH1R or PTH3R receptor activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having PTH1R or PTH3R receptor activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having PTH1R or PTH3R receptor activity include, *inter alia,* (1) isolating the PTH1R or PTH3R receptor gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the PTH1R or PTH3R receptor gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting PTH1R or PTH3R receptor mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having PTH1R or PTH3R receptor activity. By "a polypeptide having PTH1R or PTH3R receptor activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the PTH1R or PTH3R receptor of the invention, as measured in a particular biological assay. For example, PTH1R or PTH3R receptor activity can be measured using competition binding experiments of labeled PTH or PTHrP to cells expressing the candidate PTH1R or PTH3R polypeptide as described in Treanor *et al., Nature 382*:80-83 (1996) or Jing *et al., Cell 85*: 1113-1124 (1996).

As demonstrated in Examples 3 and 4 herein, assays to address PTH1R and PTH3R function are well known in the art. Any cell line expressing the PTH1R or PTH3R receptor, or variants thereof, may be used to assay ligand binding and second messenger activation as described in Examples 3 and 4. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNAs or the nucleic acid sequence shown in Figure 1C (SEQ ID NO:1) or Figure 1D (SEQ ID NO:3) will encode a polypeptide "having PTH1R or PTH3R receptor activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having PTH1R or PTH3R protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

### *Vectors and Host Cells*

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of PTH1R or PTH3R polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5-receptor has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The PTH1R or PTH3R receptor can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

***PTH1R and PTH3R Polypeptides and Fragments***

The invention further provides an isolated PTH1R or PTH3R polypeptide having the amino acid sequence encoded by the deposited cDNAs, or the amino acid sequence in Figure 2A (SEQ ID NO:2) or Figure 2B (SEQ ID NO:4), or a peptide or polypeptide comprising a portion of the above polypeptides.

It will be recognized in the art that some amino acid sequences of the PTH1R or PTH3R receptor can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the PTH1R or PTH3R receptor which show substantial PTH1R or PTH3R receptor activity or which include regions of PTH1R or PTH3R protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.,* "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of Figure 2A (SEQ ID NO:2) or Figure 2B (SEQ ID NO:4), or that encoded by the deposited cDNAs, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the PTH1R or PTH3R protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol. 2*:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the PTH1R or PTH3R receptor of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

| | |
|---|---|
| Aromatic | Phenylalanine<br>Tryptophan<br>Tyrosine |
| Hydrophobic | Leucine<br>Isoleucine<br>Valine |
| Polar | Glutamine<br>Asparagine |
| Basic | Arginine<br>Lysine<br>Histidine |
| Acidic | Aspartic Acid<br>Glutamic Acid |
| Small | Alanine<br>Serine<br>Threonine<br>Methionine<br>Glycine |

Amino acids in the PTH1R or PTH3R protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the antimicrobial peptide polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the PTH1R or PTH3R receptor can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention also include the polypeptide encoded by the deposited PTH1R cDNA including the leader, the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 2A (SEQ ID NO:2) including the leader, the polypeptide of Figure 2A (SEQ ID NO:2) minus the leader, the extracellular domain, the transmembrane domain, a polypeptide comprising amino acids about 1 to about 536 in SEQ ID NO:2, and a polypeptide comprising amino acids about 2 to about 536 in SEQ ID NO:2, as well as polypeptides which are at least 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

The polypeptides of the present invention also include the polypeptide encoded by the deposited PTH3R cDNA including the leader, the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 2 (SEQ ID NO:4) including the leader, the polypeptide of Figure 4 (SEQ ID NO:4) minus the leader, the extracellular domain, the transmembrane domain, a polypeptide comprising amino acids about 1 to about 523 in SEQ ID NO:4, and a polypeptide comprising amino acids about 2 to about 523 in SEQ ID NO:4, as well as polypeptides which are at least 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a PTH1R or PTH3R polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the PTH1R or PTH3R receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figure 2A (SEQ ID NO:2) or Figure 2B (SEQ ID NO:4) or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting PTH1R or PTH3R expression as described below or as agonists and antagonists capable of enhancing or inhibiting PTH1R or PTH3R receptor function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" PTH1R or PTH3R receptor binding proteins which are also candidate agonist and antagonist according to the present invention. The yeast two hybrid system is described in Fields and Song, *Nature 340:*245-246 (1989).

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Thus, one skilled in the art will have the requisite knowledge to select antigenic epitope bearing regions from the polypeptides in Figure 2A (SEQ ID NO:2) and Figure 2B (SEQ ID NO:4).

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. For example, Houghten provides a general method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids Houghten, R. A., *Proc. Natl. Acad. Sci. USA 82*:5131-5135 (1985)). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate, PTH1R or PTH3R polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al., Nature 331*:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric PTH1R or PTH3R protein or protein fragment alone (Fountoulakis *et al., J. Biochem 270*:3958-3964 (1995)).

### *Diagnosis and Prognosis*

It is believed that certain tissues in mammals with certain diseases and disorders express significantly decreased levels of the PTH1R or PTH3R receptor and mRNA encoding the PTH1R or PTH3R receptor when compared to a corresponding "standard" mammal, i.e., a mammal of the same species not having the disorder. Further, it is believed that enhanced levels of the PTH1R or PTH3R receptor can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with cancer when compared to sera from mammals of the same species not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of diseases and disorders, for example, which involves assaying the expression level of the gene encoding the PTH1R or PTH3R receptor in mammalian cells or body fluid and comparing the gene expression level with a standard PTH1R or PTH3R receptor gene expression level, whereby an decrease in the gene expression level over the standard is indicative of certain disorders.

Where a diagnosis of a disorder has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting decreased PTH1R or PTH3R gene expression will experience a worse clinical outcome relative to patients expressing the gene at a higher level.

By "assaying the expression level of the gene encoding the PTH1R or PTH3R protein" is intended qualitatively or quantitatively measuring or estimating the level of the PTH1R or PTH3R protein or the level of the mRNA encoding the PTH1R or PTH3R receptor in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the PTH1R or PTH3R protein level or mRNA level in a second biological sample).

Preferably, the PTH1R or PTH3R protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard PTH1R or PTH3R protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the cancer. As will be appreciated in the art, once a standard PTH1R or PTH3R protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains PTH1R or PTH3R protein or mRNA. Biological samples include mammalian body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain PTH1R or PTH3R protein, and ovarian, prostate, heart, placenta, pancreas liver, spleen, lung, breast, neural, and umbilical tissue.

The present invention is useful for detecting disorders in mammals. In particular the invention is useful during diagnosis of diseases and disorders in mammals involving PTH1R or PTH3R receptor expression or function. Mutations that affect PTH1R or PTH3R sequence and/or expression levels of PTH1R or PTH3R could be diagnostic for patients with diseases or disorders of a developmental, physiological or neurological nature. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Total cellular RNA can be isolated from a biological sample using the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the PTH1R or PTH3R receptor are then assayed using any appropriate method. These include Northern blot analysis (Harada *et al., Cell 63:*303-312 (1990)), S1 nuclease mapping (Fujita *et al., Cell 49:*357- 367 (1987)), the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR) (Fujita *et al., Cell 49:*357- 367 (1987)), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying PTH1R or PTH3R protein levels in a biological sample can occur using antibody-based techniques. For example, PTH1R or PTH3R protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell. Biol. 105:*3087-3096 (1987)). Other antibody-based methods useful for detecting PTH1R or PTH3R receptor gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable labels are known in the art and include enzyme labels, such as glucose oxidase, and radioisotopes, such as iodine ($^{125}I$, $^{121}I$), carbon ($^{14}C$), sulfur ($^{35}S$), tritium ($^{3}H$), indium ($^{112}In$), and technetium ($^{99m}Tc$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

***Agonists and Antagonists of the PTH1R or PTH3R***

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by the PTH1R or PTH3R receptor, which involves contacting cells which express the PTH1R or PTH3R receptor with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a screening assay for agonists and antagonists is provided which involves determining the effect a candidate compound has on PTH or PTHrP binding to the PTH1R or PTH3R receptor. In particular, the method involves contacting the PTH1R or PTH3R receptor with a PTH or a

PTHrP polypeptide and a candidate compound and determining whether PTH or PTHrP polypeptide binding to the PTH1R or PTH3R receptor is increased or decreased due to the presence of the candidate compound.

By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating PTH1R or PTH3R receptor response (*e.g.*, signaling through the cAMP or inositol phosphate pathway). By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting PTH1R or PTH3R receptor response (*e.g.*, signaling through the cAMP or inositol phosphate pathway). Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit PTH1R or PTH3R receptor activity can be determined using art-known competition binding assays, including those described in more detail below.

One such screening procedure involves the use of melanophores which are transfected to express a receptor of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) activation of the PTH1R or PTH3R receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both PTH or PTHrP as a ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

Other screening techniques include the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science 246*:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the PTH1R or PTH3R receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the PTH1R or PTH3R receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing in cells a construct wherein the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the PTH1R or PTH3R receptor or inhibition of activation of the PTH1R or PTH3R receptor from the phospholipase signal.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the PTH1R or PTH3R receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

Further screening assays for agonist and antagonist of the present invention are described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992).

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to PTH or PTHrP. The method involves contacting cells which express the PTH1R or PTH3R polypeptide with a candidate compound and the

PTH or PTHrP ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or PTH or PTHrP (e.g., determining or estimating an increase or decrease in cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the PTH1R or PTH3R polypeptide can be contacted with either an endogenous or exogenously administered PTH or PTHrP.

Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, PTH or PTHrP peptide fragments, or other known compounds that behave as PTH or PTHrP agonist. Preferred agonist include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and β-amyloid peptide. (*Science 267*:1457-1458 (1995)). Further preferred agonist include polyclonal and monoclonal antibodies raised against the PTH1R or PTH3R polypeptide, or a fragment thereof.

Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *ElB*, Baculovirus *p35* and *IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1, LMP-1,* African swine fever virus *LMW5-HL,* and Herpesvirus yl 34.5), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and α-Hexachlorocyclohexane).

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem. 56*:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al., Nucleic Acids Research 6*:3073 (1979); Cooney *et al., Science 241*:456 (1988); and Dervan *et al., Science 251*:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptor.

Further antagonist according to the present invention include soluble forms of PTH1R or PTH3R fragments that include the ligand binding domain from the extracellular region of the full length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize PTH1R or PTH3R mediated signaling by competing with the cell surface PTH1R or PTH3R for binding to PTH or PTHrP. These are preferably expressed as dimers or trimers, since these have been shown to be superior to monomeric forms of soluble receptor as antagonists, e.g., IgGFc-PTH1R or IgGFc-PTH3R receptor family fusions.

***Modes of administration***

It will be appreciated that conditions caused by a decrease in the standard or normal level of PTH1R or PTH3R receptor activity in an individual, can be treated by administration of PTH1R or PTH3R protein. Thus, the invention further provides a method of treating an individual in need of an increased level of PTH1R or PTH3R receptor activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an isolated PTH1R or PTH3R polypeptide of the invention, effective to increase the PTH1R or PTH3R receptor activity level in such an individual.

The invention also relates to a method of treating an individual in need of an increased level of PTH1R or PTH3R receptor activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an agonist for PTH1R or PTH3R. The invention further relates to a method of treating an individual in need of a decreased level of PTH1R or PTH3R receptor activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an antagonist for PTH1R or PTH3R.

As a general proposition, the total pharmaceutically effective amount of PTH1R or PTH3R polypeptide or its agonists or antagonists administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the PTH1R or PTH3R polypeptide is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Pharmaceutical compositions containing the PTH1R or PTH3R polypeptide(s)of the invention or its agonists or antagonsits may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

***Chromosome Assays***

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a PTH1R or PTH3R receptor gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose. In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3′ untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

## *Examples*

### *Example 1: Isolation of Genomic DNA Clones Encoding the Zebrafish PTH1R and a novel type-3 receptor, PTH3R*

Using zebrafish genomic DNA and several degenerate forward and reverse primers (Figure 1), two distinct products of approximately 200 and 840 bp, respectively were obtained under stringent conditions. Forward (For) and reverse (Rev) degenerate primers (synthesized by the MGH Polymer core facility) for nested PCR (nPCR) were based on previously isolated mammalian and frog PTH1R sequences. Primers were located in exon M6/7 which encodes in mammals the third extracellular loop and the amino-terminal portion of transmembrane (TM) helix 7, and exon M7 which encodes the carboxy-terminal portion of TM7 and the beginning of the intracellular tail (Kong; Schipani) (Figure 1).

The primers for the first PCR reaction were the following: For M6a (5′TTYGGIGTSCAYTAYATHGTVTT; (SEQ ID NO:5) 576-fold degenerate), or For M6b (5′GTSYTBRTGCCICTHYTYGG; (SEQ ID NO:6) 1152-fold degenerate), and Rev M7 (CTCDCCATTRCAGWARCAGTADAT; (SEQ ID NO:7) 72-fold degenerate) (Fig. 1). PCR was performed using 1 mg of zebrafish (*Danio rerio*) genomic DNA, Gibco Taq (5 units), and the following PCR profile [initial denaturation at 95° for 3 min, (denature at 94° for 1 min, anneal at 50° for 1 min, and polymerize at 72° for 4 or 6 min) for 35 cycles] on an MJ research thermal cycler (Watertown, MA). nPCR was performed using 2 μl of a 1:100 dilution of the initial PCR product with a degenerate nested primer Rev M7#2 (5′GTADATRATDGMMACAAARAADCC; (SEQ ID NO:8) 432-fold degenerate), and either For M6a or For M6b using the same PCR profile as before (Fig. 1). nPCR products were identified on a 1.5% agarose gel with ethidium bromide, and DNA species of approximately 210 bp and 850 bp were excised, spun through a spin column (Bio101, La Jolla, CA), ethanol precipitated overnight [(1/10 vol of 3M sodium acetate was added, 2 vol of ethanol, 1 μl of glycogen (Pharmacia, Uppsala, Sweden)], and ligated into pGEM-T (Promega, Madison, WI). After transformation of competent DH5a *E. coli* cells (Gibco, Grand Island, NY), plasmid DNA from single colonies was purified using standard protocols, and sequenced by $^{33}P$ cycle sequencing (Amersham, Arlington Heights, IL) on an 8M urea 6% polyacrylamide field gradient gel (Rubin *et al.*, 1998). The DNA sequences, from the nested PCRs using For M6a or For M6b, were analyzed by the GCG package program (GCG, Univ. WI) and subsequently refered to as zPTH1R(TM6/7)/GEM-T and zPTH3R(TM6/7)/GEM-T, respectively.

Nucleotide sequence analysis revealed that both clones showed significant homology at their 5′ and 3′ ends with the hPTH1R (78% and 73% identity, respectively). While clone #1, zPTH1R(TM6/7), contained 84 bp of intronic sequence (compared to 81 bp of the hPTH1R) (Schipani), clone #2, zPTH3R(TM6/7), contained an intron of more than 700 bp. These findings indicated that portions of two distinct genes had been isolated which both share higher homology with PTH1R than with PTH2R (64% and 70% identity, respectively).

### *Example 2: Isolation of partial cDNA clones encoding zPTH1R and zPTH3R*

Total RNA from adult zebrafish was isolated as previously described (Rubin *et al.*, in press) and used as a template for RT-PCR (Gibco). For and Rev primers for RT-PCR were based on either zPTH1R or zPTH3R genomic DNA sequences corresponding to the mammalian exons M6/7 and M7, zPTH1R(TM6/7)/GEM-T and zPTH3R(TM6/7)/GEM-T, respectively (Fig. 1). Reverse transcription (RT) was performed using Superscript II RNAase $H^-$ reverse transcriptase and 5 μg total RNA at 42° C with zPTH1R/Rev 6(1) (5'GCATTTCATAATGCATCTGGATTTG) (SEQ ID NO:9), or zPTH3R/Rev 6(1) (5'CTGTGAAGAATTGAAGAGCATCTC) (SEQ ID NO:10), respectively. PCR, using the For 313 (5'ACMAACTACTAYTGGATYCTGGTG (SEQ ID NO:11); 8-fold degenerate) and either of the two Rev 6(1) primers was performed using Gibco Taq (5 units) and the following PCR profile [initial denaturation at 95° for 3 min, (denature at 94° for 1 min, anneal at 56° for 1 min, and polymerize at 72° for 2 min) for 35 cycles]. nPCR was performed using 2 μl of a 1:100 dilution from the initial zPTH1R or zPTH3R RT-PCR product, and either zPTH1R/Rev 6(2) (5'AGAAACTTCTGTGTAAGGCATCGC) (SEQ ID NO:12) or zPTH3R/Rev 6(2) (5'AAGAGCCATGAACAGCATGTAATG) (SEQ ID NO:13), and the For 313 primer using the previous PCR profile except for 35 cycles.

zPTH1R and zPTH3R PCR products were identified on a 1.5% agarose gel with ethidium bromide, cDNA species of approximately 450 bp were cloned, as described above, into pGEM-T to yield zPTH1R(TM3/6)/pGEMT and zPTH3R(TM3/6)/pGEMT, respectively.

### *Example 3: Isolation of full-length cDNAs encoding the zPTH1R*

RT-PCR using For313 and primers specific for zPTH1R(TM6/7) produced a 450 bp cDNA, zPTH1R(TM3/6), corresponding to TM3 through TM6 of the mammalian PTH1R. Subsequently, 5′ and 3′ RACE reactions were performed to generate overlapping sequences, and a full-length zPTH1R clone was constructed using a unique MfeI endonuclease restriction site (Fig. 1).

5′ and 3′ RACE reactions (Gibco) were performed using total RNA, and primers based on zPTH1R(TM3/6)/pGEMT nucleotide sequence. RT for 5′ RACE was performed, as described above, except that Rev 6(2) was used. The first 5′RACE PCR was performed using Rev 6(3) (5′GAAGACTATGTAGTGAACACCGAA) (SEQ ID NO:14), Gibco Taq (2.5 units) and the following PCR profile [initial denaturation at 95° for 3 min, (denature at 94° for 1 min, anneal at 55° for 1 min, and polymerize at 72° for 2 min) for 7 cycles, followed by 28 cycles with annealing at 64°. The first nPCR was performed using 5 μl of a 1:100 dilution from the previous PCR, Rev TM5 primer (5′ATATTGTTGTCTGGTGTCACATCT) (SEQ ID NO:15), (KlenTaq, 5.0 units) (Clontech, CA), and the following PCR profile [initial denaturation at 95° for 3 min, [(denature at 94° for 1 min, anneal at 62° for 1 min, and polymerize at 72° for 2 min) for 35 cycles} with a final extension of 10 min at 72°]. A second nPCR was performed using 5 μl of a 1:100 dilution from the first nPCR, Rev 4x primer (5′CGCATTTGTTTCTCGAAGTTTTGTTGC) (SEQ ID NO:16), Gibco Taq (5.0 units) and the same PCR profile as the first nPCR. The second nPCR products were purified as described above and ligated into pGEM-T EASY (Promega) to yield zPTH1R(5′)/pGEMTeasy, for transformation of TOP10 *E. coli* cells (Invitrogen).

For 3′ RACE, RT was performed as before but with an oligo-dT anchor primer (Gibco). PCR was performed using the For TM3 primer (5′ATCTTCATGACCTTCTTCTCAGAC) (SEQ ID NO:17), Gibco Taq (2.5 units) and the following profile [initial denaturation at 95° for 3 min, [(denature at 94° for 1 min, anneal at 64° for 1 min, and polymerize at 72° for 3 min) for 35 cycles} with a final extension of 10 min at 72°]. nPCR was performed using the previous PCR profile with 5 µl of a 1:100 dilution from the previous PCR, and For 4(1) (5'AGGAAGTACCTCTGGGGCTTCA) (SEQ ID NO:18). The 3' RACE nPCR products were purified and cloned to yield zPTH1R(3')/pGEMTeasy.

Midiprep DNA of zPTH1R(5')/pGEMTeasy and zPTH1R(3')/pGEMTeasy were digested with Mfe I and Nde I (New England Biolabs, Beverly, MA), and a 1.0 Kb fragment from zPTH1R(3') was ligated into an approximately 4.5 Kb fragment from zPTH1R(5')/pGEMTeasy to yield the full length zPTH1R clone, zPTH1R(FL)/pGEMTeasy. The zPTH1R(FL)/pGEMTeasy plasmid DNA was digested with EcoRI and SacI, and cloned into the corresponding sites in pGEM-3 (Promega) to yield zPTH1R(FL)/pGEM3. Subsequently, an EcoRI/SphI fragment, from the insert of zPTH1R(FL)/pGEM3 which comprises the entire coding region, of the zPTH1R was cloned into the corresponding sites of pcDNAI/Amp (Invitrogen) to yield zPTH1R(FL)/pcDNAI/Amp (zPTH1R).

The 5' RACE reactions generated two PCR products which contained an identical Kozak sequence and coding region (including the putative signal sequence) but varied in the length of the 5' UT; 5'RACE#29 was 149 bp and 5'RACE#25 was 391 bp. Both clones, zPTH1R#25 and zPTH1R#29, were characterized by radioligand assay, total IP generation, and cAMP accumulation.

The amino acid sequence encoded by zPTH1R cDNA (536 residues, Fig. 2A) showed highest sequence homology to the frog and mammalian PTH1Rs (Bergwitz; Kong). The overall amino acid sequence homology with the hPTH1R was 76% but only 68% when compared to the hPTH2R. Similar to the mammalian PTH1Rs, the 3' non-coding region of zPTH1R did not contain a typical polyadenylation signal sequence, however, an imperfect sequence was found (TATAAA) 49 bp upstream of the poly $A_{(n)}$ tail.

### *Example 4: Isolation of full-length cDNAs encoding the zPTH3R*

The genomic clone, zPTH3R(TM6/7), contained, at the 5′ and 3′ end, nucleotide sequences which were similar to but distinct from zPTH1R and zPTH2R, and contained approximately 700 bp, rather than 84 bp, of intronic sequence. This information indicated that portions of a novel gene had been isolated, subsequently refered to as zPTH3R. RT-PCR using For313 and primers specific for zPTH3R(TM6/7) produced a 450 bp cDNA clone, zPTH3R(TM3/6), which encodes TM3 through TM6 of the zPTH3R.

A zebrafish l gt11 cDNA library (Clontech) was screened by plaque hybridization using a 450 bp $^{32}$P-radiolabeled cDNA probe which was generated by PCR from zPTH3R(TM3/6)/pGEMT. Filters containing 1.5 x $10^6$ pfu were hybridized (42° for 18 hrs) in 50% formamide (Rubin *et al.*, 1996), and washes were performed for 30 min each at RT, 50°, and at 55°, respectively, with 1x SSC/0.1% SDS. Autoradiography was performed for 5 days at -70° with a DuPont Cronex intensifying screen and Kodak XAR film. A single phage was plaque-purified and subcloned into the EcoRI site of pcDNAI/Amp using the λ TRAP phage kit (Clontech) to yield zeb3-3′/pcDNAI/Amp.

5' RACE reactions (Gibco) were performed as above using three successive reverse zPTH3R primers [TM1 (5′ GAAGAGGTGGATGTGGATGTAGTT) (SEQ ID NO:19), G (5′ GCAGTGGAGACGTTTGAAATA) (SEQ ID NO:20), and E3 (5′ CCAGTTACCTGATGCATCACAGTG) (SEQ ID NO:21)]. The cDNA products were ligated to pGEMT-EASY (yielding zeb3-5′/pGEMT), minipreped, and their sequences analysed for homology to the known PTH receptors using GCG. Inserts which were determined to contain a nucleotide sequence with homology to the signal sequence of the mammalian PTH1Rs were ligated into zeb3-3′/pcDNAI/Amp using sites for BamHI, ApaLI, and NotI to yield zPTH3R(FL)/pcDNAI/Amp (zPTH3R).

From 1.5 x $10^6$ screened PFUs, a single phage clone was identified and the 2.5 Kb insert was subcloned to yield zeb3-3′/pcDNAI/Amp. Sequence analysis showed that the clone was closely related to the known PTH1Rs from the region corresponding to the mammalian exon E1 through the carboxy-terminal region encoded by exon T (Kong). However, the cDNA portion encoding the amino-terminal, extracellular domain and most of the 3′ untranslated region immediately following the termination codon were missing. 5′RACE on total zebrafish RNA revealed the presence of several putative splice variants which is similar to the findings with the zPTH2R (Rubin *et al.*, 1998). Seven of the ten zeb3-5′ clones were identified as containing cDNA sequences similar to the mammalian exons E3 and E1, which encode portions of the amino-terminal extracellular domain of the PTH1Rs. These clones also contained a Kozak sequence and a nucleotide sequence with homology to the signal peptide sequence found in the mammalian PTH1Rs (Schipani, Kong). Three other zeb3-5′ clones, which also contained the E3 and E1 equivalent, had different 5′ ends and could therefore represent putative splice variants. Similar to the zPTH2R (Rubin et a., 1998) and the human PTH1R (Joun, Bettoun), one of these zPTH3R putative splice variants lacked a signal peptide sequence but did contain an initiator AUG two codons upstream of the exon E1 equivalent. The second putative splice variant lacked a Kozak sequence, an initiator AUG, and contained a highly charged sequence upstream of the equivalent of E1 which is unlikel;y to represent a signal peptide. Only those clones which contained a Kozak sequence, an inframe AUG, and a 5′ coding region with homology to the zPTH1R, were ligated into the ApaLI site of zeb3-3′/pcDNAI/Amp to yield the full-length zPTH3R (Fig. 1).

Overall, the sequence encoded by this novel receptor (523 residues, Fig. 2B) shared 66% AA similarity and 59% AA identity with zPTH1R, but only 55% similarity with the zPTH2R. Similar to the frog PTH1Rs, zPTH1R and zPTH3R lacked the equivalent of the cDNA encoded by exon E2, suggesting that the appearance of this non-essential exon (Lee and Gardella) represents a mammalian evolutionary innovation (Lauder and Liem).

In contrast to this mammalian E2 apomorphy, zPTH1R and zPTH3R contain the same eight extracellular cysteines as all known mammalian and non-mammalian members of this family of G protein-coupled receptors as well as several other "signature residues" (G protein coupled receptor database: Http://www.gcrdb.uthacsa.edu). However, there are differences in the number of consensus sequences (N-X-S or N-X-T) for potential N-glycosylation between zPTH1R and zPTH3R; only two are conserved for all zebrafish PTH receptors (Fig. 2C). Furthermore, analysis of the intracellular tail residues indicates that this region has a higher rate of sequence variation between PTH receptor subtypes and was therefore used for further comparison (Table 2). For example, the homology between zPTH1R and hPTH1R in this region is 56%, which is similar to the 58% AA similarity for zPTH2R and hPTH2R. In contrast, the homology between the tail regions of zPTH3R and hPTH1R is 38%, and 28% when compared to hPTH2R (Table 2). The zPTH3R was therefore identified as a novel member within the PTH/PTHrP receptor family.

### *Example 5: Functional characterization of zPTH1R and zPTH3R in COS-7 cells*

Plasmid DNAs encoding the two full length zPTH1Rs (#25 and #29) and the zPTH3R were transiently expressed in COS-7 cells. COS-7 African green monkey kidney cells (approximately 200,000 cells/well in a 24-well plate) were cultured and transfected with plasmid DNA (200 ng/well) as described (Rubin *et al.*, 1998). After transfection, cells were cultured for 72 hrs at 37° with daily exchanges of medium, followed by an additional 24 hrs at 33° (Gardella *et al.*, 1997) until they were functionally evaluated after 96 hrs.

Radioligand studies with COS-7 cells expressing either zPTH1R or zPTH3R were performed as described using either $^{125}$I-labeled [Nle$^{8,21}$,Tyr$^{34}$]rPTH-(1-34)amide (rPTH) (SEQ ID NO:22) or $^{125}$I-labeled [Tyr$^{36}$]hPTHrP-(1-36)amide (PTHrP) (SEQ ID NO:23), and increasing concentrations of either [Tyr$^{34}$]hPTH(1-34)amide (PTH) (SEQ ID NO:24), or

[$Tyr^{36}$]hPTHrP(1-36)amide (PTHrP) (SEQ ID NO:X) (Bergwitz). Peptides were synthesized by the MGH polymer core facility as described (Gardella *et al.*, 1995 and 1996). Specific binding was calculated by subtracting radioligand binding in the presence of excess unlabeled peptide from the total binding. All points represent mean ± S.E.M. of two to three replicates from two or more independent experiments (Tallarida and Jacob, 1979). $IC_{50}$ values (dose of a competing ligand which resulted in 50% inhibition of radioligand binding) were calculated as previously described (Gardella *et al.*, 1996).

In order to assess agonist-dependent cAMP accumulation of COS-7 cells expressing zPTH1R and zPTH3R, experiments were done in 24-well plates with COS-7 cells stimulated in the presence of increasing concentrations of either PTH or PTHrP, and intracellular cAMP was determined as described (Bergiwtz *et al.*,) $EC_{50}$ values were determined as previously described (Gardella *et al.*, 1996).

In order to determine total inositol phosphate turnover for COS-7 cells expressing zPTH1R, zPTH3R, and hPTH1R, cells were grown in 6-well plates with COS-7 cells (approximately 200,00 cells/well transfected with either 1 μg/well of zPTH1R, zPTH3R, or hPTH1R (stina)). Cells were cultured for 3 days in DMEM/7% fetal bovine serum (FBS) at 37°C with daily exchanges of medium. The cells were then preloaded with 3 μCi/ml myo-[$^3$H]inositol (New England Nuclear, Boston, MA) in inositol-free DMEM (Gibco)/7% FBS (33°C for 18 hr). The following day, plates were rinsed and then incubated with $10^{-6}$ M of either PTH, PTHrP in DMEM/0.1% BSA, or with DMEM/0.1% BSA alone (40 min at 37°C), in the presence of 30 mM LiCl. Total inositol phosphate (IPs) were isolated by anion exchange column chromatography as previously described (Iida-Klein *et al.*, 1995 and 1997), and 1 ml of the eluate (1/8th of total) was counted in a liquid scintillation counter (model LS 6000IC, Beckman, Fullerton, CA). All points represent mean ± S.E.M. of two to three replicates from two or more independent experiments (Tallarida and Jacob, 1979).

Both zPTH1R clones showed high affinity binding of radiolabeled rPTH and PTHrP (Table 2, Fig. 4). Apparently, due to the shorter 5′ UT, zPTH1R#29 showed in comparison to #25 slightly higher expression levels and higher maximal cAMP accumulation (101.6 pmole/well for hPTHrP and 106.2 pmole/well for hPTH versus 92.4 pmole/well for hPTHrP and 90.4 pmole/well for hPTH; *see* Table 2), but otherwise both clones were indistinguishable. Only the functional data for zPTH1R#29 are therefore presented (figure 3, Table 3). Interestingly, zPTH3R showed a higher specific binding for radiolabeled PTHrP than rPTH. Furthermore, the zPTH3R showed a higher apparent Kd for PTHrP than PTH (approximately 3nM versus approximately 100nM, respectively). These results indicate that zPTH3R preferentially interacts with PTHrP.

Similar to these binding data, COS-7 cells expressing the zPTH1R showed very similar $EC_{50s}$ for cAMP accumulation in response to either PTH or PTHrP which is similar to the findings with mammalian PTH1Rs ($EC_{50}$: 0.8 ± 0.03 nM for PTH and 0.3 ± 0.04 nM for PTHrP, Table 2). The zPTH3R showed a higher maximal cAMP accumulation (285.4 nM for PTH and 227.4 for PTHrP nM), but in contrast to the zPTH1R, zPTH3R showed a reduced efficacy for PTH ($EC_{50s}$: 5.98 ± 0.24 nM for PTH and 0.49 ± 0.17 nM for PTHrP, Table 2, Fig. 4). Furthermore, in addition to being more efficiently activated by PTHrP, the zPTH3R showed a significant activation at $10^{-11}$ M (Fig. 4). These results confirmed the radioreceptor studies which had indicated that the zPTH3R interacts preferentially with PTHrP.

Similar to the mammalian PTH1Rs, COS-7 cells expressing the zPTH1R showed an equivalent increase of IP accumulation (2-fold) when stimulated with either PTH or PTHrP. In contrast, despite higher expression levels, no IP accumulation was detectable when challenged with either ligand (Fig. 5). The lack of signaling through this second messenger may be related to significant structural alterations in the second intracellular loop of zPTH3R (Fig. 4). Previous studies with the rat PTH1R have shown that this portion of the receptor is important for IP signaling, since replacement of some residues in this "EKKY" cassette either impaired or abolished phospholipase C activation (Akiko). While the zPTH1R contains a conserved DRKY sequence instead of the mammalian

EKKY, the corresponding AA residues of the zPTH3R are DKNC (Fig. 3). The two most important residues are therefore altered in the novel receptor which could explain the signaling selectivity of the zPTH3R. The zPTH3R is therefore a naturally occurring PTH/PTHrP receptor which appears to be incapable of signaling through IP.

***Example 6: Southern Blot Analysis of Zebrafish Genomic DNA***

To confirm that zPTH1R and zPTH3R are encoded by distinct genes, three infrequently cutting restriction endonucleases were utilized to digest zebrafish genomic DNA to completion (Fig. 6). Approximately 16 μg of zebrafish genomic DNA was digested to completion with either BamHI, EcoRI, or HindIII, split into two equal aliquots for electrophoresis through a 0.8% agarose gel containing ethidium bromide, and transferred onto a nitrocellulose membrane (MSI, Westborough, MA). After baking *in vacuo* for 2 hr at 80° C, the blots were hybridized in 50% formamide (42° for 18 hrs) with PCR-generated $^{32}$P-labeled probes (Schowalter and Sommer, 1989) encoding either the carboxy-terminal tail of zPTH1R or zPTH3R (240 and 335 bp, respectively). Washes were performed for 30 min each at RT and 42° in 1x SSC/0.1% SDS, and at 50° in 0.5x SSC/0.1% SDS followed by autoradiography at -70° for 7 days with a DuPont Cronex intensifying screen and Kodak XAR film.

Initial Southern blot data using probes corresponding to TM3 through TM6 of either receptor showed multiple hybridizing DNA species, indicating that these probes cross hybridized with each others gene or with closely related genes (data not shown). To increase the specificity for either subtype, hybridizations were performed with radiolabeled probes comprising only the tail region of each receptor, zPTH1R/tail or zPTH3R/tail, which showed the highest rates of sequence variation between PTH receptor subtypes. For each digest the tail probes hybridized, under stringent conditions, to a single but different genomic

DNA fragment indicating that distinct genes encode zPTH1R and zPTH3R (Fig. 6).

### *Example 7: Phylogenetic and Structural Analyses of All Known PTHRs*

Alignment of all known PTH1Rs, PTH2Rs, the goldfish VIP receptor (#U56391), and the human CRF receptor (#P34998) sequences was performed as previously described (Rubin *et al.*, 1988). Sequences were subsequently aligned within MacClade 3.0 (Maddison and Maddison, 1992) and gaps were entered to maximize the homology of the native proteins. Each AA was treated as an unweighted character when analyzed using the branch-and-bound search option of PAUP 3.1 (Swofford, 1993). A bootstrapping analysis using the branch-and-bound option on 100 replicates (Hedges, 1992; Felsenstein and Kishino, 1993) was performed and only groups which were compatible with the 50% majority-rule consensus were retained (Swofford and Olsen, 1990; Swofford, 1993).

GCG was used for comparing the tail regions of zPTH1R, zPTH2R, zPTH3R, hPTH1R, and hPTH2R. A further analysis was performed within MacClade to determine unambiguous residues which may be character-dependent for each PTH/PTHrP receptor subtype (Madison and Maddison, 1992; Swofford, 1993; Rubin *et al.*, 1998).

The single most parsimonious Bootstrap consensus tree revealed two statistically significant PTH/PTHrP receptor clades; the PTH1R/PTH3R clade and the PTH2R clade (Fig. 7). Furthermore, within the PTH1R/PTH3R clade, the PTH1R groups significantly different from PTH3R and, at least for the PTH1R, the terminal branches contained within are congruent with morphologically based phylogenies (Pough, 1989).

Whereas the overall amino acid conservation between zPTH1R and zPTH3R is relatively high, particularly within the transmembrane region, multivariate analysis led to the identification of amino acid residues that may be specific for PTH3Rs (Fig. 3). Although additional PTH3R sequences from other species are required to confirm the receptor specificity of these residues, the limited number of amino acid changes may already allow mutational studies to explore their functional importance, particularly with regard to phospholipase C activation.

Table 2. ***Comparisons of the Amino Acid sequences of the Intracellular, Carboxy-Terminal Tail of Different PTH Receptors.***
Residues comprising the tail region of the zPTH1R and zPTH3R were compared to corresponding regions of the zPTH2R, and the human PTH1R or PTH2R. %similarity/%identity are indicated.

**Table 2**

| | zPTH1R | zPTH2R | zPTH3R | hPTH1R |
|---|---|---|---|---|
| zPTH2R | 38/31 | | | |
| zPTH3R | 36/33 | 26/23 | | |
| hPTH1R | 56/50 | 35/28 | 38/33 | |
| hPTH2R | 38/29 | 58/53 | 28/25 | 38/31 |

Table 3. ***Binding and cAMP Signaling Properties of PTH and PTHrP Analogs on the zPTH1R and the zPTH3R.***

Competitive binding and cAMP stimulation assays were performed at room temperature with intact COS-7 cells that expressed either the zPTH1R or the zPTH3R, as described in Materials and Methods. Homologous binding reactions utilized $^{125}$I-rPTH with unlabeled rPTH, and $^{125}$I-hPTHrP with unlabeled hPTHrP, heterologous binding reactions utilized $^{125}$I-hPTH with unlabeled rPTH. $EC_{50}$ and $IC_{50}$ values were determined as previously described (Gardella *et al.*, 1996). Values are the mean ± SEM of at least three independent transfections. ND, not determined.

**Table 3**

| | zPPR3 | | | zPPR1#29 | | | zPPR1#25 | | |
|---|---|---|---|---|---|---|---|---|---|
| Ligand | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) | cAMP Maximum (pmol/well) | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) | cAMP Maximum (pmol/well) | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) | cAMP Maximum (pmol/well) |
| hPTHrP | ~3.0 | 0.49 ± 0.17 | 285.4 ± 3.4 | ~3.0 | 0.3 ± 0.04 | 101.6 ± 3.6 | ~3.0 | 0.90 ± 0.09 | 92.4 ± 8.00 |
| hPTH | ~100 | 5.98 ± 0.24 | 227.4 ± 41.6 | ~3.0 | 0.8 ± 0.03 | 106.2 ± 4.8 | ~3.0 | ~3.0 | 90.4 ± 11.4 |

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## *What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the PTH1R receptor having the complete amino acid sequence at positions from about 1 to about 536 in SEQ ID NO:2;

(b) a nucleotide sequence encoding the PTH1R receptor having the amino acid sequence at positions from about 2 to about 536 in SEQ ID NO:2;

(c) a nucleotide sequence encoding the mature PTH1R receptor having the amino acid sequence at positions from about 25 to about 536 in SEQ ID NO:2;

(d) a nucleotide sequence encoding the PTH1R receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(e) a nucleotide sequence encoding the mature PTH1R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(f) a nucleotide sequence encoding the PTH1R extracellular domain;

(g) a nucleotide sequence encoding the PTH1R transmembrane domain;

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 1C (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1C (SEQ ID NO:1) encoding the PTH1R receptor having the complete amino acid sequence in Figure 2A (SEQ ID NO:2).

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1C (SEQ ID NO:1) encoding the mature PTH1R receptor having the amino acid sequence in Figure 2A (SEQ ID NO:2).

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. ____.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the PTH1R receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature PTH1R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____.

8. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

9. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a PTH1R receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1.

10. The isolated nucleic acid molecule of claim 1, which encodes the PTH1R receptor extracellular domain.

11. The isolated nucleic acid molecule of claim 1, which encodes the PTH1R receptor transmembrane domain.

12. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

13. A recombinant vector produced by the method of claim 12.

14. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 13 into a host cell.

15. A recombinant host cell produced by the method of claim 14.

16. A recombinant method for producing a PTH1R polypeptide, comprising culturing the recombinant host cell of claim 15 under conditions such that said polypeptide is expressed and recovering said polypeptide.

17. An isolated PTH1R polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the PTH1R polypeptide having the complete amino acid sequence at positions from about 1 to about 536 in SEQ ID NO:2;

(b) the amino acid sequence of the PTH1R polypeptide having the amino acid sequence at positions from about 2 to about 536 in SEQ ID NO:2;

(c) the amino acid sequence of the mature PTH1R polypeptide having the amino acid sequence at positions from about 25 to about 536 in SEQ ID NO:2;

(d) the amino acid sequence of the PTH1R polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(e) the amino acid sequence of the mature PTH1R polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(f) the amino acid sequence of the PTH1R receptor extracellular domain;

(g) the amino acid sequence of the PTH1R receptor transmembrane domain; and

(h) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), or (g).

18. An isolated polypeptide comprising an epitope-bearing portion of the PTH1R receptor protein.

19. An isolated antibody that binds specifically to a PTH1R receptor polypeptide of claim 17.

20. A method of treating diseases and disorders associated with the decreased PTH1R activity comprising administering an effective amount of the polypeptide as claimed in claim 17, or an agonist thereof to a patient in need thereof.

21. A method of treating diseases and disorders associated with increased PTH1R activity comprising administering an effective amount an antagonist of the polypeptide as claimed in claim 17 to a patient in need thereof.

22. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the PTH3R receptor having the complete amino acid sequence at positions from about 1 to about 523 in (SEQ ID NO:4);

(b) a nucleotide sequence encoding the PTH3R receptor having the amino acid sequence at positions from about 2 to about 523 in (SEQ ID NO:4);

(c) a nucleotide sequence encoding the mature PTH3R receptor having the amino acid sequence at positions from about 22 to about 523 in (SEQ ID NO:4);

(d) a nucleotide sequence encoding the PTH3R receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(e) a nucleotide sequence encoding the mature PTH3R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ___;

(f) a nucleotide sequence encoding the PTH3R extracellular domain;

(g) a nucleotide sequence encoding the PTH3R transmembrane domain;

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

23. The nucleic acid molecule of claim 22 wherein said polynucleotide has the complete nucleotide sequence in Figure 1D (SEQ ID NO:3).

24. The nucleic acid molecule of claim 22 wherein said polynucleotide has the nucleotide sequence in Figure 1D (SEQ ID NO:3) encoding the PTH3R receptor having the complete amino acid sequence in Figure 2B (SEQ ID NO:4).

25. The nucleic acid molecule of claim 22 wherein said polynucleotide has the nucleotide sequence in Figure 1D (SEQ ID NO:3) encoding the mature PTH3R receptor having the amino acid sequence in Figure 2B (SEQ ID NO:4).

26. The nucleic acid molecule of claim 22 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No._____.

27. The nucleic acid molecule of claim 22 wherein said polynucleotide has the nucleotide sequence encoding the PTH3R receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ______.

28. The nucleic acid molecule of claim 22 wherein said polynucleotide has the nucleotide sequence encoding the mature PTH3R receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No.______.

29. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), or (h) of claim 22 wherein said polynucleotide which hybridizes

does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

30. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a PTH3R receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f), (g), or (h) of claim 22.

31. The isolated nucleic acid molecule of claim 22, which encodes the PTH3R receptor extracellular domain.

32. The isolated nucleic acid molecule of claim 22, which encodes the PTH3R receptor transmembrane domain.

33. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 22 into a vector.

34. A recombinant vector produced by the method of claim 33.

35. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 34 into a host cell.

36. A recombinant host cell produced by the method of claim 35.

37. A recombinant method for producing a PTH3R polypeptide, comprising culturing the recombinant host cell of claim 36 under conditions such that said polypeptide is expressed and recovering said polypeptide.

38. An isolated PTH3R polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the PTH3R polypeptide having the complete amino acid sequence at positions from about 1 to about 523 in (SEQ ID NO:4);

(b) the amino acid sequence of the PTH3R polypeptide having the amino acid sequence at positions from about 2 to about 523 in (SEQ ID NO:4);

(c) the amino acid sequence of the mature PTH3R polypeptide having the amino acid sequence at positions from about 22 to about 523 in(SEQ ID NO:4);

(d) the amino acid sequence of the PTH3R polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(e) the amino acid sequence of the mature PTH3R polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ___;

(f) the amino acid sequence of the PTH3R receptor extracellular domain;

(g) the amino acid sequence of the PTH3R receptor transmembrane domain;

(h) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), or (g).

39. An isolated polypeptide comprising an epitope-bearing portion of the PTH3R receptor protein.

40. An isolated antibody that binds specifically to a PTH3R receptor polypeptide of claim 38

41. A method of treating diseases and disorders associated with the decreased PTH3R activity comprising administering an effective amount of the

polypeptide as claimed in claim 41, or an agonist thereof to a patient in need thereof.

42. A method of treating diseases and disorders associated with increased PTH3R activity comprising administering an effective amount an antagonist of the polypeptide as claimed in claim 38 to a patient in need thereof.

43. An isolated polynucleotide encoding a modified PTH3R protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids 1 to 523 of SEQ ID NO:4;
(b) amino acids 2 to 523 of SEQ ID NO:4; and
(c) amino acids 22 to 523 of SEQ ID NO:4.

44. A modified PTH3R protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids 1 to 523 of SEQ ID NO:4;
(b) amino acids 2 to 523 of SEQ ID NO:4; and
(c) amino acids 22 to 523 of SEQ ID NO:4.

45. An isolated polynucleotide encoding a modified PTH1R protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids 1 to 536 of SEQ ID NO:2;
(b) amino acids 2 to 536 of SEQ ID NO:2; and
(c) amino acids 25 to 536 of SEQ ID NO:2.

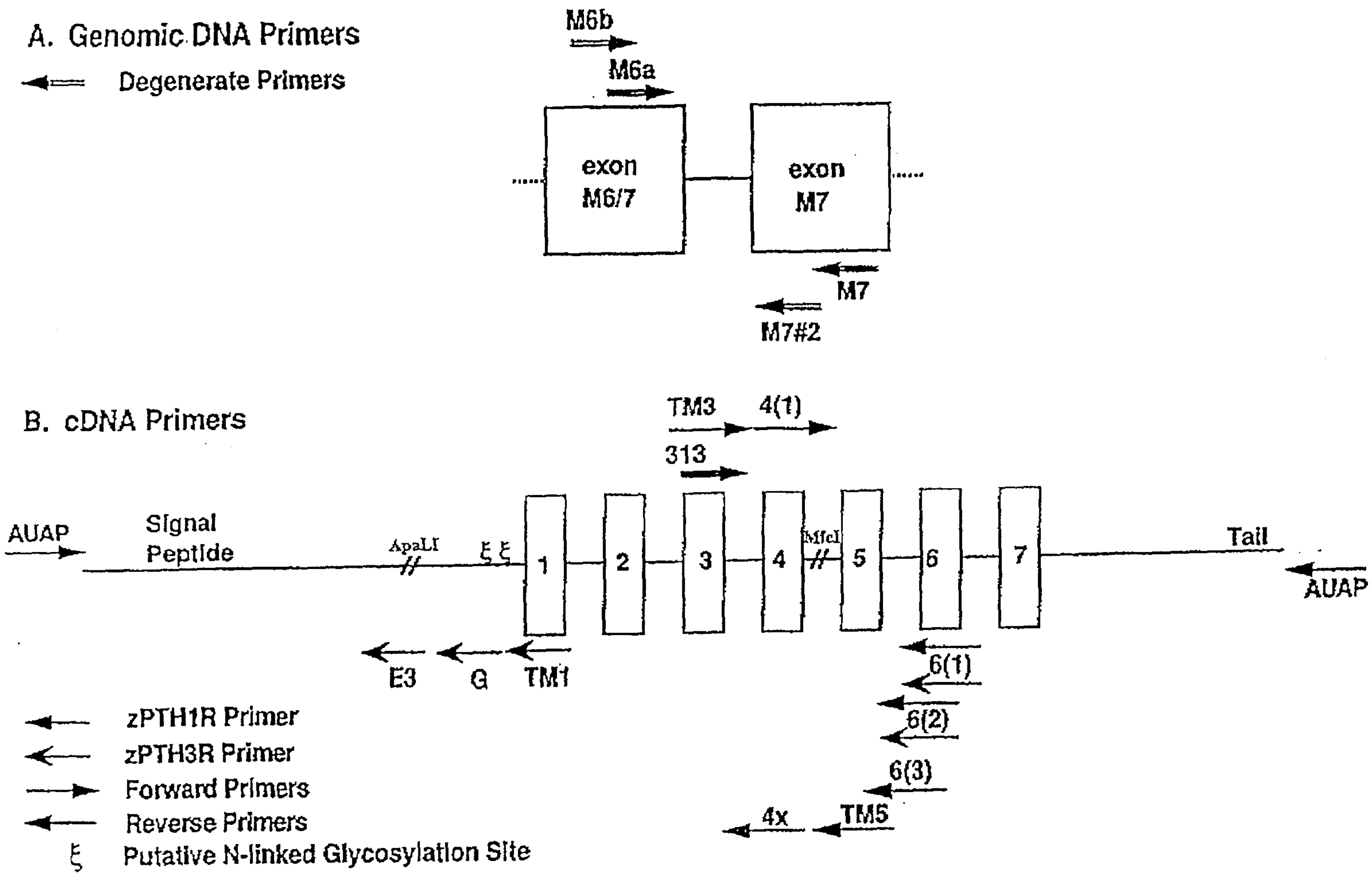
46. A modified PTH1R protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids 1 to 536 of SEQ ID NO:2;

(b) amino acids 2 to 536 of SEQ ID NO:2; and

(c) amino acids 25 to 536 of SEQ ID NO:2.

FIGURE 1


A. Genomic DNA Primers
Degenerate Primers
M6b
M6a
exon M6/7
exon M7
M7
M7#2
B. cDNA Primers
TM3
4(1)
313
AUAP
Signal Peptide
ApaLI
MfeI
1
2
3
4
5
6
7
Tail
AUAP
E3
G
TM1
6(1)
6(2)
6(3)
TM5
4x
zPTH1R Primer
zPTH3R Primer
Forward Primers
Reverse Primers
Putative N-linked Glycosylation Site

# FIGURE 1C

(PTH1R Nucleotide seQ)

1 TTCTCTCCAA TCGACGCGAC TGCCATGTCC TGAAGAGAAA CAGGAGCTCT

51 CTGGAGAGCA GGAGTTCTGG AAAAGGTCAA AGGTCCTGGG TTAAGCATGG

101 TGTCAGTGGA GGTCTCTGTG GCTTTAGTGC TGTGCTGTGT TTTGATGGGA

151 GCCAGAGCTC TGATTGATTC AGATGATGTC ATCACAAGAG ATGAACAGAT

201 CTTTCTCCTC ATTGGTGCGC GGTCGAGGTG TGAGAGAACC ATCCGTGCAC

251 AGTCAGACGT GGTCAGAGAG AATAACTGCG CTCCTGAGTG GGATGGGATC

301 ATTTGCTGGC CCACAGGAAA ACCCAATCAG ATGGTGGCAG TTCTGTGTCC

351 TGAGTACATC TATGACTTCA ACCACAGAGG ATACGCGTAT CGACACTGTG

401 ATGCATCAGG TAACTGGGAG CAGGTGTCCA TTATAAACCG GACGTGGGCA

451 AACTACACGG AATGCACCAC TTACCTGCAC ACCAACCACA GTGATCAGGA

501 GGAAGTGTTT GAGCGCCTTT ACCTCATGTA CACTATTGGA TACTCCATAT

551 CACTGGCAGC GTTACTGGTG GCGGTCTCTA TCCTTTGCTA TTTCAAACGT

601 CTCCACTGCA CTCGTAACTA CATCCACATC CACCTCTTCA CCTCGTTCAT

651 ATGTCGAGCA ATCAGTATTT TTGTGAAAGA CGCCGTTCTT TACGCCGTCA

701 CGAATGATGG AGAACTAGAA GATGGGGCAG TGGAACAAAG ACCCATGGTG

751 GGCTGCAAGG CTGCTGTGAC CCTCTTCCTG TATCTGTTGG CGACCAATCA

801 TTATTGGATC CTGGTGGAGG GTTTGTACTT GCATAGTCTG ATCTTCATGG

851 CCTTCCTGTC TGATAAGAAC TGCCTGTGGG CTTTGACAAT CATAGGCTGG

901 GGGATCCCAG CAGTGTTTGT GTCTATATGG GTCAGTGCCA GGGTGTCTCT

951 GGCAGACACA CAGTGCTGGG ATATCAGTGC AGGCAATTTG AAATGGATTT

1001 ATCAAGTACC AATCCTGGCA GCCATTGTTG TAAACTTCTT CCTCTTCCTC

1051 AATATCATCA GGGTTTTGGC CTCTAAGTTG TGGGAAACAA ACACGGGAAA

1101 ACTGGACCCT AGACAGCAGT ACAGGAAGCT GCTGAAGTCA ACAATGGTGC

1151 TGATGCCACT GTTTGGAGTT CATTACATGC TGTTCATGGC TCTTCCGTAC

1201 ACTGATGTGA CTGGTTTGCT GTGGCAGATT CTGATGCATT ACGAGATGCT

1251 CTTCAATTCT TCACAGGGTT TCTTTGTGGC GTTTATTTAC TGCTTCTGCA

# FIGURE 1C

(PTH1R Nucleotide SEQ.)

1301 ATGGGGAGGT GCAGGCAGAG GTGAAGAAGG CCTGGTTGCG AAGCAGTCTT

1351 GCGTTAGACC AGAAGCAGAA GGCTCGAGTC CACAGCAGTG CAGGATGTGG

1401 AAGTGGTTAC TATGGAGGAA TGATGTCCCA CACCACCACA CAGAGCGTGT

1451 GTCTCAGTGT CAGTGGTGCT AAAGGCGGTC ATTCTCTGCA CACCATAGGA

1501 GCCAAAGGAC AATCCCGTCT ACAACATTCA GGAAACTTAC CCGGTACGCG

1551 CCTCAGGGCG CATAGACTTT GTTTTACCCA GTGGTCCCAA AGCAGAAAGA

1601 GACTCCATGC AGACAGAGCA GCAGGAATGC AGAGGAAAGC GAGCATGATT

1651 TTGAGCCATA TTTCGTAGCG G. (SEQ ID NO:1)

# FIGURE 1 D

(PTH3R Nucleotide SEQ.)

1 acttttttat ataccagcaa acgatgtact aatgggaggg gaggagatgg

51 aagcgaaatg actgcccctt tgaaggcgct taaacagacg taaacaccag

101 agaaatccca ttttaaactg acagcacatt tgcgtgaact tgcgtcgggA

151 TGGGAGCCAC GCTGATCGTA CGCACTTTAG GCTTTCTCTT CTGCGGCACC

201 TTGCTGAGTT TCGTCTATGG TCTGGTCGAT GCAGATGATG TCCTCACAAA

251 GGAGGAGCAA ATCTATCTTC TGTTCAACGC AAAACGAAAA TGTGAGCGAG

301 CAATCAAGTC CAAGCATAAA ACGTCTGAGG GATCCTGTCT GCCAGAGTGG

351 GATGGCATCC TATGTTGGCC CGAGGGAGTT CCTGGAAAGA TGGTGTCCAC

401 TTCATGCCCA GAGTACATAT ATGACTTCAA CCACAAAGGT CATGCCTACC

451 GGCGCTGCGA CCTGAACGGG ACCTGGGAAC TGGCCTCACA TAACAACAAA

501 ACCTGGGCTA ATTACAGCGA ATGTGCCAAA TTCTTCCCCC ATTATAACCA

551 GAACCAGGAG AGGGAGGTTT TCGACAGACT TTACCTGATC TACACAGTGG

601 GCTACTCCAT CTCTCTGGGA TCACTTATGG TGGCCACAGT CATCCTCGGA

651 TACTTTCGAC GGCTCCACTG CACCAGGAAC TACATCCACA TGCACCTGTT

701 TCTATCGTTC ATGTTGAGGG CCATTAGTAT CTTCGTGAAG GATGTGGTGC

751 TGTACTCTGG TTCGGCGCTG CAGGAAATGG AACGAATCAC TGTGGAGGAT

801 CTCAAATCCA TCACTGAAGC CCCTCCTGCC AACAAAACCC AGTTTATCGG

851 CTGTAAGGTG GCGGTGAGGC TCTTCTTGTA CTTCTTGGCC ACTAATTATT

901 ACTGGATTCT GGTGGAAGGC CTGTACCTGC ACAGCCTTAT CTTCATGACC

951 TTCTTCTCAG ACAGGAAGTA CCTCTGGGGC TTCACTCTGA TTGGTTGGGG

1001 TGTTCCTGCG ATGTTTGTCA CCATCTGGGC GAGTGTTAGA GCCACACTTG

1051 CTGACACTGA GTGCTGGGAT TTGAGTGCAG GAAACCTGAA ATGGATTGTG

1101 CAGATCCCCA TTCTTACTGC AATTGTTGTC AATTTTTTGT TGTTCCTGAA

1151 TATAATTCGA GTCTTGGCAA CAAAACTTCG AGAAACAAAT GCGGGCAGAT

1201 GTGACACCAG ACAACAATAT AGGAAGCTGC TGAAGTCGAC TCTGGTCCTC

1251 ATGCCGTTGT TCGGTGTTCA CTACATAGTC TTCATGGCGA TGCCTTACAC

FIGURE 1D
(PTH3R Nucleotide SEQ.)

1301 AGAAGTTTCT GGAGTACTGT GGCAAATCCA GATGCATTAT GAAATGCTCT
1351 TTAACTCAGT CCAGGGATTC TTTGTTGCGA TTATATATTG CTTCTGCAAC
1401 GGAGAGGTCC AAGCGGAAAT CAAGAAGGCC TGGAACAGAA GGACTCTTGC
1451 TCTGGACTTC AAGAGAAAAG CCAGGAGCGG CAGTAACACA TACAGCTATG
1501 GACCCATGGT TTCTCACACC AGTGTTACCA ATGTGACGGC GCGGGGGCCG
1551 CTGGCCCTTC ACCTCACCAA CCGACTGGGG CACGTCACCA CTAACGGCCA
1601 CAGAAACCTT CCGGGATACA TAAAAAACGG CTCCGTTTCA GAAAACTCCA
1651 TCCCGTCCTC GGGTCACGAG CTTCACATTC AGGAGGAAGA GCCTTCGAAG
1701 ACCTTCCAGA TGGAGAAAAC CATCCAGGTG GTGGAGGAGG AAAGAGAAAC
1751 CGTCATGTGA CTTTACTGAC GGACAAATGC TAGATATATT GAAGGATTCA
1801 AGAGTCCTTC GCTTCTCAGG ATTTCCTTAC AATTGCTGtt gttggaggca
1851 cagagaaaga ccggagaaat gagccacgag tctggaagct actgcagcat
1901 cttcaccagc agcatttggt aaaggagaac agactttgct ttggattttt
1951 ttaaatatta gatattcgca ctgtagacaa gcaactgcgg tgctttcagg
2001 tcgtcatatc acctccatac acgaagaacg gtgagtttgc atcaggatca
2051 cgtcatataa agcattgtgg ggacgtgaat tgtacacaaa gaagacactc
2101 gtgttatttt taaaaaaaaa aaaaaaaaa (SEQ ID NO: 3)

# FIGURE 2A

(PTH1R A.A. SeQ)

```
        ATGGGAGCCACGCTGATCGTACGCACTTTAGGCTTTCTCTTCTGCGGCACCTTGCTGAGT
        +---------+---------+---------+---------+---------+---------
        TACCCTCGGTGCGACTAGCATGCGTGAAATCCGAAAGAGAAGACGCCGTGGAACGACTCA

a    1  M  G  A  T  L  I  V  R  T  L  G  F  L  F  C  G  T  L  L  S        Signal peptide

        TTCGTCTATGGTCTGGTCGATGCAGATGATGTCCTCACAAAGGAGGAGCAAATCTATCTT
        +---------+---------+---------+---------+---------+---------
        AAGCAGATACCAGACCAGCTACGTCTACTACAGGAGTGTTTCCTCCTCGTTTAGATAGAA

a   21  F  V  Y  G| L  V  D  A  D  D  V  L  T  K  E  E  Q  I  Y  L

        CTGTTCAACGCAAAACGAAAATGTGAGCGAGCAATCAAGTCCAAGCATAAAACGTCTGAG
        +---------+---------+---------+---------+---------+---------
        GACAAGTTGCGTTTTGCTTTTACACTCGCTCGTTAGTTCAGGTTCGTATTTTGCAGACTC

a   41  L  F  N  A  K  R  K  C  E  R  A  I  K  S  K  H  K  T  S  E

        GGATCCTGTCTGCCAGAGTGGGATGGCATCCTATGTTGGCCCGAGGGAGTTCCTGGAAAG
        +---------+---------+---------+---------+---------+---------
        CCTAGGACAGACGGTCTCACCCTACCGTAGGATACAACCGGGCTCCCTCAAGGACCTTTC

a   61  G  S  C  L  P  E  W  D  G  I  L  C  W  P  E  G  V  P  G  K

        ATGGTGTCCACTTCATGCCCAGAGTACATATATGACTTCAACCACAAAGGTCATGCCTAC
        +---------+---------+---------+---------+---------+---------
        TACCACAGGTGAAGTACGGGTCTCATGTATATACTGAAGTTGGTGTTTCCAGTACGGATG

a   81  M  V  S  T  S  C  P  E  Y  I  Y  D  F  N  H  K  G  H  A  Y

        CGGCGCTGCGACCTGAACGGGACCTGGGAACTGGCCTCACATAACAACAAAACCTGGGCT
        +---------+---------+---------+---------+---------+---------
        GCCGCGACGCTGGACTTGCCCTGGACCCTTGACCGGAGTGTATTGTTGTTTTGGACCCGA

a  101  R  R  C  D  L  N  G  T  W  E  L  A  S  H  N  N  K  T  W  A

        AATTACAGCGAATGTGCCAAATTCTTCCCCCATTATAACCAGAACCAGGAGAGGGAGGTT
        +---------+---------+---------+---------+---------+---------
        TTAATGTCGCTTACACGGTTTAAGAAGGGGGTAATATTGGTCTTGGTCCTCTCCCTCCAA

a  121  N  Y  S  E  C  A  K  F  F  P  H  Y  N  Q  N  Q  E  R  E  V

        TTCGACAGACTTTACCTGATCTACACAGTGGGCTACTCCATCTCTCTGGGATCACTTATG
        +---------+---------+---------+---------+---------+---------
        AAGCTGTCTGAAATGGACTAGATGTGTCACCCGATGAGGTAGAGAGACCCTAGTGAATAC

a  141  F  D  R  L  Y  L  I| Y  T  V  G  Y  S  I  S  L  G  S  L  M     TM1

        GTGGCCACAGTCATCCTCGGATACTTTCGACGGCTCCACTGCACCAGGAACTACATCCAC
        +---------+---------+---------+---------+---------+---------
        CACCGGTGTCAGTAGGAGCCTATGAAAGCTGCCGAGGTGACGTGGTCCTTGATGTAGGTG
```

FIGURE 2A
(PTH1R A.A. SEQ.)

```
a 161 V  A  T  V  I  L  G  Y  F |R  R  L  H  C  T  R  N  Y |I  H
      ATGCACCTGTTTCTATCGTTCATGTTGAGGGCCATTAGTATCTTCGTGAAGGATGTGGTG      TM2
      +---------+---------+---------+---------+---------+---------
      TACGTGGACAAAGATAGCAAGTACAACTCCCGGTAATCATAGAAGCACTTCCTACACCAC

a 181 M  H  L  F  L  S  F  M  L  R  A  I  S  I  F  V  K |D  V  V
      CTGTACTCTGGTTCGGCGCTGCAGGAAATGGAACGAATCACTGTGGAGGATCTCAAATCC
      +---------+---------+---------+---------+---------+---------
      GACATGAGACCAAGCCGCGACGTCCTTTACCTTGCTTAGTGACACCTCCTAGAGTTTAGG

a 201 L  Y  S  G  S  A  L  Q  E  M  E  R  I  T  V  E  D  L  K  S
      ATCACTGAAGCCCCTCCTGCCAACAAAACCCAGTTTATCGGCTGTAAGGTGGCGGTGACG
      +---------+---------+---------+---------+---------+---------
      TAGTGACTTCGGGGAGGACGGTTGTTTTGGGTCAAATAGCCGACATTCCACCGCCACTGC

a 220 I  T  E  A  P  P  A  N  K  T  Q  F  I  G  C  K |V  A  V  T
      CTCTTCTTGTACTTCTTGGCCACTAATTATTACTGGATTCTGGTGGAAGGCCTGTACCTG      TM3
      +---------+---------+---------+---------+---------+---------
      GAGAAGAACATGAAGAACCGGTGATTAATAATGACCTAAGACCACCTTCCGGACATGGAC

a 241 L  F  L  Y  F  L  A  T  N  Y  Y  W  I  L  V  E  G  L  Y  L
      CACAGCCTTATCTTCATGACCTTCTTCTCAGACAGGAAGTACCTCTGGGGCTTCACTCTG
      +---------+---------+---------+---------+---------+---------
      GTGTCGGAATAGAAGTACTGGAAGAAGAGTCTGTCCTTCATGGAGACCCCGAAGTGAGAC

a 261 H  S |L  I  F  M  T  F  F  S  D  R  K  Y  L  W |G  F  T  L
      ATTGGTTGGGGTGTTCCTGCGATGTTTGTCACCATCTGGGCGAGTGTTAGAGCCACACTT      TM4
      +---------+---------+---------+---------+---------+---------
      TAACCAACCCCACAAGGACGCTACAAACAGTGGTAGACCCGCTCACAATCTCGGTGTGAA

a 281 I  G  W  G  V  P  A  M  F  V  T  I  W  A  S  V |R  A  T  L
      GCTGACACTGAGTGCTGGGATTTGAGTGCAGGAAACCTGAAATGGATTGTGCAGATCCCC
      +---------+---------+---------+---------+---------+---------
      CGACTGTGACTCACGACCCTAAACTCACGTCCTTTGGACTTTACCTAACACGTCTAGGGG

a 301 A  D  T  E  C  W  D  L  S  A  G  N  L  K  W  I  V  Q |I  P
      ATTCTTACTGCAATTGTTGTCAATTTTTTGTTGTTCCTGAATATAATTCGAGTCTTGGCA      TM5
      +---------+---------+---------+---------+---------+---------
      TAAGAATGACGTTAACAACAGTTAAAAAACAACAAGGACTTATATTAAGCTCAGAACCGT

a 321 I  L  T  A  I  V  V  N  F  L  L  F  L  N  I  I  R  V  L |A
      ACAAAACTTCGAGAAACAAATGCGGGCAGATGTGACACCAGACAACAATATAGGAAGCTG
      +---------+---------+---------+---------+---------+---------
```

# FIGURE 2A
(PTH1R AA SEQ.)

```
       TGTTTTGAAGCTCTTTGTTTACGCCCGTCTACACTGTGGTCTGTTGTTATATCCTTCGAC

a 341  T  K  L  R  E  T  N  A  G  R  C  D  T  R  Q  Q  Y  R  K  L

       CTGAAGTCGACTCTGGTCCTCATGCCGTTGTTCGGTGTTCACTACATAGTCTTCATGGCG
       +---------+---------+---------+---------+---------+---------
       GACTTCAGCTGAGACCAGGAGTACGGCAACAAGCCACAAGTGATGTATCAGAAGTACCGC

a 361  L  K  S  T |L  V  L  M  P  L  F  G  V  H  Y  I  V  F  M  A|      TM6

       ATGCCTTACACAGAAGTTTCTGGAGTACTGTGGCAAATCCAGATGCATTATGAAATGCTC
       +---------+---------+---------+---------+---------+---------
       TACGGAATGTGTCTTCAAAGACCTCATGACACCGTTTAGGTCTACGTAATACTTTACGAG

a 381  M  P  Y  T  E  V  S  G  V  L  W  Q  I  Q  M  H  Y  E |M  L

       TTTAACTCAGTCCAGGGATTCTTTGTTGCCATTATATATTGCTTCTGCAACGGAGAGGTC      TM7
       +---------+---------+---------+---------+---------+---------
       AAATTGAGTCAGGTCCCTAAGAAACAACGGTAATATATAACGAAGACGTTGCCTCTCCAG

a 401  F  N  S  V  Q  G  F  F  V  A  I  I  Y  C  F  C| N  G  E  V

       CAAGCGGAAATCAAGAAGGCCTGGAACAGAAGGACTCTTGCTCTGGACTTCAAGAGAAAA
       +---------+---------+---------+---------+---------+---------
       GTTCGCCTTTAGTTCTTCCGGACCTTGTCTTCCTGAGAACGAGACCTGAAGTTCTCTTTT

a 421  Q  A  E  I  K  K  A  W  N  R  R  T  L  A  L  D  F  K  R  K

       GCCAGGAGCGGCAGTAACACATACAGCTATGGACCCATGGTTTCTCACACCAGTGTTACC
       +---------+---------+---------+---------+---------+---------
       CGGTCCTCGCCGTCATTGTGTATGTCGATACCTGGGTACCAAAGAGTGTGGTCACAATGG

a 441  A  R  S  G  S  N  T  Y  S  Y  G  P  M  V  S  H  T  S  V  T

       AATGTGACGGCGCGGGGCCCGCTGGCCCTTCACCTCACCAACCGACTGGGGCACGTCACC
       +---------+---------+---------+---------+---------+---------
       TTACACTGCCGCGCCCCGGGCGACCGGGAAGTGGAGTGGTTGGCTGACCCCGTGCAGTGG

a 461  N  V  T  A  R  G  P  L  A  L  H  L  T  N  R  L  G  H  V  T

       ACTAACGGCCACAGAAACCTTCCGGGATACATAAAAAACGGCTCCGTTTCAGAAAACTCC
       +---------+---------+---------+---------+---------+---------
       TGATTGCCGGTGTCTTTGGAAGGCCCTATGTATTTTTTGCCGAGGCAAAGTCTTTTGAGG

a 481  T  N  G  H  R  N  L  P  G  Y  I  K  N  G  S  V  S  E  N  S

       ATCCCGTCCTCGGGTCACGAGCTTCACATTCAGGAGGAAGAGCCTTCGAAGACCTTCCAG
       +---------+---------+---------+---------+---------+---------
       TAGGGCAGGAGCCCAGTGCTCGAAGTGTAAGTCCTCCTTCTCGGAAGCTTCTGGAAGGTC

a 501  I  P  S  S  G  H  E  L  H  I  Q  E  E  E  P  S  K  T  F  Q

       ATGGAGAAAACCATCCAGGTGGTGGAGGAGGAAAGAGAAACCGTCATGT
```

FIGURE 2A
(PTHIR A.A. SEQ.)

```
+---------+---------+---------+---------+--------
TACCTCTTTTGGTAGGTCCACCACCTCCTCCTTTCTCTTTGGCAGTACA

a 521 M E K T I Q V V E E E R E T V M -   (SEQ ID NO:2)
```

# FIGURE 2B
(PTH3R A.A SEQ.)

```
        ATGGTGTCAGTGGAGGTCTCTGTGGCTTTAGTGCTGTGCTGTGTTTTGATGGGAGCCAGA
        ---+---------+---------+---------+---------+---------+------
        TACCACAGTCACCTCCAGAGACACCGAAATCACGACACGACACAAAACTACCCTCGGTCT

a   1   M  V  S  V  E  V  S  V  A  L  V  L  C  C  V  L  M  G  A  R      signal peptide

        GCTCTGATTGATTCAGATGATGTCATCACAAGAGATGAACAGATCTTTCTCCTCATTGGT
        ---+---------+---------+---------+---------+---------+------
        CGAGACTAACTAAGTCTACTACAGTAGTGTTCTCTACTTGTCTAGAAAGAGGAGTAACCA

a  21   A |L  I  D  S  D  D  V  I  T  R  D  E  Q  I  F  L  L  I  G

        GCGCGGTCGAGGTGTGAGAGAACCATCCGTGCACAGTCAGACGTGGTCAGAGAGAATAAC
        ---+---------+---------+---------+---------+---------+------
        CGCGCCAGCTCCACACTCTCTTGGTAGGCACGTGTCAGTCTGCACCAGTCTCTCTTATTG

a  41   A  R  S  R  C  E  R  T  I  R  A  Q  S  D  V  V  R  E  N  N

        TGCGCTCCTGAGTGGGATGGGATCATTTGCTGGCCCACAGGAAAACCCAATCAGATGGTG
        ---+---------+---------+---------+---------+---------+------
        ACGCGAGGACTCACCCTACCCTAGTAAACGACCGGGTGTCCTTTTGGGTTAGTCTACCAC

a  61   C  A  P  E  W  D  G  I  I  C  W  P  T  G  K  P  N  Q  M  V

        GCAGTTCTGTGTCCTGAGTACATCTATGACTTCAACCACAGAGGATACGCGTATCGACAC
        ---+---------+---------+---------+---------+---------+------
        CGTCAAGACACAGGACTCATGTAGATACTGAAGTTGGTGTCTCCTATGCGCATAGCTGTG

a  81   A  V  L  C  P  E  Y  I  Y  D  F  N  H  R  G  Y  A  Y  R  H

        TGTGATGCATCAGGTAACTGGGAGCAGGTGTCCATTATAAACCGGACGTGGGCAAACTAC
        ---+---------+---------+---------+---------+---------+------
        ACACTACGTAGTCCATTGACCCTCGTCCACAGGTAATATTTGGCCTGCACCCGTTTGATG

a 101   C  D  A  S  G  N  W  E  Q  V  S  I  I  N  R  T  W  A  N  Y

        ACGGAATGCACCACTTACCTGCACACCAACCACAGTGATCAGGAGGAAGTGTTTGAGCGC
        ---+---------+---------+---------+---------+---------+------
        TGCCTTACGTGGTGAATGGACGTGTGGTTGGTGTCACTAGTCCTCCTTCACAAACTCGCG

a 121   T  E  C  T  T  Y  L  H  T  N  H  S  D  Q  E  E  V  F  E  R

        CTTTACCTCATGTACACTATTGGATACTCCATATCACTGGCAGCGTTACTGGTGGCGGTC
        ---+---------+---------+---------+---------+---------+------
        GAAATGGAGTACATGTGATAACCTATGAGGTATAGTGACCGTCGCAATGACCACCGCCAG

a 141   L  Y  L  M  Y |T  I  G  Y  S  I  S  L  A  A  L  L  V  A  V      TM 1

        TCTATCCTTTGCTATTTCAAACGTCTCCACTGCACTCGTAACTACATCCACATCCACCTC
        ---+---------+---------+---------+---------+---------+------
        AGATAGGAAACGATAAAGTTTGCAGAGGTGACGTGAGCATTGATGTAGGTGTAGGTGGAG
```

# FIGURE 2B (PTH3R A.A. SEQ.)

```
a  161 S  I  L  C  Y  F |K  R  L  H  C  T  R  N  Y  I  H |I  H  L
       TTCACCTCGTTCATATGTCGAGCAATCAGTATTTTTGTGAAAGACGCCGTTCTTTACGCC
       ---+---------+---------+---------+---------+---------+------        TM2
       AAGTGGAGCAAGTATACAGCTCGTTAGTCATAAAAACACTTTCTGCGGCAAGAAATGCGG

a  181 F  T  S  F  I  C  R  A  I  S  I  F  V  K |D  A  V  L  Y  A
       GTCACGAATGATGGAGAACTAGAAGATGGGGCAGTGGAACAAAGACCCATGGTGGGCTGC
       ---+---------+---------+---------+---------+---------+------
       CAGTGCTTACTACCTCTTGATCTTCTACCCCGTCACCTTGTTTCTGGGTACCACCCGACG

a  201 V  T  N  D  G  E  L  E  D  G  A  V  E  Q  R  P  M  V  G  C
       AAGGCTGCTGTGACCCTCTTCCTGTATCTGTTGGCGACCAATCATTATTGGATCCTGGTG
       ---+---------+---------+---------+---------+---------+------
       TTCCGACGACACTGGGAGAAGGACATAGACAACCGCTGGTTAGTAATAACCTAGGACCAC

a  221 K |A  A  V  T  L  F  L  Y  L  L  A  T  N  H  Y  W  I  L  V         TM3
       GAGGGTTTGTACTTGCATAGTCTGATCTTCATGGCCTTCCTGTCTGATAAGAACTGCCTG
       ---+---------+---------+---------+---------+---------+------
       CTCCCAAACATGAACGTATCAGACTAGAAGTACCGGAAGGACAGACTATTCTTGACGGAC

a  241 E  G  L  Y  L  H  S |L  I  F  M  A  F  L  S  D  K  N  C  L
       TGGGCTTTGACAATCATAGGCTGGGGGATCCCAGCAGTGTTTGTGTCTATATGGGTCAGT
       ---+---------+---------+---------+---------+---------+------
       ACCCGAAACTGTTAGTATCCGACCCCCTAGGGTCGTCACAAACACAGATATACCCAGTCA

a  261 W |A  L  T  I  I  G  W  G  I  P  A  V  F  V  S  I  W  V  S         TM4
       GCCAGGGTGTCTCTGGCAGACACACAGTGCTGGGATATCAGTGCAGGCAATTTGAAATGG
       ---+---------+---------+---------+---------+---------+------
       CGGTCCCACAGAGACCGTCTGTGTGTCACGACCCTATAGTCACGTCCGTTAAACTTTACC

a  281 A |R  V  S  L  A  D  T  Q  C  W  D  I  S  A  G  N  L  K  W
       ATTTATCAAGTACCAATCCTGGCAGCCATTGTTGTAAACTTCTTCCTCTTCCTCAATATC
       ---+---------+---------+---------+---------+---------+------
       TAAATAGTTCATGGTTAGGACCGTCGGTAACAACATTTGAAGAAGGAGAAGGAGTTATAG

a  301 I  Y  Q |V  P  I  L  A  A  I  V  V  N  F  F  L  F  L  N  I
       ATCAGGGTTTTGGCCTCTAAGTTGTGGGAAACAAACACGGGAAAACTGGACCCTAGACAG
       ---+---------+---------+---------+---------+---------+------        TM5
       TAGTCCCAAAACCGGAGATTCAACACCCTTTGTTTGTGCCCTTTTGACCTGGGATCTGTC

a  321 I  R  V  L |A  S  K  L  W  E  T  N  T  G  K  L  D  P  R  Q
       CAGTACAGGAAGCTGCTGAAGTCAACAATGGTGCTGATGCCACTGTTTGGAGTTCATTAC
       ---+---------+---------+---------+---------+---------+------
```

# FIGURE 2B

## PTH3R Amino Acid sequence

```
          GTCATGTCCTTCGACGACTTCAGTTGTTACCACGACTACGGTGACAAACCTCAAGTAATG

a  341    Q  Y  R  K  L  L  K  S  T |M  V  L  M  P  L  F  G  V  H  Y  -         TN6

          ATGCTGTTCATGGCTCTTCCGTACACTGATGTGACTGGTTTGCTGTGGCAGATTCTGATG
          ---+---------+---------+---------+---------+---------+------ 1236
          TACGACAAGTACCGAGAAGGCATGTGACTACACTGACCAAACGACACCGTCTAAGACTAC

a  361    M  L  F  M  A| L  P  Y  T  D  V  T  G  L  L  W  Q  I  L  M  -

          CATTACGAGATGCTCTTCAATTCTTCACAGGGTTTCTTTGTGGCGTTTATTTACTGCTTC
          ---+---------+---------+---------+---------+---------+------ 1296
          GTAATGCTCTACGAGAAGTTAAGAAGTGTCCCAAAGAAACACCGCAAATAAATGACGAAG

a  381    H  Y  E |M  L  F  N  S  S  Q  G  F  F  V  A  F  I  Y  C  F  -         TN7

          TGCAATGGGGAGGTGCAGGCAGAGGTGAAGAAGGCCTGGTTGCGACGCAGTCTTGCGTTA
          ---+---------+---------+---------+---------+---------+------ 1356
          ACGTTACCCCTCCACGTCCGTCTCCACTTCTTCCGGACCAACGCTGCGTCAGAACGCAAT

a  401    C |N  G  E  V  Q  A  E  V  K  K  A  W  L  R  R  S  L  A  L  -

          GACCAGAAGCAGAAGGCTCGAGTCCACAGCAGTGCGGGATGTGGAAGTGGTTACTATGGA
          ---+---------+---------+---------+---------+---------+------ 1416
          CTGGTCTTCGTCTTCCGAGCTCAGGTGTCGTCACGCCCTACACCTTCACCAATGATACCT

a  421    D  Q  K  Q  K  A  R  V  H  S  S  A  G  C  G  S  G  Y  Y  G  -

          GGAATGATGTCCCACACCACCACACAGAGCGTGTGTCTCAGTGTCAGTGGTGCTAAAGGC
          ---+---------+---------+---------+---------+---------+------ 1476
          CCTTACTACAGGGTGTGGTGGTGTGTCTCGCACACAGAGTCACAGTCACCACGATTTCCG

a  441    G  M  M  S  H  T  T  T  Q  S  V  C  L  S  V  S  G  A  K  G  -

          GGTCATTCTCTGCACACCATAGGAGCCAAAGGACAATCCCGTCTACAACATTCAGGAAAC
          ---+---------+---------+---------+---------+---------+------ 1536
          CCAGTAAGAGACGTGTGGTATCCTCGGTTTCCTGTTAGGGCAGATGTTGTAAGTCCTTTG

a  461    G  H  S  L  H  T  I  G  A  K  G  Q  S  R  L  Q  H  S  G  N  -

          TTACCCGGTACGCGCCTCAGGGCGCATAGACTTTGTTTTACCCAGTGGTCCCAAAGCAGA
          ---+---------+---------+---------+---------+---------+------ 1596
          AATGGGCCATGCGCGGAGTCCCGCGTATCTGAAACAAAATGGGTCACCAGGGTTTCGTCT

a  481    L  P  G  T  R  L  R  A  H  R  L  C  F  T  Q  W  S  Q  S  R  -

          AAGAGACTCCATGCAGACAGAGCAGCAGGAATGCAGAGGAAAGCGAGCATGATTTTGAGC
          ---+---------+---------+---------+---------+---------+------ 1656
          TTCTCTGAGGTACGTCTGTCTCGTCGTCCTTACGTCTCCTTTCGCTCGTACTAAAACTCG

a  501    K  R  L  H  A  D  R  A  A  G  M  Q  R  K  A  S  M  I  L  S  -

          CATATTTCG
```

# FIGURE 2B
(PTH3R A.A. SEQ.)

521 ---+------
GTATAAAGC

a H I S - (SEQ ID NO:4)

```
1        Signal Peptide                                                                                          100
zPTH3R  M VSVEVSV    ALVLCC VLMGARA                                         LIDSDDVITRDEQIFLLIGARSRCERTIR
zPTH1R  M GATLIVRTLGF   LFCGTLLSFVYG                                        LVDADDVLTKEEQIYLLFNAKRKCERAIK
zPTH2R  M  LTVSL       LILCKP SSSPSPVKIIPVDDLPATAELRASVLRVSLPKTFIKSFLNHLLQAGEDGEITAEEQVQMLLDAKLQC  LQKVSSDDPA

101                                                                        #    #                                    200
zPTH3R  AQSDVVRENNCAPEWDGIICWPTGKPNQMVAVLCPEYIYDFNHRGYAYRHCDASGNWEQVSIINRTWANYTECTTYL HTNHSDQEEVFERLYLMYTIGY
zPTH1R   SKHKTSEGSCLPEWDGILCWPEGVPGKMVSTSCPEYIYDFNHKGHAYRRCDLNGTWELASHNNKTWANYSECAKFFPHYNQNQEREVFDRLYLIYTVGY
zPTH2R           VGVCVPEWDGLICWPQGFPGTLTKTPCPGYIYDFNHAAHAYRRCDSNGSSVLAESSNKTWVNYTECIK  SPEPNKKRQVFFERLHIMYTVGY

201                                                                                                              300
zPTH3R  SISLAALLVAVSILCYFKRLHCTRNYIHIHLFTSFIICRAISIFVKDAVLYAVTNDGELEDGAVEQRP              MVGCKAAVTLFLYILATN
zPTH1R  SISLGSLMVATVILGYFRRLHCTRNYIHMHLFLSFMLRAISIFVKDVVLYSGSALQEMERITVEDLKSITEA    PPANKTQFIGCKVAVTLFLYFLATN
zPTH2R  AVSFSSLLVAIFIIGYFRRLHCTRNYIHMHLFVSFMLRAASIFVKDHVVHTSAGLQESDAVLM N  -NFTNAVDVAPVDTSQYMGCKVTVLLFIYFLATN

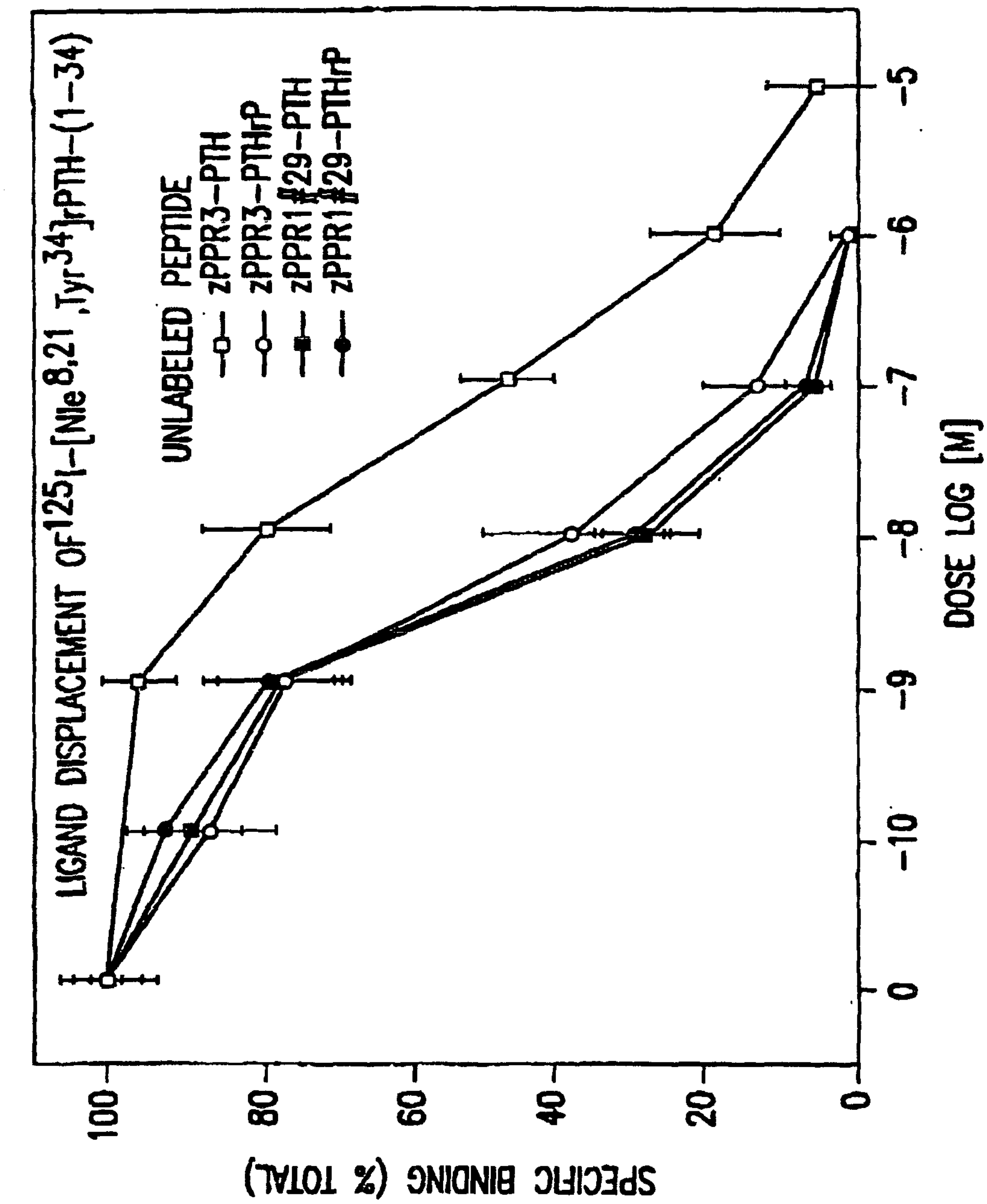
301                                                                                                              400
zPTH3R  HYWILVEGLYLHSLIFMAFLSDKNCLWALTIIGWGIPAVFVSIWVSARVSLADTQCWDISAGNLKWIYQVPILAAIVVNFFLFLNIIRVLASKLWETNTG
zPTH1R  YYWILVEGLYLHSLIFMTFFSDRKYLWGFTLIGWGVPAMFVTIWASVRATLADTECWDLSAGNLKWIVQIPILTAIVVNFLLFLNIIRVLATKLRETNAG
zPTH2R  YYWILVEGLYLHSLIFMAFLSDSKYLWGFTLIGWGVPAVFVAAWAVVRATLADARCWELSAGNIKWIYQEPILTAIGLNFILFVNIVRVLATKIRETNGG

401                                                                                                              500
zPTH3R  KLDPRQQYRKLLKSTMVLMPLFGVHYMLFMALPYTDVTGLLWQILMHYEMLFNSSQGFFVAFIYCFCNGEVQAEVKKAWLRRSLALDQKQKARVHSSAGC
zPTH1R  RCDTRQQYRKLLKSTLVLMPLFGVHYIVFMAMPYTEVSGVLWQIQMHYEMLFNSVQGFFVAIIYCFCNGEVQAEIKKAWNRRTLALDFKRKARSGSNT Y
zPTH2R  RYDTRKQYRKLAKSTQVLVFVFGVHYIVFVGMPHT FEGLGWEERMYCELFFNSFQGFFVSIIYCYCNGEVQTEIKKTWTRWNLAFDWKGPVVCGSNR Y

501                                                                                                              600
zPTH3R  CSGYYGGMMSHTTTQSVC  LSVSG AKGGHSLHTIGAKGQSRLQHSGNLPGTRLRAHRLCFT QWSQSRKRLHA DRAAGMQRKASM  ILSHIS
zPTH1R  S   YGPMVSHTSVTNV    TARG PLALHLTNRLGHV   TTNGHRNLPGYIKNGSVS ENSIPSSGHE LHIQEEEPSKT         FQMEKTI  Q
zPTH2R  GSVLTGLNNS TSSQSQLAAGGPGTRSTTLFSSRVYRSSGGPTVSTHATLPGYVL    NSD    ADSLPP     SIPEEP EDSAKQVDDILLKESLPTR

601
zPTH3R                    (SEQ ID NO:4)
zPTH1R  VVEEERETVM        (SEQ ID NO:2)
zPTH2R  PSSGLEDDEETL      (SEQ ID NO:5)
```

FIG.3



LIGAND DISPLACEMENT OF 125I-[Nle8,21,Tyr34]rPTH-(1-34)
UNLABELED PEPTIDE
zPPR3-PTH
zPPR3-PTHrP
zPPR1#29-PTH
zPPR1#29-PTHrP
SPECIFIC BINDING (% TOTAL)
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
-5
DOSE LOG [M]


FIG. 4A

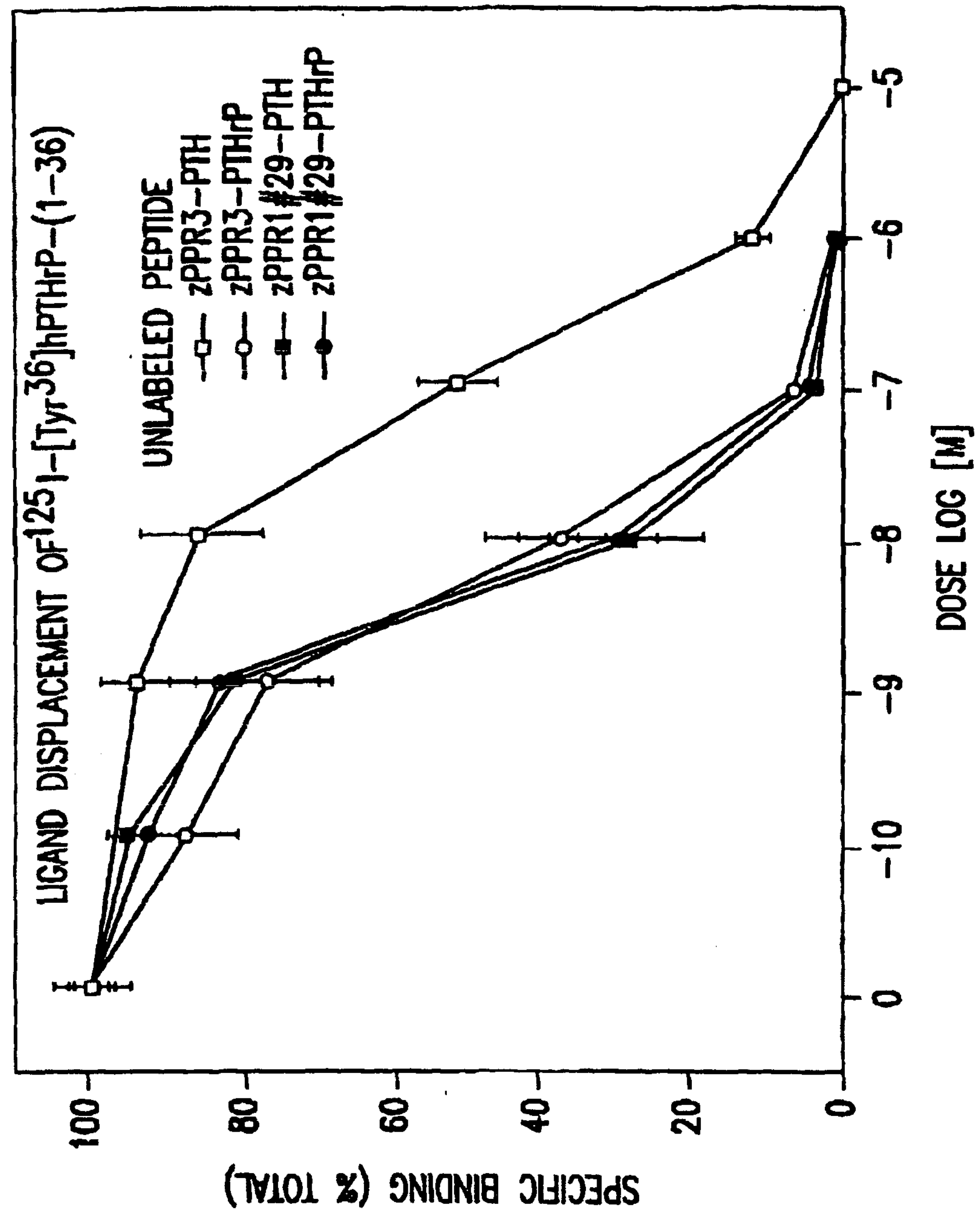

LIGAND DISPLACEMENT OF 125I-[Tyr36]hPTHrP-(1-36)
UNLABELED PEPTIDE
zPPR3-PTH
zPPR3-PTHrP
zPPR1#29-PTH
zPPR1#29-PTHrP
SPECIFIC BINDING (% TOTAL)
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
-5
DOSE LOG [M]

FIG. 4B

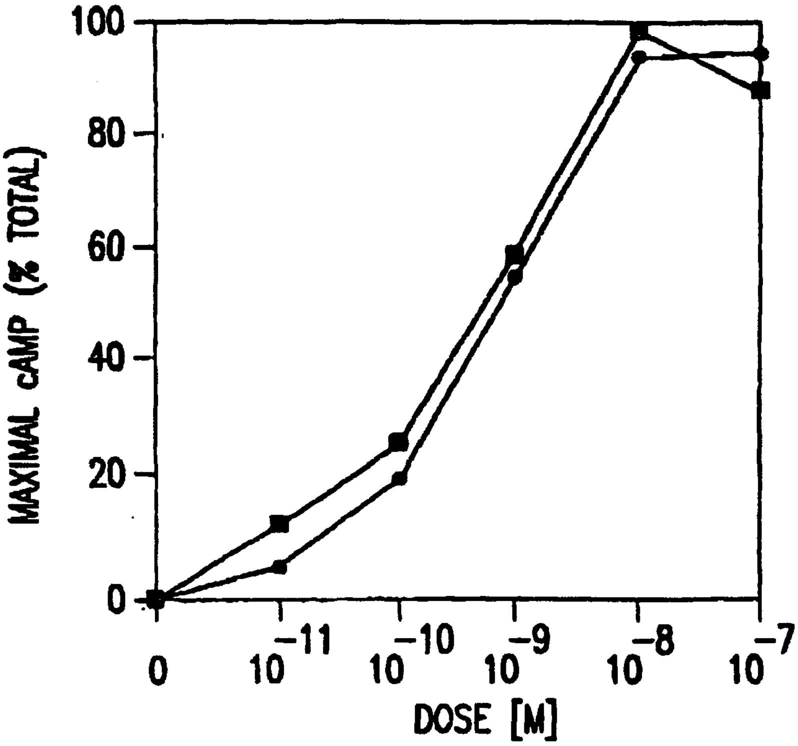

100
80
60
40
20
0
MAXIMAL cAMP (% TOTAL)
0
$10^{-11}$
$10^{-10}$
$10^{-9}$
$10^{-8}$
$10^{-7}$
DOSE [M]

FIG. 4C

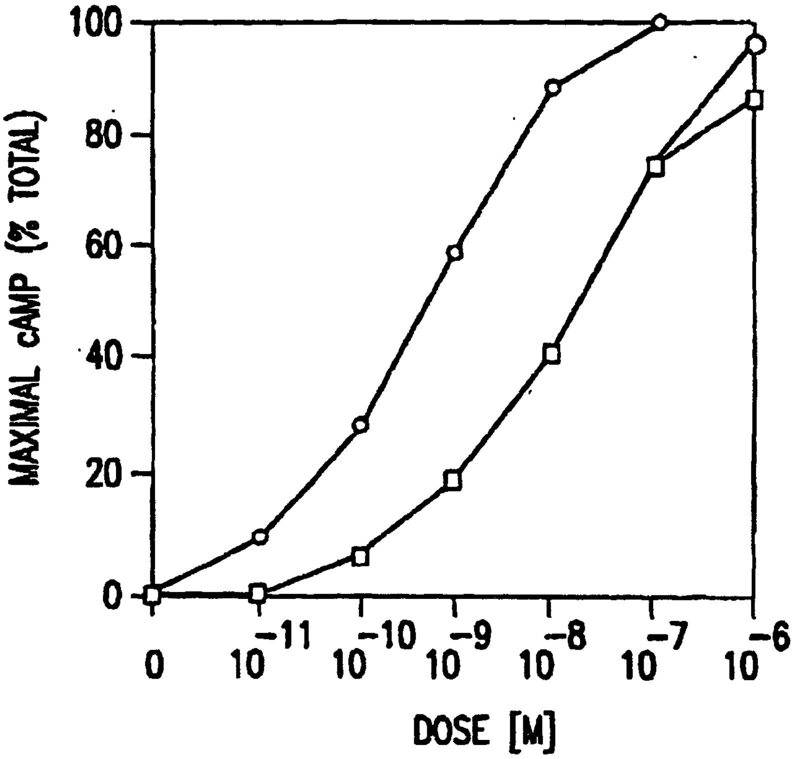

100
80
60
40
20
0
MAXIMAL cAMP (% TOTAL)
0
$10^{-11}$
$10^{-10}$
$10^{-9}$
$10^{-8}$
$10^{-7}$
$10^{-6}$
DOSE [M]

FIG. 4D

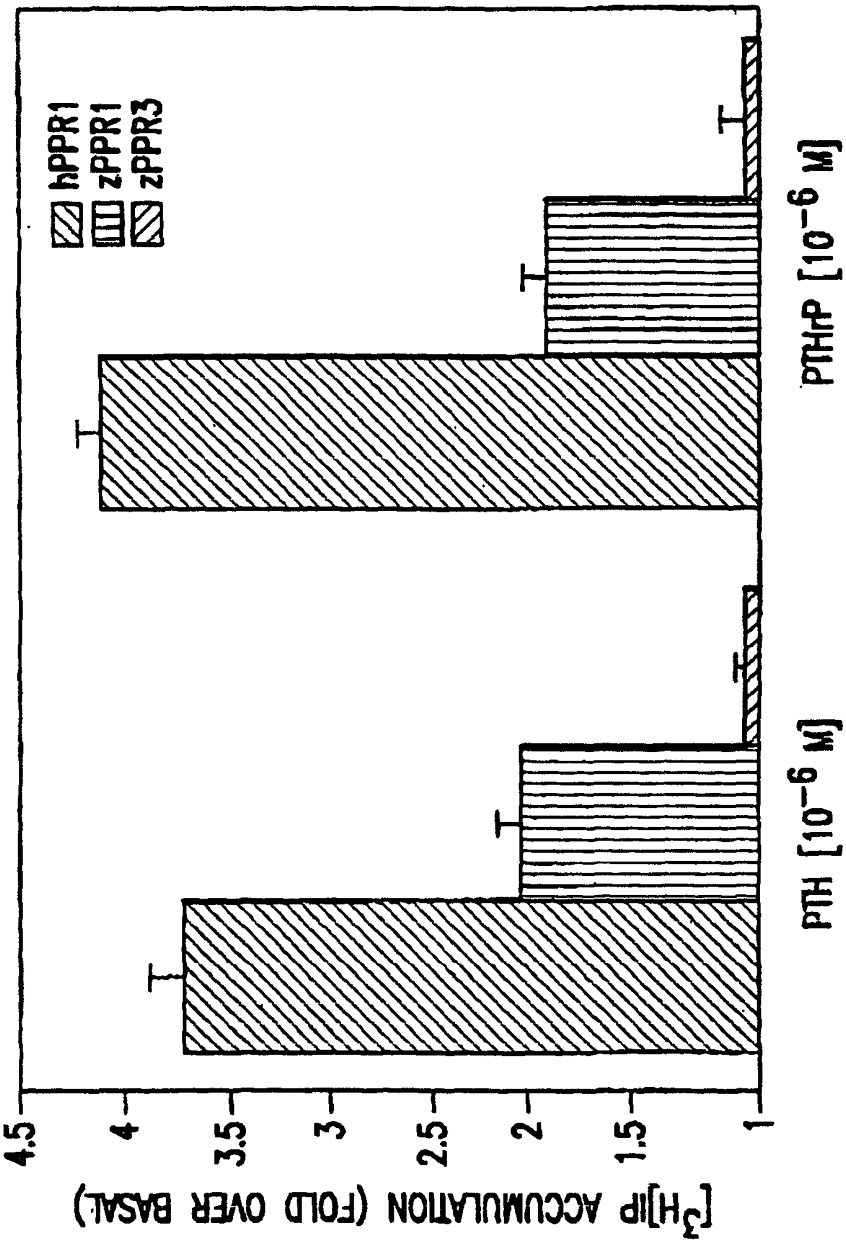
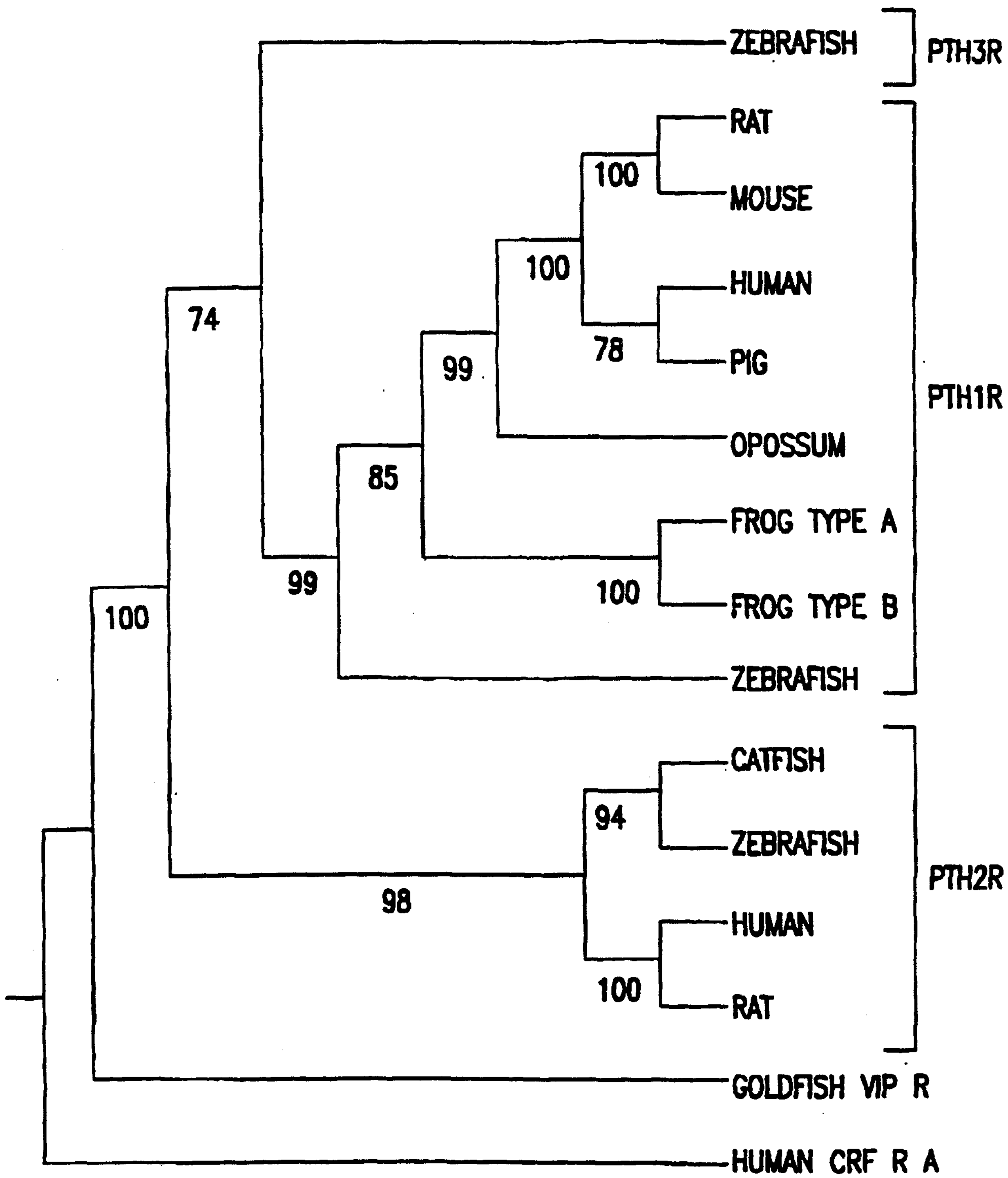

hPPR1
zPPR1
zPPR3
PTH [$10^{-6}$ M]
PTHrP [$10^{-6}$ M]
4.5
4
3.5
3
2.5
2
1.5
1
[$^3$H]IP ACCUMULATION (FOLD OVER BASAL)

FIG. 5


ZEBRAFISH
PTH3R
RAT
100
MOUSE
100
HUMAN
74
78
PIG
99
PTH1R
OPOSSUM
85
FROG TYPE A
99
100
FROG TYPE B
100
ZEBRAFISH
CATFISH
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ZEBRAFISH
PTH2R
98
HUMAN
100
RAT
GOLDFISH VIP R
HUMAN CRF R A

FIG. 6

SEQUENCE LISTING

<110> Jueppner, Harald
Rubin, David A.

<120> PTH1R and PTH3R Receptors, Methods and Uses Thereof

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Thr Leu Leu Ser Phe Val Tyr Gly Leu Val Asp Ala Asp Asp Val Leu
20 25 30

aca aag gag gag caa atc tat ctt ctg ttc aac gca aaa cga aaa tgt 144
Thr Lys Glu Glu Gln Ile Tyr Leu Leu Phe Asn Ala Lys Arg Lys Cys
35 40 45

gag cga gca atc aag tcc aag cat aaa acg tct gag gga tcc tgt ctg 192
Glu Arg Ala Ile Lys Ser Lys His Lys Thr Ser Glu Gly Ser Cys Leu
50 55 60

cca gag tgg gat ggc atc cta tgt tgg ccc gag gga gtt cct gga aag 240
Pro Glu Trp Asp Gly Ile Leu Cys Trp Pro Glu Gly Val Pro Gly Lys
65 70 75 80

atg gtg tcc act tca tgc cca gag tac ata tat gac ttc aac cac aaa 288
Met Val Ser Thr Ser Cys Pro Glu Tyr Ile Tyr Asp Phe Asn His Lys

85 90 95

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ggt cat gcc tac cgg cgc tgc gac ctg aac ggg acc tgg gaa ctg gcc   336
Gly His Ala Tyr Arg Arg Cys Asp Leu Asn Gly Thr Trp Glu Leu Ala
            100                 105                 110

tca cat aac aac aaa acc tgg gct aat tac agc gaa tgt gcc aaa ttc   384
Ser His Asn Asn Lys Thr Trp Ala Asn Tyr Ser Glu Cys Ala Lys Phe
        115                 120                 125

ttc ccc cat tat aac cag aac cag gag agg gag gtt ttc gac aga ctt   432
Phe Pro His Tyr Asn Gln Asn Gln Glu Arg Glu Val Phe Asp Arg Leu
    130                 135                 140

tac ctg atc tac aca gtg ggc tac tcc atc tct ctg gga tca ctt atg   480
Tyr Leu Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser Leu Met
145                 150                 155                 160

gtg gcc aca gtc atc ctc gga tac ttt cga cgg ctc cac tgc acc agg   528
Val Ala Thr Val Ile Leu Gly Tyr Phe Arg Arg Leu His Cys Thr Arg
                165                 170                 175

aac tac atc cac atg cac ctg ttt cta tcg ttc atg ttg agg gcc att   576
Asn Tyr Ile His Met His Leu Phe Leu Ser Phe Met Leu Arg Ala Ile
            180                 185                 190

agt atc ttc gtg aag gat gtg gtg ctg tac tct ggt tcg gcg ctg cag   624
Ser Ile Phe Val Lys Asp Val Val Leu Tyr Ser Gly Ser Ala Leu Gln
        195                 200                 205

gaa atg gaa cga atc act gtg gag gat ctc aaa tcc atc act gaa gcc   672
Glu Met Glu Arg Ile Thr Val Glu Asp Leu Lys Ser Ile Thr Glu Ala
    210                 215                 220

cct cct gcc aac aaa acc cag ttt atc ggc tgt aag gtg gcg gtg acg   720
Pro Pro Ala Asn Lys Thr Gln Phe Ile Gly Cys Lys Val Ala Val Thr
225                 230                 235                 240

ctc ttc ttg tac ttc ttg gcc act aat tat tac tgg att ctg gtg gaa   768
Leu Phe Leu Tyr Phe Leu Ala Thr Asn Tyr Tyr Trp Ile Leu Val Glu
                245                 250                 255

ggc ctg tac ctg cac agc ctt atc ttc atg acc ttc ttc tca gac agg   816
Gly Leu Tyr Leu His Ser Leu Ile Phe Met Thr Phe Phe Ser Asp Arg
            260                 265                 270

aag tac ctc tgg ggc ttc act ctg att ggt tgg ggt gtt cct gcg atg   864
Lys Tyr Leu Trp Gly Phe Thr Leu Ile Gly Trp Gly Val Pro Ala Met
        275                 280                 285

ttt gtc acc atc tgg gcg agt gtt aga gcc aca ctt gct gac act gag   912
Phe Val Thr Ile Trp Ala Ser Val Arg Ala Thr Leu Ala Asp Thr Glu
```

290 295 300

tgc tgg gat ttg agt gca gga aac ctg aaa tgg att gtg cag atc ccc 960
Cys Trp Asp Leu Ser Ala Gly Asn Leu Lys Trp Ile Val Gln Ile Pro
305 310 315 320

att ctt act gca att gtt gtc aat ttt ttg ttg ttc ctg aat ata att 1008
Ile Leu Thr Ala Ile Val Val Asn Phe Leu Leu Phe Leu Asn Ile Ile
325 330 335

cga gtc ttg gca aca aaa ctt cga gaa aca aat gcg ggc aga tgt gac 1056
Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg Cys Asp
340 345 350

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Thr Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Leu Val Leu Met
355 360 365

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Pro Leu Phe Gly Val His Tyr Ile Val Phe Met Ala Met Pro Tyr Thr
370 375 380

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385 390 395 400

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405 410 415

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420 425 430

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Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Asn Thr Tyr
435 440 445

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Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val Thr Ala
450 455 460

cgg ggg ccg ctg gcc ctt cac ctc acc aac cga ctg ggg cac gtc acc 1440
Arg Gly Pro Leu Ala Leu His Leu Thr Asn Arg Leu Gly His Val Thr
465 470 475 480

act aac ggc cac aga aac ctt ccg gga tac ata aaa aac ggc tcc gtt 1488
Thr Asn Gly His Arg Asn Leu Pro Gly Tyr Ile Lys Asn Gly Ser Val
485 490 495

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Ser Glu Asn Ser Ile Pro Ser Ser Gly His Glu Leu His Ile Gln Glu

500 505 510

gaa gag cct tcg aag acc ttc cag atg gag aaa acc atc cag gtg gtg 1584
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530 535

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35 40 45

Glu Arg Ala Ile Lys Ser Lys His Lys Thr Ser Glu Gly Ser Cys Leu
50 55 60

Pro Glu Trp Asp Gly Ile Leu Cys Trp Pro Glu Gly Val Pro Gly Lys
65 70 75 80

Met Val Ser Thr Ser Cys Pro Glu Tyr Ile Tyr Asp Phe Asn His Lys
85 90 95

Gly His Ala Tyr Arg Arg Cys Asp Leu Asn Gly Thr Trp Glu Leu Ala
100 105 110

Ser His Asn Asn Lys Thr Trp Ala Asn Tyr Ser Glu Cys Ala Lys Phe
115 120 125

Phe Pro His Tyr Asn Gln Asn Gln Glu Arg Glu Val Phe Asp Arg Leu
130 135 140

Tyr Leu Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser Leu Met
145 150 155 160

Val Ala Thr Val Ile Leu Gly Tyr Phe Arg Arg Leu His Cys Thr Arg
165 170 175

Asn Tyr Ile His Met His Leu Phe Leu Ser Phe Met Leu Arg Ala Ile
180 185 190

Ser Ile Phe Val Lys Asp Val Val Leu Tyr Ser Gly Ser Ala Leu Gln
195 200 205

Glu Met Glu Arg Ile Thr Val Glu Asp Leu Lys Ser Ile Thr Glu Ala
210 215 220

Pro Pro Ala Asn Lys Thr Gln Phe Ile Gly Cys Lys Val Ala Val Thr
225 230 235 240

Leu Phe Leu Tyr Phe Leu Ala Thr Asn Tyr Tyr Trp Ile Leu Val Glu
245 250 255

Gly Leu Tyr Leu His Ser Leu Ile Phe Met Thr Phe Phe Ser Asp Arg
260 265 270

Lys Tyr Leu Trp Gly Phe Thr Leu Ile Gly Trp Gly Val Pro Ala Met
275 280 285

Phe Val Thr Ile Trp Ala Ser Val Arg Ala Thr Leu Ala Asp Thr Glu
290 295 300

Cys Trp Asp Leu Ser Ala Gly Asn Leu Lys Trp Ile Val Gln Ile Pro
305 310 315 320

Ile Leu Thr Ala Ile Val Val Asn Phe Leu Leu Phe Leu Asn Ile Ile
325 330 335

Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg Cys Asp
340 345 350

Thr Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Leu Val Leu Met
355 360 365

Pro Leu Phe Gly Val His Tyr Ile Val Phe Met Ala Met Pro Tyr Thr
370 375 380

Glu Val Ser Gly Val Leu Trp Gln Ile Gln Met His Tyr Glu Met Leu
385 390 395 400

Phe Asn Ser Val Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys Phe Cys
405 410 415

Asn Gly Glu Val Gln Ala Glu Ile Lys Lys Ala Trp Asn Arg Arg Thr
420 425 430

Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Asn Thr Tyr
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Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val Thr Ala
450 455 460

Arg Gly Pro Leu Ala Leu His Leu Thr Asn Arg Leu Gly His Val Thr
465 470 475 480

Thr Asn Gly His Arg Asn Leu Pro Gly Tyr Ile Lys Asn Gly Ser Val
485 490 495

Ser Glu Asn Ser Ile Pro Ser Ser Gly His Glu Leu His Ile Gln Glu
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Glu Glu Pro Ser Lys Thr Phe Gln Met Glu Lys Thr Ile Gln Val Val
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atg gga gcc aga gct ctg att gat tca gat gat gtc atc aca aga gat 96
Met Gly Ala Arg Ala Leu Ile Asp Ser Asp Asp Val Ile Thr Arg Asp
20 25 30

gaa cag atc ttt ctc ctc att ggt gcg cgg tcg agg tgt gag aga acc 144
Glu Gln Ile Phe Leu Leu Ile Gly Ala Arg Ser Arg Cys Glu Arg Thr
35 40 45

atc cgt gca cag tca gac gtg gtc aga gag aat aac tgc gct cct gag 192
Ile Arg Ala Gln Ser Asp Val Val Arg Glu Asn Asn Cys Ala Pro Glu
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tgg gat ggg atc att tgc tgg ccc aca gga aaa ccc aat cag atg gtg 240
Trp Asp Gly Ile Ile Cys Trp Pro Thr Gly Lys Pro Asn Gln Met Val
65 70 75 80

gca gtt ctg tgt cct gag tac atc tat gac ttc aac cac aga gga tac 288
Ala Val Leu Cys Pro Glu Tyr Ile Tyr Asp Phe Asn His Arg Gly Tyr
85 90 95

gcg tat cga cac tgt gat gca tca ggt aac tgg gag cag gtg tcc att 336
Ala Tyr Arg His Cys Asp Ala Ser Gly Asn Trp Glu Gln Val Ser Ile

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ata aac cgg acg tgg gca aac tac acg gaa tgc acc act tac ctg cac    384
Ile Asn Arg Thr Trp Ala Asn Tyr Thr Glu Cys Thr Thr Tyr Leu His
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acc aac cac agt gat cag gag gaa gtg ttt gag cgc ctt tac ctc atg    432
Thr Asn His Ser Asp Gln Glu Glu Val Phe Glu Arg Leu Tyr Leu Met
    130                 135                 140

tac act att gga tac tcc ata tca ctg gca gcg tta ctg gtg gcg gtc    480
Tyr Thr Ile Gly Tyr Ser Ile Ser Leu Ala Ala Leu Leu Val Ala Val
145                 150                 155                 160

tct atc ctt tgc tat ttc aaa cgt ctc cac tgc act cgt aac tac atc    528
Ser Ile Leu Cys Tyr Phe Lys Arg Leu His Cys Thr Arg Asn Tyr Ile
                165                 170                 175

cac atc cac ctc ttc acc tcg ttc ata tgt cga gca atc agt att ttt    576
His Ile His Leu Phe Thr Ser Phe Ile Cys Arg Ala Ile Ser Ile Phe
            180                 185                 190

gtg aaa gac gcc gtt ctt tac gcc gtc acg aat gat gga gaa cta gaa    624
Val Lys Asp Ala Val Leu Tyr Ala Val Thr Asn Asp Gly Glu Leu Glu
        195                 200                 205

gat ggg gca gtg gaa caa aga ccc atg gtg ggc tgc aag gct gct gtg    672
Asp Gly Ala Val Glu Gln Arg Pro Met Val Gly Cys Lys Ala Ala Val
    210                 215                 220

acc ctc ttc ctg tat ctg ttg gcg acc aat cat tat tgg atc ctg gtg    720
Thr Leu Phe Leu Tyr Leu Leu Ala Thr Asn His Tyr Trp Ile Leu Val
225                 230                 235                 240

gag ggt ttg tac ttg cat agt ctg atc ttc atg gcc ttc ctg tct gat    768
Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Leu Ser Asp
                245                 250                 255

aag aac tgc ctg tgg gct ttg aca atc ata ggc tgg ggg atc cca gca    816
Lys Asn Cys Leu Trp Ala Leu Thr Ile Ile Gly Trp Gly Ile Pro Ala
            260                 265                 270

gtg ttt gtg tct ata tgg gtc agt gcc agg gtg tct ctg gca gac aca    864
Val Phe Val Ser Ile Trp Val Ser Ala Arg Val Ser Leu Ala Asp Thr
        275                 280                 285

cag tgc tgg gat atc agt gca ggc aat ttg aaa tgg att tat caa gta    912
Gln Cys Trp Asp Ile Ser Ala Gly Asn Leu Lys Trp Ile Tyr Gln Val
    290                 295                 300

cca atc ctg gca gcc att gtt gta aac ttc ttc ctc ttc ctc aat atc    960
Pro Ile Leu Ala Ala Ile Val Val Asn Phe Phe Leu Phe Leu Asn Ile
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305 310 315 320

atc agg gtt ttg gcc tct aag ttg tgg gaa aca aac acg gga aaa ctg 1008
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325 330 335

gac cct aga cag cag tac agg aag ctg ctg aag tca aca atg gtg ctg 1056
Asp Pro Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Met Val Leu
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atg cca ctg ttt gga gtt cat tac atg ctg ttc atg gct ctt ccg tac 1104
Met Pro Leu Phe Gly Val His Tyr Met Leu Phe Met Ala Leu Pro Tyr
355 360 365

act gat gtg act ggt ttg ctg tgg cag att ctg atg cat tac gag atg 1152
Thr Asp Val Thr Gly Leu Leu Trp Gln Ile Leu Met His Tyr Glu Met
370 375 380

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Leu Phe Asn Ser Ser Gln Gly Phe Phe Val Ala Phe Ile Tyr Cys Phe
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tgc aat ggg gag gtg cag gca gag gtg aag aag gcc tgg ttg cga cgc 1248
Cys Asn Gly Glu Val Gln Ala Glu Val Lys Lys Ala Trp Leu Arg Arg
405 410 415

agt ctt gcg tta gac cag aag cag aag gct cga gtc cac agc agt gcg 1296
Ser Leu Ala Leu Asp Gln Lys Gln Lys Ala Arg Val His Ser Ser Ala
420 425 430

gga tgt gga agt ggt tac tat gga gga atg atg tcc cac acc acc aca 1344
Gly Cys Gly Ser Gly Tyr Tyr Gly Gly Met Met Ser His Thr Thr Thr
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cag agc gtg tgt ctc agt gtc agt ggt gct aaa ggc ggt cat tct ctg 1392
Gln Ser Val Cys Leu Ser Val Ser Gly Ala Lys Gly Gly His Ser Leu
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cac acc ata gga gcc aaa gga caa tcc cgt cta caa cat tca gga aac 1440
His Thr Ile Gly Ala Lys Gly Gln Ser Arg Leu Gln His Ser Gly Asn
465 470 475 480

tta ccc ggt acg cgc ctc agg gcg cat aga ctt tgt ttt acc cag tgg 1488
Leu Pro Gly Thr Arg Leu Arg Ala His Arg Leu Cys Phe Thr Gln Trp
485 490 495

tcc caa agc aga aag aga ctc cat gca gac aga gca gca gga atg cag 1536
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<212> PRT
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Glu Gln Ile Phe Leu Leu Ile Gly Ala Arg Ser Arg Cys Glu Arg Thr
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Ile Arg Ala Gln Ser Asp Val Val Arg Glu Asn Asn Cys Ala Pro Glu
    50                  55                  60

Trp Asp Gly Ile Ile Cys Trp Pro Thr Gly Lys Pro Asn Gln Met Val
65                  70                  75                  80

Ala Val Leu Cys Pro Glu Tyr Ile Tyr Asp Phe Asn His Arg Gly Tyr
                85                  90                  95

Ala Tyr Arg His Cys Asp Ala Ser Gly Asn Trp Glu Gln Val Ser Ile
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Ile Asn Arg Thr Trp Ala Asn Tyr Thr Glu Cys Thr Thr Tyr Leu His
        115                 120                 125

Thr Asn His Ser Asp Gln Glu Glu Val Phe Glu Arg Leu Tyr Leu Met
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Tyr Thr Ile Gly Tyr Ser Ile Ser Leu Ala Ala Leu Leu Val Ala Val
145                 150                 155                 160

Ser Ile Leu Cys Tyr Phe Lys Arg Leu His Cys Thr Arg Asn Tyr Ile
                165                 170                 175

His Ile His Leu Phe Thr Ser Phe Ile Cys Arg Ala Ile Ser Ile Phe
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Val Lys Asp Ala Val Leu Tyr Ala Val Thr Asn Asp Gly Glu Leu Glu
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Asp Gly Ala Val Glu Gln Arg Pro Met Val Gly Cys Lys Ala Ala Val
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Thr Leu Phe Leu Tyr Leu Leu Ala Thr Asn His Tyr Trp Ile Leu Val
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Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Leu Ser Asp
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Lys Asn Cys Leu Trp Ala Leu Thr Ile Ile Gly Trp Gly Ile Pro Ala
            260                 265                 270

Val Phe Val Ser Ile Trp Val Ser Ala Arg Val Ser Leu Ala Asp Thr
        275                 280                 285

Gln Cys Trp Asp Ile Ser Ala Gly Asn Leu Lys Trp Ile Tyr Gln Val
    290                 295                 300

Pro Ile Leu Ala Ala Ile Val Val Asn Phe Phe Leu Phe Leu Asn Ile
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Ile Arg Val Leu Ala Ser Lys Leu Trp Glu Thr Asn Thr Gly Lys Leu
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Asp Pro Arg Gln Gln Tyr Arg Lys Leu Leu Lys Ser Thr Met Val Leu
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Met Pro Leu Phe Gly Val His Tyr Met Leu Phe Met Ala Leu Pro Tyr
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Thr Asp Val Thr Gly Leu Leu Trp Gln Ile Leu Met His Tyr Glu Met
    370                 375                 380

Leu Phe Asn Ser Ser Gln Gly Phe Phe Val Ala Phe Ile Tyr Cys Phe
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Cys Asn Gly Glu Val Gln Ala Glu Val Lys Lys Ala Trp Leu Arg Arg
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Ser Leu Ala Leu Asp Gln Lys Gln Lys Ala Arg Val His Ser Ser Ala
            420                 425                 430

Gly Cys Gly Ser Gly Tyr Tyr Gly Gly Met Met Ser His Thr Thr Thr
        435                 440                 445

Gln Ser Val Cys Leu Ser Val Ser Gly Ala Lys Gly Gly His Ser Leu
    450                 455                 460

His Thr Ile Gly Ala Lys Gly Gln Ser Arg Leu Gln His Ser Gly Asn
465                 470                 475                 480

Leu Pro Gly Thr Arg Leu Arg Ala His Arg Leu Cys Phe Thr Gln Trp
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Arg Lys Ala Ser Met Ile Leu Ser His Ile Ser
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<220>
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<210> 17
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gcagtggaga cgtttgaaat a 21

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ccagttacct gatgcatcac agtg 24

Statement Concerning Non-Prejudicial Disclosure or Exception to Lack of Novelty

Due to a public disclosure on November 30, 1998, the applicant respectfully requests that the subject International application be granted the respective provisions under national laws concerning Exceptions to Lack of Novelty in each of the designated countries. This is not an admission that the subject invention is not novel. Exception to Lack of Novelty is hereby requested for purposes of disclosure and precautionary measures.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/11883

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12N15/12 C12N5/10 C07K14/72 C07K16/28 A61K38/17
//C07K14/635

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | JÜPPNER H ET AL.: "A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide" SCIENCE , vol. 254, 15 November 1991 (1991-11-15), pages 1024-1026, XP002115899 the whole document --- | 8,9,18, 29,30, 39,43-46 |
| Y | WO 92 17602 A (GEN HOSPITAL CORP) 15 October 1992 (1992-10-15) abstract page 3, line 17 -page 7, line 21 page 49, line 12 - line 17 claims 1-49 --- | 1-46 |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 September 1999 | 06/10/1999 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Galli, I |

Form PCT/ISA/210 (second sheet) (July 1992)

## INTERNATIONAL SEARCH REPORT

.ernational application No.
PCT/US 99/ 11883

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 20,21,41,42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-21,45,46

   An isolated nucleic acid molecule encoding the PTH1R receptor of sequence ID 2.

   Related polypeptides (Seq. ID 1), mutants, vectors, recombinant cells, antibodies and uses.

2. Claims: 22-44

   Idem as subject matter 1 but limited to PTH3R (Seq. IDs 3 and 4).

# INTERNATIONAL SEARCH REPORT

**Information on patent family members**

International Application No
PCT/US 99/11883

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
| --- | --- | --- | --- |
| WO 9217602 A | 15-10-1992 | CA 2107569 A | 06-10-1992 |
| | | EP 0579758 A | 26-01-1994 |
| | | JP 6506598 T | 28-07-1994 |
| | | US 5886148 A | 23-03-1999 |
| | | US 5840853 A | 24-11-1998 |
| | | US 5494806 A | 27-02-1996 |