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# Identical Complementary Deoxyribonucleic Acids Encode a Human Renal and Bone Parathyroid Hormone (PTH)/PTH-Related Peptide Receptor*

ERNESTINA SCHIPANI, HELEN KARGA, ANDREW C. KARAPLIS, JOHN T. POTTS, JR., HENRY M. KRONENBERG, GINO V. SEGRE, ABDUL-BADI ABOU-SAMRA, AND HARALD JÜPPNER

*Endocrine Unit, Departments of Medicine and Pediatrics, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114*

**ABSTRACT**

Identical complementary DNAs (cDNAs) that encode a 593-amino acid human PTH (PTH)/PTH-related peptide (PTHrP) receptor were isolated by hybridization techniques from two cDNA libraries which had been constructed from human kidney and human osteoblast-like osteosarcoma cells (SaOS-2). Northern blot analysis of total RNA from human bone- and kidney-derived tissue revealed one single major messenger RNA species of about 2.5 kilobases in both tissues. The human PTH/PTHrP receptor has 91% and 81% identity, respectively, with the previously cloned rat and opossum receptors, indicating a high degree of conservation among mammals. Despite this striking degree of amino-acid conservation, the human PTH/PTHrP receptor has several unique biological properties when transiently expressed in COS-7 cells. The apparent dissociation constants for [$Nle^{8,18}$,$Tyr^{34}$] bovine PTH(1–34) amide [bPTH(1–34)] are similar for the human and the rat receptor (~8 *vs.* ~15 nM) whereas [$Tyr^{36}$]PTHrP(1–36) amide has a slightly lower affinity for the human (15–40 nM) than for the rat receptor (~15 nM). Both ligands stimulate efficiently and with similar efficacy the accumulation of intracellular cAMP. The affinities for the antagonists [$Nle^{8,18}$,$Tyr^{34}$] bPTH(3–34) amide [bPTH(3–34)] and in particular for [$Nle^{8,18}$,$Tyr^{34}$] bPTH(7–34) amide [bPTH(7–34)] are considerably higher for the human receptor, *e.g.* approximately 8 nM *vs.* 30 nM for bPTH(3–34) and approximately 100 nM *vs.* 5000 nM for bPTH(7–34), respectively. Similar biological findings were previously attributed to differences in species- and/or organ-specific PTH/PTHrP receptors. The expression of the recombinant, highly homologous rat and human receptors in a uniform environment indicate that the moderate differences in the primary receptor structure have profound consequences for the receptor binding affinity of amino-terminally truncated PTH analogs. Furthermore, the molecular cloning of identical cDNAs encoding a human PTH/PTHrP receptor from the two major target organs for PTH, bone and kidney, provides strong evidence for one single PTH/PTHrP receptor in both organs, although additional and/or alternatively spliced receptors cannot be excluded. (*Endocrinology* **132:** 2157–2165, 1993)

PTH is the major regulator of calcium and phosphate metabolism. The 84-amino acid polypeptide acts through guanyl nucleotide-binding protein (G-protein)-coupled, plasma membrane receptors in bone- and kidney-derived tissue (1). The first 34 amino acids of PTH are sufficient to mediate the hormone's actions on mineral ion homeostasis, whereas the carboxyl-terminal portion of the intact PTH(1–84) may serve other, yet largely undefined functions (2–4). The PTH-related peptide (PTHrP), which causes the syndrome of humoral hypercalcemia of malignancy, shares 8 of 13 amino-terminal residues with PTH, whereas its remaining peptide sequence is distinct (5). Amino-terminally truncated PTHrP analogs that lack sequence homology with PTH are capable of binding to the PTH/PTHrP receptor, albeit with low affinity, indicating that the secondary structure of these unrelated peptides is sufficiently similar to that of correspondingly truncated PTH analogs to allow specific interaction with the same receptor (6, 7). As previously suggested, the 14–34 region must therefore contribute considerably to the proper folding of PTH and PTHrP, and thus to the overall receptor affinity of either ligand (8–10).

Considerable variation in the pharmacological properties of PTH/PTHrP receptors for various PTH and PTHrP ligands have suggested species- and/or organ-specific differences in PTH/PTHrP receptors. For example, in comparison to clonal rat osteoblast-like osteosarcoma cells (ROS 17/2.8), analogs of PTH(7–34) bind with up to 10-fold higher affinity to bovine renal membranes (11, 12). Preliminary data indicate that these analogs have an even higher affinity when using the clonal human osteosarcoma cell line B10, which is a subclone of the SaOS-2 cells used in the present study (13). Furthermore, PTHrP-activated PTH/PTHrP receptors in canine renal membranes appear to be less efficiently coupled to intracellular effector systems than renal membranes or clonal cells from other species (14–17). These data could imply that organ-specific PTH/PTHrP receptors are responsible for the observed differences in ligand binding to the PTH receptor and/or the subsequent activation of signal transduction systems. However, recent observations suggest that PTH/PTHrP receptors in human bone and kidney may not be different (18).

Highly homologous complementary DNAs (cDNAs) encoding the rat and the opossum PTH/PTHrP receptor were recently isolated independently by expression-cloning tech-

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Address all correspondence and requests for reprints to: Dr. Ernestina Schipani, Massachusetts General Hospital, Departments of Medicine and Pediatrics, Endocrine Unit, Harvard Medical School, Boston, Massachusetts 0211.
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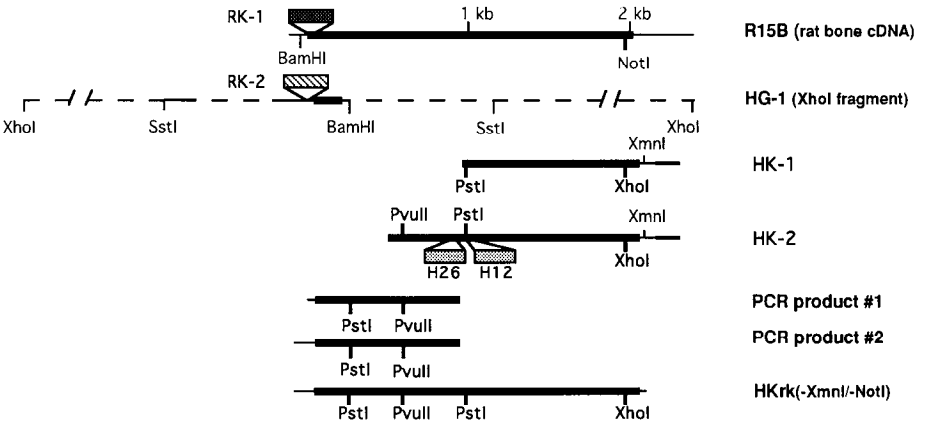


FIG. 1. Schematic representation of the rat PTH/PTHrP receptor cDNA, the human genomic DNA clone HG-1, the cDNA clones (HK-1 and HK-2), and the PCR products encoding the 5′ end of the human PTH/PTHrP receptor. ■, coding region; —, noncoding region; —, phage arm; - -, intervening sequence; ▩, degenerate primer; ▧, human genomic primer; □, human cDNA primer.

niques (19, 20). Together with the receptors for secretin (21) and calcitonin (CT) (22), these proteins represent the first members of a new subfamily of G protein-linked receptors, which signal both through adenylate cyclase and phospholipase C [so far documented only for the cloned PTH/PTHrP and CT receptors (20, 23) but observed with uncloned secretin receptors (24) as well]. PTHrP is expressed in multiple fetal and adult tissues (25–27), and the cloned PTH/PTHrP receptor is expressed in the early mouse embryo (28). Since the deletion of both PTHrP alleles in mice results in a unique lethal phenotype (29), the common PTH/PTHrP receptor is likely to play an important role in human development and disease. Its tissue-specific abundance and ligand sensitivity may be altered in diseases of calcium metabolism such as renal failure and primary hyperparathyroidism, and a subgroup of patients with selective resistance to PTH (pseudohypoparathyroidism, type Ib) (30) may well express mutant PTH receptors. To facilitate investigations addressing these questions, and to establish that PTH/PTHrP receptors in bone and kidney are either different or identical, we have isolated human PTH/PTHrP receptor homologs from human kidney and human osteoblast-like (SaOS-2) cells and found them to be identical. Amino-terminally truncated PTH analogs have distinctly different affinities for the cloned rat and human receptors, despite a high degree of conservation between both sequences. This finding could have significant implications for the design and, in particular, for the evaluation of potential *in vivo* PTH and PTHrP antagonists.

## Materials and Methods

### *General*

[Nle$^{8,18}$,Tyr$^{34}$] bovine PTH (1–34)amide [bPTH(1–34)], [Nle$^{8,18}$,Tyr$^{34}$] bPTH(3–34)amide [bPTH(3–34)], and [Nle$^{8,18}$,Tyr$^{34}$]bPTH(7–34)amide [bPTH(7–34)] were from Bachem Fine Chemicals (Torrance, CA); [Tyr$^{36}$] hPTHrP(1–36)amide [PTHrP(1–36)] was synthesized as described (31) by H. T. Keutmann (Endocrine Unit, Massachusetts General Hospital), using an Applied Biosystems (Foster City, CA) synthesizer 420A. Peptide concentrations were determined by amino acid analysis. Dulbecco's modified Eagle's medium, EDTA/trypsin, and gentamycin were from GIBCO (Grand Island, NY); fetal bovine serum was from Hyclone Laboratory (Logan, UT). COS-7 cells were kindly provided by B. Seed (Laboratory of Molecular Biology, Massachusetts General Hospital). The human osteosarcoma cell line SaOS-2 was kindly provided by A. H. Tashjian, Jr. (Harvard School of Public Health, Boston, MA). Oligonucleotide primers were synthesized using an Applied Biosystems 380B DNA synthesizer. Restriction enzymes, Klenow enzyme, T4 polynucleotide kinase, and T4 DNA ligase were from New England Biolabs (Beverly, MA). Calf alkaline phosphatase was from Boehringer Mannheim (Mannheim, Germany). All other reagents were of highest purity available.

### *Isolation of cDNA and genomic DNA clones encoding the human PTH/PTHrP receptor*

A human kidney oligo-dT-primed cDNA library ($1.7 \times 10^6$ independent clones) in λgt10 and a genomic library from human placental DNA ($2.5 \times 10^6$ independent clones) in EMBL3 (Sp6/T7; Clontech, Palo Alto, CA) were screened with the $^{32}$P-labeled 1.8-kilobase (kb) *Bam*HI/*Not*I fragment encoding most of the coding sequence of the rat PTH/PTHrP receptor (20) (Fig. 1). Labeling was by random-priming using a kit from Boehringer Mannheim. Hybridizations were performed at 42 C for 18–24 h in 50% formamide, 4× saline sodium citrate (SSC; 1× SSC = 300 mM NaCl, 30 mM NaCitrate, pH 7.0), 2× Denhardt's solution, 10% Dextran sulfate, 100 μg/ml salmon sperm DNA (final concentration). Filters were washed with 2× SSC/0.1% sodium dodecyl sulfate (SDS) for 30 min at room temperature and then with 1× SSC/0.1% SDS for 30 min at 45 C. The films were exposed at −80 C for 18–24 h using intensifying screens. About 1,000,000 clones were screened from each library. Positive clones were plaque-purified, and λ-phage DNA was isolated (32). Cloned inserts were excised from phage DNA by digestion with the restriction endonucleases, *Hin*dIII and *Eco*RI (λgt10 library), or *Xho*I or *Sst*I (EMBL3 library), and the inserts were then subcloned into pcDNAI (Invitrogen, San Diego, CA) using the appropriate, dephosphorylated restriction sites.

An oligo-dT- and random-primed cDNA library from SaOS-2 cells ($2 \times 10^6$ independent clones) was constructed in λ-*Zap*II (Stratagene, La Jolla, CA) (32). This cDNA library was screened with the $^{32}$P-labeled *Pst*I/*Xho*I fragment from the human kidney clone HK-1 (see *Results*), which corresponded to nucleotides 670–1669 of the coding region of the human kidney PTH/PTHrP receptor cDNA. Hybridizations were performed as described above. After identification of a positive clone, the pBluescript SK(−) phagemid containing the cloned positive insert was excised and recircularized (32). The 5′ end of the cDNA encoding the human bone PTH/PTHrP receptor was isolated by polymerase chain reaction (PCR) as described below using primer RK-2, which is based on a nucleotide sequence in the human genomic clone that corresponds to the 5′-nontranslated region of the rat PTH/PTHrP receptor cDNA (20).

Sequencing of the $CsCl_2$-purified subclones was performed according to Sanger *et al.* (33) by the dideoxy-termination method (Sequenase version 2 sequencing kit, United States Biochemical Corporation, Cleveland, OH).

### *Reverse transcription and PCR*

Poly (A)$^+$ RNA (3 μg) from human kidney (Clontech) in 73.5 μl $H_2O$ was incubated at 100 C for 30 sec, quenched on ice, and then added to

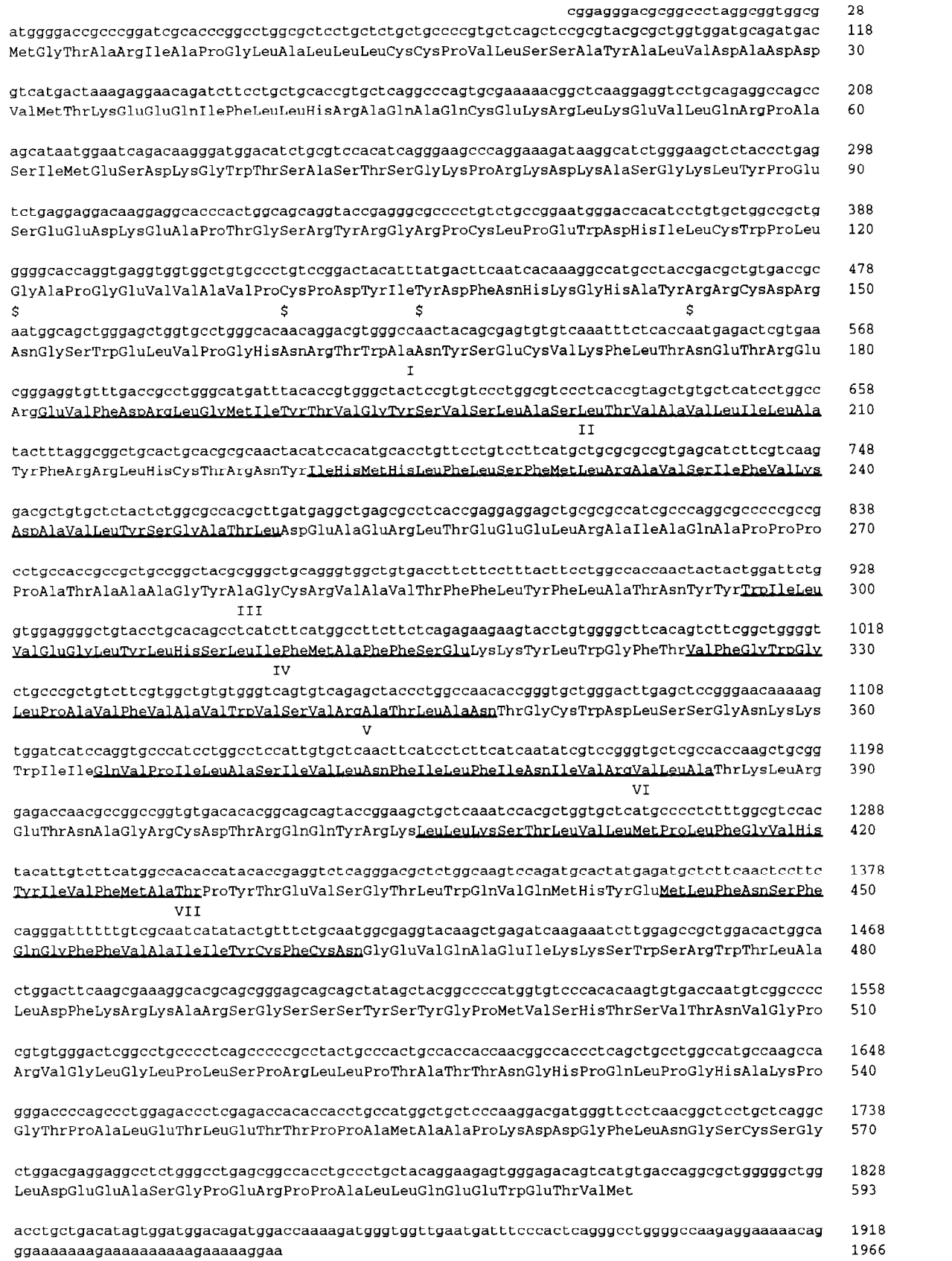


FIG. 2. Nucleotide sequence and deduced amino acid sequence of the human PTH/PTHrP receptor. Putative membrane-spanning domains I–VII are *underlined*; $, potential extracellular N-linked glycosylation sites; numbering of the amino acid residues and the nucleotides is on the *right*.

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                         **                                        *
H  MGTARIAPGL ALLLCCPVLS SAYALVDADD VMTKEEQIFL LHRAQAQCEK   50
R  ..A.....S. .......... .......... .F........ ........D.   50
O  ..AP..SHS. ......S... .V........ .I......I. .RN......Q   50

H  RLKEVLQRPA SIMESDKGWT SASTSGKPRK DKASGKLYPE SEEDKEAPTG  100
R  L.....HTA. N......... P......... E.....F... .K.N.DV...  100
O  ......RV.  ELA..A.D.   M.R.A.TK. E.PAE...SQ A..SR.VSDR   97
         *          *                *                  *
H  SRYRGRPCLP EWDHILCWPL GAPGEVVAVP CPDYIYDFNH KGHAYRRCDR  150
R  ..R.......  ...N.V.... .......... .......... ..........  150
O  ..LQDGF...  ...N.V...A .V..K..... .......... ..R......S  147
   $           $    $    *       $                  I
H  NGSWELVPGH NRTWANYSEC VKFLTNETRE REVFDRLGMI YTVGYSVSLA  200
R  .....V....  .......... L..M...... .......... ......M...  200
O  .........N  .......... .......... .......... ......I..G  197
                                          II
H  SLTVAVLILA YFRRLHCTRN YIHMHLFLSF MLRAVSIFVK DAVLYSGATL  250
R  ..........  .......... .....M.... ....A..... .......F..  250
O  .........G  .......... .......V.. .........I. .......VST  247
                                        *
H  DEAERLTEEE LRAIAQAPPP PATAAAGYAG CRVAVTFFLY FLATNYYWIL  300
R  ..........  .HI...V... ..A..V.... .......... ..........  300
O  ..I..I....  ...FTE  .. .. DK..FV. ......V... ..T.......  294
      III                                IV
H  VEGLYLHSLI FMAFFSEKKY LWGFTVFGWG LPAVFVAVWV SVRATLANTG  350
R  ..........  .......... .....I.... .......... G.........  350
O  ..........  .......... .....L.... .......... T........E  344
   *                            V
H  CWDLSSGNKK WIIQVPILAS IVLNFILFIN IVRVLATKLR ETNAGRCDTR  400
R  .......H..  .......... V......... .I........ ..........  400
O  ..........  .........A ..V....... .I........ ......  ...  394
                    VI
H  QQYRKLLKST LVLMPLFGVH YIVFMATPYT EVSGTLWQVQ MHYEMLFNSF  450
R  .......R..  ...V...... .T....L... .........I. ..........  450
O  ..........  .......... .......... ....I..... ..........  444
   VII
H  QGFFVAIIYC FCNGEVQAEI KKSWSRWTLA LDFKRKARSG SSSYSYGPMV  500
R  ..........  .......... R......... .......... ..........  500
O  ..........  .......... .......... .......... ..T.......  494

H  SHTSVTNVGP RVGLGLPLSP RLLPTAT  T NGHPQLPGHA KPGTPALETL  548
R  ..........  .A..S..... ..P. ..  . ...S...... ...A..T..  546
O  ..........  .G..A.S... ..A.G.GASA ...H....YV .H.SISENS.  544

H  ETTPPAMAAP KDDGFLNGSC SGLDEEASGP ERPPALLQEE WETVM       593
R  ..L.VT..V.  .......... .........  A...P..... .....      591
O  PSSG.E PGT  ....Y....   ..Y.PMV.  .Q..P..E.. R....      585
```

FIG. 3. Alignment of the amino acid sequences of the human (H), rat (R), and opossum (O) PTH/PTHrP receptor. ·, Identical amino acid either between the human and the rat receptor or between the human and the opossum receptor, respectively; *, conserved, extracellular cysteines; $, conserved, extracellular N-linked glycosylation sites; putative transmembrane domains I–VII are *underlined*; numbering of the amino acid residues is on the *right*. Modifications of the amino acid sequences of the rat (residue 575) and the opossum (residues 86 and 132) PTH/PTHrP receptors were introduced after revision of the two nucleotide sequences.

20 μl 5× RT buffer (1× RT buffer = 40 mM Tris-HCl, pH 8.2, 40 mM KCl, 6.6 mM $MgCl_2$, 10 mM dithiothreitol, and deoxynucleotide triphosphates at 0.5 mM each), 2 μl (4 U) RNasin (Promega Biotec, Madison, WI), 1 μl (80 pmol/μl) human cDNA primer H12 (5′-AGATGAGGCTGTGCAGGT-3′), and 80 U avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL). The reaction mixture was incubated for 40 min at 42 C. One-tenth of the first strand synthesis reaction was then amplified by PCR in a final vol of 100 μl containing

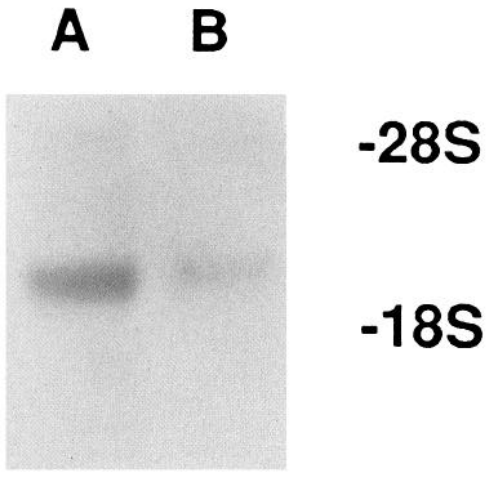


FIG. 4. Northern blot analysis of total RNA (~10 μg/lane) prepared from human kidney (A) and SaOS-2 cells (B). The blot was hybridized with the full-length cDNA encoding the human PTH/PTHrP receptor and exposed for 18 h at −80 C; positions of 28S and 18S ribosomal RNA bands are indicated.

3 mM $MgSO_4$, 200 μM deoxynucleotide triphosphates, 2 U Vent polymerase (New England Biolabs), and 2 μM of the forward and reverse primers (PCR conditions: denaturing for 1 min at 94 C, annealing for 1 min at 50 C, and extension at 72 C for 3 min; 40 cycles). Independent PCRs were performed using two different forward primers: 1) degenerate primer RK-1 (5′-GGAATTCCATGGG$^{G}_{A}$GC$^{C}_{G}$C$^{C}_{G}$CCCGGAT-3′) based on the 5′-coding end of the two previously cloned PTH/PTHrP receptors (19, 20); and 2) primer RK-2 (5′-CGGGATCCCGCGGCCCT-AGGCGGT-3′) based on the 5′-untranslated region of the human genomic clone HG-1. Both PCR reactions used the reverse primer H26 (5′-AGTATAGCGTCCTTGACGA-3′), which is complementary to nucleotides 713–731 of the coding region of the human PTH/PTHrP receptor (Fig. 1). The 5′ end of the cDNA encoding the PTH/PTHrP receptor from SaOS-2 cells was amplified by an analogous PCR approach using the forward primer RK-2. PCR products were blunt-ended using Klenow enzyme and cloned into dephosphorylated pcDNAI digested with *Eco*RV.

### *Northern blot analysis*

Total RNA was extracted from SaOS-2 cells and from human kidney by the guanidine isothiocyanate/$CsCl_2$ method (34). For Northern blot analysis, approximately 10 μg total RNA were electrophoresed on a 1.5%/37% agarose/formaldehyde gel, were then blotted onto nitrocellulose filters (Schleicher and Schuell, Keene, NH), and probed with the full-length human PTH/PTHrP receptor cDNA. The hybridization conditions were the same as those for screening the phage libraries (see above). The filters were washed at a final stringency of 0.5× SSC/0.1% SDS for 30 min at 60 C and exposed for autoradiography (18 h at −80 C).

### *Transient expression of PTH/PTHrP receptors in COS-7 cells, RRA and stimulation of cAMP production*

COS-7 cells were grown in 15-cm plates in Dulbecco's modified Eagle's medium, 10% heat-inactivated fetal bovine serum, 10 mg/liter gentamycin until 80–90% confluent. Twenty-four hours after transfection by the diethylaminoethyl/Dextran method (19, 20), with 1–2 μg plasmid DNA encoding the full-length human (HKrk) or rat PTH/PTHrP receptor (R15B) (20), respectively, the cells were trypsinized and replated into 24-well plates at a density of about 50,000 cells per well. Radioreceptor and cAMP accumulation bioassays were performed 48 h later, as previously described (20).

## Results

### *Molecular cloning of the human PTH/PTHrP receptor cDNA*

To isolate the human PTH/PTHrP receptor, we screened cDNA libraries from human bone and kidney. Since many G protein-coupled receptors are encoded by intronless genes (35–39), we also screened a human genomic library. Clone HK-1 was isolated after screening of 500,000 plaques from the human kidney cDNA library, with a probe (*Bam*HI/*Not*I) representing most of the coding region of the rat PTH/PTHrP receptor (20) (Fig. 1). Since one of the two *Eco*RI cloning sites of λgt10 had been eliminated during the library construction, an *Hin*dIII/*Eco*RI fragment containing the cDNA insert and approximately 250 base pairs (bp) of the 37-kb (*left*) λ-arm were subcloned into the corresponding restriction sites in pcDNAI. Comparison of the DNA sequence with the rat cDNA revealed that this cDNA contained the last approximately 1000 bp of the coding region and approximately 200 bp of the 3-noncoding region, which included an A-rich 3′ end. The coding region 5′ to the *Xho*I site of HK-1 subsequently was used to rescreen the library and led to the isolation of another clone, HK-2, which, after subcloning into pcDNAI, proved to contain approximately 1400 bp of the coding region. A third screening of the library, using the *Pvu*II/*Pst*I fragment of HK-2, led to the isolation of clone HK-3, which was identical to HK-2.

Clone HB-1 was isolated after screening of approximately 300,000 plaques from the human SaOS-2 cell cDNA library using the *Pst*I/*Xho*I fragment of HK-1; it contained the last 1550 nucleotides of the coding region and approximately 200 bp of the 3′-noncoding region.

Four independent clones were isolated from the genomic library (~$10^6$ plaques); the library was screened with the same *Bam*HI/*Not*I fragment of the rat PTH/PTHrP receptor that was used to screen the cDNA library. The approximately 13-kb genomic clone HG-1 contains an approximately 2.3-kb *Sst*I fragment that hybridizes to the cDNA encoding the human receptor upon Southern blot analysis. An *Sst*I fragment that is indistinguishable in size (2.3 kb) was also detected by Southern hybridization of an *Sst*I digest of human genomic DNA (data not shown). The 2.3-kb genomic *Sst*I fragment was therefore subcloned into pcDNAI. Southern blot and sequence analysis of the *Sst*I DNA fragment showed that similar to the rat gene (40), this genomic fragment contains two exons; one encodes the putative signal peptide and part of the 5′-nontranslated region, whereas the second exon contains an upstream portion of the 5′-untranslated region (Fig. 1).

To isolate the remaining approximately 450 nucleotides of the coding region located between the putative signal peptide and the 5′ end of HK-2, poly $(A)^+$ RNA from human kidney was reverse transcribed after priming with H12 (Fig. 1). After single-strand synthesis, two independent PCRs were performed using two different forward primers: 1) a degenerate primer, RK-1, based on the 5′-coding end of the two previously cloned PTH/PTHrP receptors, OK-O and R15B (19, 20); and 2) primer, RK-2, based on the 5′-noncoding region of HG-1. H26 was used as the reverse primer for both reactions. Since the resulting PCR products overlap with HK-

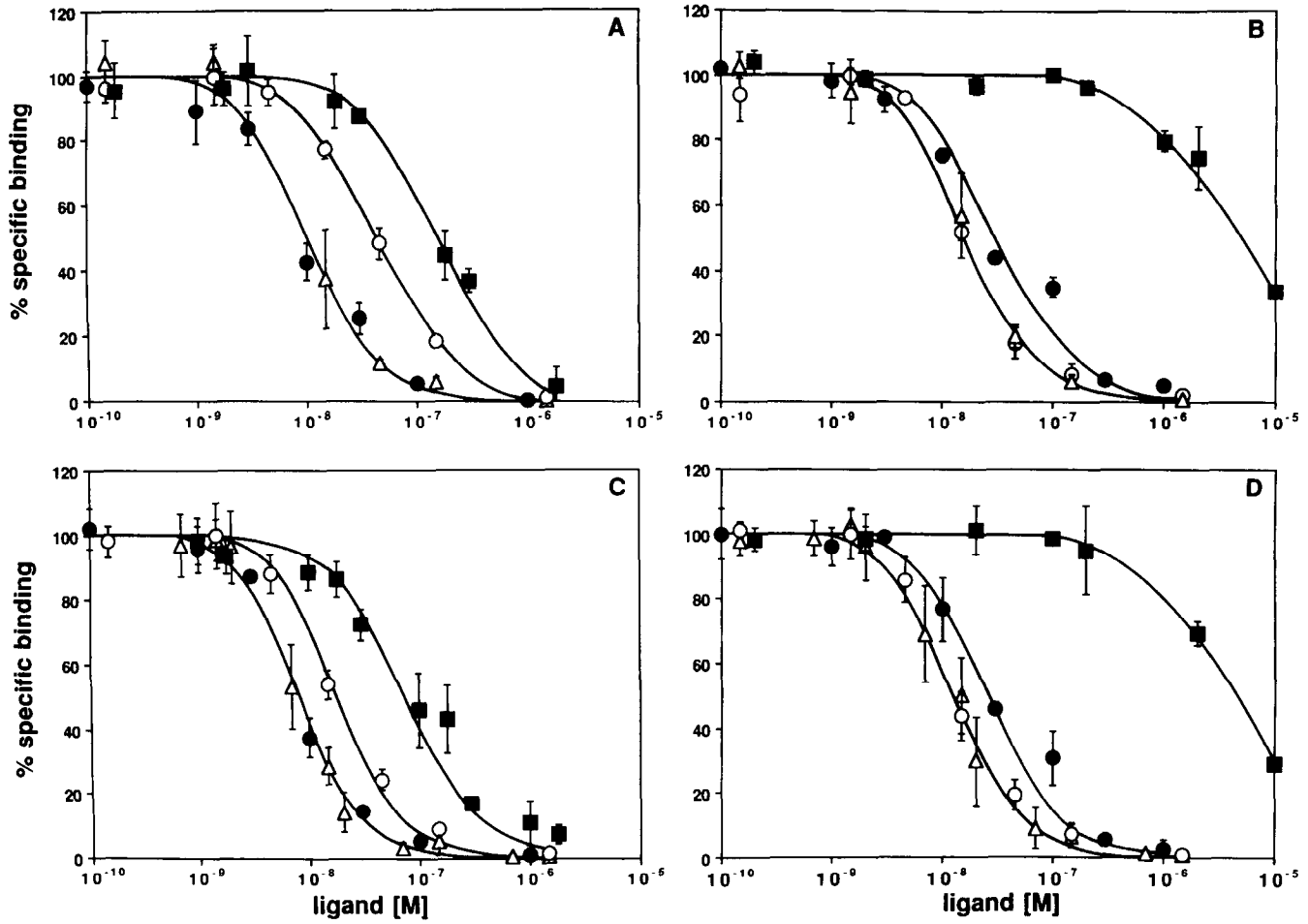


FIG. 5. Binding of $^{125}$I-labeled bPTH(1-34) (A and B) and $^{125}$I-labeled PTHrP(1-36) (C and D) to COS-7 cells transiently expressing the human (A and C) and the rat (B and D) PTH/PTHrP receptor. Competing ligands included bPTH(1-34) (△), PTHrP(1-36) (○), bPTH(3-34) (●), and bPTH(7-34) (■). Data are given as percent specific binding and represent the mean ± SD of at least three independent experiments.

2, Southern blot analysis, in combination with restriction enzyme mapping, was used to verify that the missing portion of human PTH/PTHrP receptor cDNA had been amplified. The blunt-ended PCR products encoding the 5′ end of the hPTH/PTHrP receptor cDNA were then cloned into pcDNAI using dephosphorylated *Eco*RV sites. An analogous approach using the forward primer RK-2 (Fig. 1) led to the isolation of the 5′ end of the cDNA that encodes the human bone PTH/PTHrP receptor. Sequence analysis of each PCR clone confirmed that both 5′ sequences were identical, except for the expected discrepancies at the 5′ end that resulted from the use of two different forward primers. The full-length human PTH/PTHrP receptor cDNA, HKrk, was constructed from PCR clone 2 and HK-2; this cDNA was then transiently expressed in COS-7 cells.

*Nucleotide and amino acid sequence of the human PTH/PTHrP receptor*

Nucleotide sequencing of both strands of the hPTH/PTHrP receptor cDNA revealed an open-reading frame encoding a 593-amino acid protein (Fig. 2) (Genbank accession number L04308). The nucleotide sequence of the cDNA clones from human kidney and SaOS-2 cells were identical with the exception of nucleotide 1414, which was either C or T in the two kidney-derived cDNAs and C in the bone-derived cDNA; this change, which could be due either to a polymorphism or to a cloning artifact, did not result in a modification of the predicted amino acid. Comparison of the predicted amino acid sequence of the human receptor with those of the opossum (585 amino acids) (19) and rat (591 amino acids) (20) revealed 81% and 91% identity, respectively (Fig. 3). All extracellular cysteines including the two cysteine residues in the presumed signal peptide are conserved, as are all potential extracellular N-linked glycosylation sites. It is interesting to note that most amino acid differences between rat and human receptors are not randomly distributed, but segregated to four areas corresponding to amino acids 57–97, 262–276, 542–558, and 579–589 of the human sequence. Comparison with the receptors for secretin (21), CT (22), vasoactive intestinal polypeptide (40), glucagon-like peptide 1 (41), and GH-releasing hormone (42) showed at least 30% amino acid identity and at least 50%

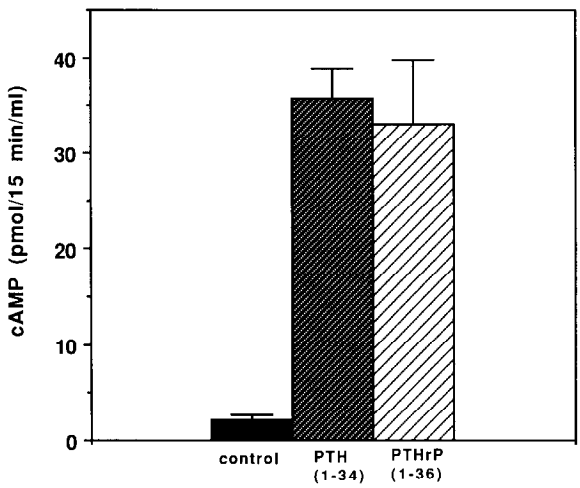


FIG. 6. Production of intracellular cAMP in COS-7 cells transiently expressing the human receptor. Control, stimulation with bPTH(1–34) and PTHrP(1–36) (both $10^{-7}$ M). Data show the mean ± SD and are representative of at least three independent experiments.

similarity, respectively. All other G protein-linked receptors had less than 10% identity with the human PTH/PTHrP receptor.

*Northern blot analysis*

Northern blot analysis of total RNA (~10 μg/lane) from human kidney and from SaOS-2 cells revealed one major hybridizing DNA species of approximately 2.5 kb, using the full-length cDNA encoding the human PTH/PTHrP receptor as probe (Fig. 4).

*Comparative characterization of the transiently expressed human and rat PTH/PTHrP receptors*

Radiolabeled bPTH(1–34) and PTHrP(1–36) (~200,000 cpm) specifically bound to a similar extent to human and rat receptors transiently expressed in COS-7 cells (specific binding: 10.1 ± 3.7% and 7.6 ± 6.0% for [$^{125}$I]bPTH(1–34), 8.1 ± 3.5% and 7.1 ± 4.1% for [$^{125}$I]PTHrP(1–36), respectively; nonspecific binding was 1.2 ± 0.7 and 1.1 ± 0.5%, respectively). The binding affinities of the rat and the human PTH/PTHrP receptors for bPTH(1–34) were similar with apparent dissociation constants of approximately 8 and 15 nM, independent of the radioligand. PTHrP(1–36) had an approximately 2-fold lower affinity for the human than the rat PTH/PTHrP receptor when using PTHrP(1–36) as the radioligand, whereas an approximately 4-fold difference in affinity was observed when using radiolabeled bPTH(1–34). Despite their substantial amino acid homology, however, the receptors showed striking differences in affinity for both bPTH(3–34) and PTH(7–34) using either radioligand (Fig. 5). The apparent binding affinities of these amino-terminally truncated PTH analogs were 4- and 50-fold higher for the human receptor than for the rat receptor [~8 nM *vs.* ~30 nM for bPTH(3–34); ~100 nM *vs.* ~5000 nM for bPTH(7–34), respectively]. Both ligands, bPTH(1–34) and PTHrP(1–36), stimulated the production of intracellular cAMP with similar efficacy and efficiency (Fig. 6).

## Discussion

The human PTH/PTHrP receptor is highly homologous to the PTH/PTHrP receptor from rat (20), a eutherian mammal, whereas the sequence identity with the PTH/PTHrP receptor from opossum, a marsupial mammal, is less striking (19). Significant structural and sequence homology is also found with the recently cloned receptors for secretin (21), CT (22), vasoactive intestinal polypeptide (40), glucagon-like peptide 1 (41), and GH-releasing hormone (42), whereas less than 10% sequence identity is shared with other G protein-linked receptors.

Recent comparative studies using human bone- and kidney-derived tissue showed no significant differences between PTH/PTHrP receptors from both organs (18). The molecular cloning of identical cDNAs (with the exception of a possible polymorphism of nucleotide 1414) encoding the human PTH/PTHrP receptor in human kidney and human bone-derived, osteoblast-like cells, now provides unequivocal molecular evidence for the presence of identical PTH/PTHrP receptors in the two major target tissues for PTH. A cDNA clone (nucleotides 321–1928) that was recently isolated through hybridization techniques from a rat kidney cDNA library showed a nucleotide sequence that is identical to the previously reported rat bone PTH/PTHrP receptor sequence (Kong, X. F., unpublished observations). This indicates that identical PTH/PTHrP receptors are also present in rat kidney and bone. The isolation of identical PTH/PTHrP receptors from both major target tissues for PTH action was not unexpected, since the PTH/PTHrP receptors from opossum kidney (19) and rat osteoblast-like (ROS 17/2.8) cells (20), both independently isolated by expression cloning, showed a high degree of amino acid sequence identity. However, additional receptors for either PTH or PTHrP, which have only limited or no nucleotide sequence homology with the common PTH/PTHrP receptor, cannot be excluded. In fact, recent Northern blot analyses of various rat tissues (43) suggest alternatively spliced receptor forms that are derived from the intron-rich PTH/PTHrP receptor gene (44), and thus could contribute to the pleiotropic biological activities of both PTH and PTHrP.

Despite the high degree of amino acid sequence homology between human and rat PTH/PTHrP receptors, the receptors, when transiently expressed in COS-7 cells, revealed striking differences in their affinities for amino-terminally truncated PTH analogs. These data confirm and extend previous binding and signaling studies using renal plasma membranes and/or clonal cell lines from different species (11–18). Since recombinant rat and human PTH/PTHrP receptors showed distinct binding affinities for bPTH(7–34) in COS-7 cells, we conclude that differences in the primary structures of the receptors from two different species, rather than organ- or species-specific differences in the cell environment, are responsible for these observations. This finding may have significant implications for the future design and the evaluation of PTH/PTHrP antagonists (11, 12) and could imply that previously reported *in vivo* PTH antagonists (45) may have significantly improved efficacy in species other than rat. Since the bPTH(7–34) analog is capable of clearly distin-

guishing between two highly homologous PTH/PTHrP receptors, this peptide may help to define those portions of the human receptor that improve antagonist binding. Our data that PTHrP(1–36) has a slightly lower affinity for human than rat PTH/PTHrP receptors are at some variance with previous reports by others (13, 18). This minor discrepancy could be related to the high copy number of receptors per COS-7 cell or the insufficient availability of G proteins in these cells to allow high affinity ligand binding. However, studies in mammalian cells stably expressing recombinant PTH/PTHrP receptors (46, 47) largely confirmed the previously reported affinity relationship of PTH and PTHrP analogs (19, 20), making such an explanation unlikely. Furthermore, recent *in vivo* studies in humans indicate that PTHrP(1–34) is 3- to 10-fold less potent than PTH(1–34) (48), and thus supports our findings.

The isolation and characterization of cDNA encoding the human PTH/PTHrP receptor will facilitate the isolation and characterization of the remaining portions of the human PTH/PTHrP receptor gene and should eventually help to define the molecular defect(s) in human disease.

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