Low resolution mutational studies have indicated that the amino-terminal extracellular domain of the rat parathyroid hormone (PTH)/PTH-related protein (PTHRP) receptor (rP1R) interacts with the carboxyterminal portion of PTH-(1–34) or PTHrP-(1–36). To further define ligand-receptor interactions, we prepared a fully functional photoreactive analog of PTHrP, [Ile5, Bpa23, Tyr36]PTHrP-(1–36)-amide (Bpa23]PTHrP, where Bpa is p-benzoyl-l-phenylalanine). Upon photolysis, radioiodinated [Bpa23]PTHrP covalently and specifically bound to the rP1R. CNBr cleavage of the broad ~80-kDa complex yielded a radiolabeled ~9-kDa nonglycosylated protein band that could potentially be assigned to rP1R residues 23–63, Tyr23 being the presumed amino-terminus of the receptor. This assignment was confirmed using a mutant rP1R (rP1R-M63I) that yielded, upon photoligand binding and CNBr digestion, a broad protein band of ~46 kDa, which was reduced to a sharp band of ~20 kDa upon deglycosylation. CNBr digestion of complexes formed with two additional rP1R double mutants (rP1R-M63I/L40M and rP1R-M63I/L41M) yielded non-glycosylated protein bands that were ~6 kDa in size, indicating that [Bpa23]PTHrP cross-links to amino acids 23–40 of the rP1R. This segment overlaps a receptor region previously identified by deletion mapping to be important for ligand binding. Alanine scanning of this region revealed two residues, Thr28 and Gln31, as being functionally involved in ligand binding. Thus, the convergence of photoaffinity cross-linking and mutational data demonstrates that the extreme amino-terminus of the rP1R participates in ligand binding.

The PTH1/PTHRP receptor (P1R) mediates the biological actions of PTH and PTHrP (1, 2); both peptides bind to this common receptor with similar affinities and stimulate the formation of cAMP and inositol phosphates with similar efficacies (3–5). In contrast, the recently isolated PTH2 receptor (P2R) (6) is activated fully by PTH and only poorly by PTHrP (6–9).

Current information suggests that PTH and PTHrP interact with the P1R through multiple sites and that these are dispersed throughout the extracellular surface of the receptor and some portions of the transmembrane helices (1). Studies with chimeras formed between P1Rs from different species or different receptor subtypes (P1R or P2R) indicate that there are interactions between the amino-terminal extracellular domain of the receptor and region 15–34 of the ligand and between the core region of the receptor and the amino-terminal portion of the ligand (9–12). Furthermore, observations from studies on other members of this peptide hormone receptor family (13, 14), and particularly with chimeras between the P1R and the calcitonin receptor (15), suggest that this general orientation of ligand-receptor interaction may apply to all members of this family of G protein-coupled receptors.

In addition to mutagenesis approaches, affinity cross-linking methods can provide valuable information on the location of ligand-receptor interactions sites in peptide hormone receptors (16, 17). For the P1R, Zhou et al. (18) recently showed that a PTH-(1–34) analog containing a photoderivatized lysine 13 cross-linked to a 17-amino acid segment of the amino-terminal extracellular receptor domain that mapped close to the junction with the first membrane-spanning helix. In related experiments, this group showed that another PTH-(1–34) analog, which contains p-benzoyl-l-phenylalanine (Bpa) (19) at position 1, cross-linked to a region of the P1R containing transmembrane helix 6 and extracellular loop 3 (20). The results of these physicochemical analyses are in agreement with previous mutational studies that functionally identified similar regions of the P1R as candidate ligand-interaction sites (12, 21–23). In addition to these two putative ligand contact regions, mutational studies also identified segments within the large (~190 amino acids) amino-terminal domain of the receptor that appear to interact with region 15–34 of the ligand (10, 24).

We have now performed cross-linking studies with a PTHrP analog that contains photoreactive Bpa at position 23 in place of the native phenylalanine, a residue recently shown to be involved in the ligand binding specificity of the PTH2 receptor (7, 11). This new photoreactive ligand, [Ile5, Bpa23, Tyr36]PTHRP-(1–36)-amide, cross-linked to a short segment between residue 40 and the amino-terminus, which is predicted to be Tyr-23. We also confirmed the importance of this amino-terminal receptor region by mutational methods and have identified two amino acid residues that contribute to ligand binding affinity.

**EXPERIMENTAL PROCEDURES**

*Materials—*

[**Nle**6,21, Ty**r**34]rPTH-(1–34)-amide (rNlePTH), [**Nle**6, **Tyr**34]hPTH-(1–34)-amide (hNH2PTH), [Ile6, Bpa23,**Tyr**36]hPTHRP-(1–36)-amide ([Bpa23]hPTHRP), [**Tyr**36]hPTHRP-(1–36)-amide (PTHRP), and [Leu1, **Nle**7, Trp31]hPTHRP-(7–34)-amide (PTHRP-(7–34)) were synthesized.**
by the Protein and Peptide Core Facility at Massachusetts General Hospital (Boston, MA) by solid-phase method on Perkin-Elmer Model 430A and 431A synthesizers. The Fastmoc version of Fmoc (N-(9-fluorenylethoxycarbonyl) chemistry was utilized, and peptides were purified by reversed-phase chromatography.

N-terminal peptide was from Pierce, and CNBr-cleaved genomic DNA was purchased from Berkeley Antibodies (Berkeley, CA). The oligonucleotide primers were synthesized on an Applied Biosystems Model 380A DNA synthesizer. Positive mutants were verified by nucleotide sequence analysis of plasmid DNA.

**Cell Culture and DNA Transfection—** COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained as described (26) in Ham's F-12 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

**Radioligand-Receptor Binding Assays and cAMP Accumulation—** Radiolabeled rNePTH, PTHrP, and [Bpa<sup>23</sup>]PTHrP were prepared by chloramine-T iodination, followed by high pressure liquid chromatography purification using a 30–50% acetonitrile in 0.1% trifluoroacetic acid gradient over 30 min. Radioligand-receptor binding assays were performed in 24-well plates as described (11). In brief, each well (final volume of 200 μl) contained 50,000 cpm radiolabeled ligand-receptor complexes from cells cultured in 150-mm dishes, we used preparative SDS-polyacrylamide gels (5–20% acrylamide, 0.75-mm spacers) in a humidified atmosphere containing 95% air and 5% CO<sub>2</sub>. Cells were seeded in 24-well plates (200,000 cells/well) for radioreceptor and cAMP assays and for preliminary cross-linking experiments; all other cross-linking experiments were performed in 150-mm dishes (6 × 10<sup>6</sup> cells). Once the cell monolayer reached 90–100% confluency, cells were transfected by the DEAE-dextran method as described (22) using 200 ng of plasmid DNA/well or 2 μg/150-mm dish. The medium was replaced daily, and 3 days after transfection, cells were used either for radioligand binding and cAMP accumulation assays or for cross-linking experiments. ROS 17/2.8 cells were maintained as described (26) in Ham's F-12 medium supplemented with 5% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

**Results**

**Cell-surface Expression of PTH/PTHrP Receptors—** Cell-surface expression was assessed as described (24) using antibody 12CA5 directed against the HA epitope in the rP1R-HA receptors and a secondary radiolabeled anti-mouse IgG Fab fragment. Relative specific binding of antibody to each mutant P1R was calculated by subtracting nonspecifically bound radioactivity (determined in mock-transfected COS cells; typically 0.1–0.2% of added radioactivity) from the total bound radioactivity divided by the radioactivity specifically bound to wild-type rP1R-HA (typically 1–2% of the added radioactivity).

**Affinity Labeling of the PTH/PTHrP Receptor—** In preliminary experiments, COS-7 cells that were grown and transfected in 24-well plates were rinsed twice with 1 ml of cold binding buffer, and the cell monolayer was then incubated for 6 h at 4°C with 12<sup>5</sup>I-[Bpa<sup>23</sup>]PTHrP (1 × 10<sup>6</sup> cpmp) diluted in 0.5 ml of binding buffer with or without unlabeled ligand (10<sup>–10</sup> M NePTH or [Bpa<sup>23</sup>]PTHrP). After incubation, cells were rinsed three times with 1 ml of cold binding buffer before adding 200 μl of binding buffer and placing the dishes on ice under a UV light source for 20 min (Blak Ray long-wave lamp; 366 nm, 7000 microwatts/cm<sup>2</sup>; UV Products, San Gabriel, CA; source-to-dish-distance of ~5 cm). After photolysis, cells were rinsed once with cold phosphate-buffered saline, twice with a cold acidic buffer (0.05 M glycine and 0.15 M NaCl [pH 2.5]) to remove noncovalently bound radioligand, and twice with cold phosphate-buffered saline and 0.5 ml of 12<sup>5</sup>I-SDS-PAGE sample buffer (4% w/v) SDS, 80 mM Tris-HCl (pH 6.8), 20% (v/v) glycerol, 0.2% bromphenol blue, and 100 mM dithiothreitol). The lysate was then passed six times through a 19-gauge needle to shear genomic DNA.

**Preparation of Labelled Ligation-Receptor Complex—** To prepare larger amounts of the cross-linked ligand-receptor complex, the final protocol was followed using COS-7 cells that were grown and transfected in 150-mm dishes. For each rinsing step, 30 ml of cold binding buffer were used, and incubation with 12<sup>5</sup>I-[Bpa<sup>23</sup>]PTHrP (2–4 × 10<sup>6</sup> cpmp) was performed in a final volume of 20 ml of binding buffer. During UV light exposure, the cell monolayer was covered with 10 ml of binding buffer, and after photolysis and rinsing, cells were solubilized with 4 ml of SDS-PAGE sample buffer.

**SDS-PAGE Analysis of the Ligand-Receptor Complex—** After heating to 70°C, samples (and appropriate size markers) were either subjected to analytical SDS-PAGE analysis (5–20% acrylamide, 0.75-mm spacers) according to the method of Laemmli (27) or loaded onto a 16.5% (v/v) Tricine gel (0.75-mm spacers) according to the method of Schägger and Von Jagow (28), with subsequent autoradiography of the dried gels (16.5% Tricine gel, 20°C with intensifying screens).

**CNBr Cleavage—** CNBr was dissolved in 100% trifluoroacetic acid and then added to the partially purified radiolabeled ligand-receptor complexes. After a 30-min incubation of 100 μg of CNBr treated with 100 μg of CNBr-treated receptor complex was stored at 20°C in elution buffer (25 mM Tris, 192 mM glycine, and 0.02% SDS) before chemical/enzymatic treatment (see below).

**Pepitide N-Glycosidase F Digestion—** The CNBr-cleaved and concentrated radioligand-receptor complex was treated with peptide N-glycosidase F (2500 units) for 1 h at 37°C in 30 μl of 50 mM sodium phosphate (pH 7.5), 0.5% SDS, 1% β-mercaptoethanol, and 1% Nonidet P-40 according to the protocol provided by the supplier.

**RESULTS**

**Functional Characterization of [Bpa<sup>23</sup>]PTHrP—** [Bpa<sup>23</sup>]PTHrP was tested in competition binding studies performed with COS-7 cells expressing the native rP1R and was found to have an apparent binding affinity that is indistinguishable from that of bNePTH and of other analogs of PTH and PTHrP (7, 10). The Bpa-containing PTHrP analog was also fully functional in cAMP accumulation assays and exhibited a potency that was indistinguishable from that of bNePTH (data not shown).

**Photoaffinity Labeling of Rat PTH/PTHrP Receptors—** After binding and photoactivation, the covalent complex formed between radioiodinated [Bpa<sup>23</sup>]PTHrP and the rP1R was visualized by analytical SDS-PAGE and subsequent autoradiography. The complex migrated as a single broad band corresponding to a glycosylated protein with a molecular mass of ~70 kDa (Fig. 1, lane 1). This size of the ligand-receptor complex is comparable to that previously seen with other photoactivatable PTH or PTHrP analogs using either cells expressing endogenous PTH/PTHrP receptors (29–32) or HEK-293 cells expressing the cloned P1R (18). Coincubation of transfected COS-7 cells with 12<sup>5</sup>I-[Bpa<sup>23</sup>]PTHrP and unlabeled bPTH (1–34) (10<sup>–6</sup> M) or unlabeled [Bpa<sup>23</sup>]PTHrP (10<sup>–6</sup> M) completely...
eliminated the formation of the radiolabeled ligand-receptor complex (data not shown).

To identify the region of the P1R that interacted with the Bpa\textsuperscript{23} moiety, we isolated larger amounts of the radiolabeled ligand-receptor complex using preparative SDS-PAGE, cleaved the partially purified complex with CNBr, and separated the cleavage products on analytical gels. After CNBr cleavage, most of the radioactivity migrated on SDS-PAGE as a single sharp protein band corresponding to a size of \( <14 \text{ kDa} \) (Fig. 1, lane 2). A minor fraction migrated as a diffuse band at the 46-kDa size marker and probably corresponded to a partially cleaved glycosylated ligand-receptor complex. Tricine/SDS-PAGE analysis was used to achieve higher resolution in the low molecular mass range, and this suggested a molecular size of \( \approx 9 \text{ kDa} \) for the principal radiolabeled CNBr-generated fragment (see also Fig. 5, lane 1). Since [Bpa\textsuperscript{23}]PTHrP has a molecular size of 4.286 kDa, the receptor fragment contributing to the complex was estimated to have a molecular size of \( \approx 5 \text{ kDa} \). The same results were obtained when analyzing the complex formed between radiolabeled [Bpa\textsuperscript{23}]PTHrP and the endogenous PTH/PTHrP receptor of ROS 17/2.8 cells (data not shown).

The above results suggested that Bpa at position 23 of PTHrP interacts with an \( \approx 5 \text{ kDa} \) non-glycosylated CNBr-generated portion of the receptor. Inspection of the amino acid sequence of the rP1R showed that several fragments delimited by methionine residues are within this molecular size range (Fig. 2). Because of the predicted overall architecture of ligand-receptor interaction (1, 11, 15), we considered the hypothesis that Bpa\textsuperscript{23} interacts with the receptor segment defined by Met\textsuperscript{63} and the amino-terminus, which is predicted to be Tyr\textsuperscript{23} by a recently developed algorithm (33). A mutant rP1R was generated, rP1R-M63I, in which methionine at position 63 was replaced by isoleucine (Fig. 3B); this mutant receptor had functional (data not shown) and cross-linking (Fig. 1, lane 3) properties that were indistinguishable from the wild-type rP1R. CNBr cleavage of the covalently labeled rP1R-M63I mutant yielded to a broad radioactive band comigrating with the 46-}

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**Fig. 1.** Analysis of the CNBr-digested [Bpa\textsuperscript{23}]PTHrP-P1R complex using COS-7 cells expressing the wild-type rat PTH/PTHrP receptor and the M63I receptor mutant. As described under "Experimental Procedures," the partially purified complex of ligand and wild-type P1R (lanes 1 and 2) or the M63I receptor mutant (lanes 3 and 4) was incubated in 70% trifluoroacetic acid in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of CNBr (100 mM). After repeated lyophilization, samples were analyzed by SDS-PAGE with subsequent autoradiography (overnight at \( 280 \, ^\circ \text{C} \)). The positions of different size markers are indicated in kilodaltons.

**Fig. 2.** Schematic model of the rat P1R showing the seven putative transmembrane domains and the locations of all 14 methionine residues in the mature protein. The amino-terminus is at the top. Potential N-linked glycosylation sites (\( \circ \)), the putative signal peptide, and the predicted first residue (Tyr\textsuperscript{23}) of the mature receptor are shown. The sites for leucine (\( L \)) to methionine (\( M \)) and for methionine (\( M \)) to isoleucine (\( I \)) substitutions and the location of methionine residues (\( \bullet \)) are indicated (see also Fig. 3).

**Fig. 3.** Schematic drawing of the amino-terminus of the wild-type rat PTH/PTHrP receptor and two receptor mutants. The predicted signal sequence cleavage site (arrow), the sites for N-linked glycosylation (\( \circ \)), and the locations of methionine residues (\( \bullet \)) and the introduced isoleucine (\( \square \)) are indicated. The calculated molecular sizes of CNBr fragments are indicated in daltons. A, wild-type P1R; B, P1R-M63I; C, P1R-M63I/L40M.
kDa marker (Fig. 1, lane 4, and Fig. 4, lane 1); this cleavage product was reduced to a sharp protein band of 20 kDa upon further digestion with peptide N-glycosidase F (Fig. 4, lane 2). The positions of different size markers are indicated in kilodaltons.

FIG. 4. Analysis of the CNBr-digested [Bpa\(^{23}\)]PTHrP-P1R-M63I complex using COS-7 cells expressing the M63I receptor mutant. As described under “Experimental Procedures,” the partially purified, CNBr-cleaved radioligand-receptor complex (lane 1) was digested with peptide N-glycosidase F (lane 2). Samples were analyzed by Tricine/SDS-PAGE with subsequent autoradiography (14 days at –80 °C). The positions of different size markers are indicated in kilodaltons.

To further define the site of cross-linking, we introduced methionine substitutions at either Leu\(^{40}\) or Leu\(^{41}\) in the rP1R-M63I mutant to yield the double mutants rP1R-M63I/L40M and rP1R-M63I/L41M, respectively (Fig. 3C). Like rP1R M63I, these two mutants had biological properties that were indistinguishable from those of the wild-type rP1R (data not shown). Leu\(^{40}\) and Leu\(^{41}\) were chosen because their substitution with methionine is a conservative replacement and because CNBr cleavage at these positions would yield ligand-receptor conjugates whose size upon SDS-PAGE analysis would easily distinguish between the two possible sites of interaction with [Bpa\(^{23}\)]PTHrP. Thus, cross-linking to a site amino-terminal of Met\(^{63}\) or Met\(^{41}\) would yield non-glycosylated, low molecular mass complexes corresponding to receptor residues 23–40 and 23–41, respectively, whereas cross-linking to a site carboxyl-terminal of either mutation would yield glycosylated, high molecular mass complexes corresponding to residues 41–174 and 42–174, respectively.
As shown in Fig. 5 (lane 3), CNBr cleavage of the affinity-labeled M63I/L40M mutant yielded a small radiolabeled, non-glycosylated complex of ~6 kDa, as did cleavage of the M63I/L41M mutant (data not shown). This indicated that the covalent interaction between Bpa23 and the rP1R occurred between the receptor’s amino-terminus and Leu 40.

Effects of Point Mutations in the Amino-terminal Extracellular Domain of the PTH/PTHrP Receptor on Ligand Binding—

The amino-terminal receptor fragment identified by the above physicochemical approach overlaps a P1R region previously shown by functional studies to be important for ligand binding (24). Two mutant receptors with deletions of residues 26–60 (the E1 region) or 31–47 (E1a) were shown to have only moderately reduced receptor expression levels in COS-7 cells (22 ± 1 and 36 ± 3% of the wild type, respectively) and little or no capacity to bind radiolabeled PTH (24). To further examine the functional importance of residues in this amino-terminal E1a region, we first made four cassette mutant receptors, termed E1a-1 through E1a-4, in which four or five adjacent residues were replaced by either alanine or valine (Fig. 6, A and B). Each mutant receptor was adequately expressed on the surface of COS-7 cells (~35% of the wild-type) (Fig. 6C). The two mutants in which residues 31–35 and 36–39 were altered displayed diminished 125I-rNlePTH binding capacity, whereas the two mutants with substitutions of residues 40–43 and 44–47, respectively, maintained high levels of PTH binding (Fig. 6D).

To further localize candidate binding residues within region 31–39 (E1a-1 and E1a-2), an alanine-scanning approach was used. Several of the individual alanine substitutions in this region, which had little or no effect on cell surface expression (Fig. 7A), resulted in small reductions in 125I-rNlePTH binding capacity (Fig. 7B). A reduction in PTH binding of ~25% occurred with two substitutions, T33A and Q37A (Fig. 7B). In addition, each of these two point mutations had a more severe effect on 125I-PTHrP binding than on 125I-rNlePTH binding (Fig. 7C). In competition binding studies with 125I-rNlePTH as tracer radioligand, the apparent binding affinity of rNlePTH for wild-type and mutant P1Rs was comparable (Fig. 8A). The apparent binding affinity of bNlePTH for the T33A and Q37A mutant receptors was 5.0- and 2.3-fold weaker, respectively, than it was for the wild-type receptor (Fig. 8B). Consistent with the reduced maximal binding of radiolabeled PTHrP, the apparent binding affinity of PTHrP-(1–36) for these two mutant receptors was 14- and 48-fold weaker, respectively, than it was for the wild-type receptor (Fig. 8C). Both receptor mutations also abolished binding of an amino-terminally truncated PTHrP analog, [Leu11,D-Trp12]rPTHrP-(7–34)-amide, indicating that Thr33 and Gln37 affect interactions with region 7–34 of the ligand rather than with region 1–6 (Fig. 8D and Table I).

**DISCUSSION**

Previous studies have suggested that the amino-terminal extracellular region of the PTH/PTHrP receptor interacts with...
carboxy-terminal region 15–34 of either PTH or PTHrP; a similar architecture of ligand-receptor interaction may well apply to other members of this family of G protein-coupled receptors (9–15). In this study, we confirmed and extended these predictions for the P1R with a PTHrP analog containing photoreactive Bpa at position 23, a residue with apparent functional significance based on its ability to determine ligand binding specificity in the P2R (7, 11). After CNBr digestion of [Bpa23]PTHrP-P1R complexes, an ~9-kDa radiolabeled protein was detected upon Tricine/SDS-PAGE analysis. This fragment was likely to represent 125I-[Bpa23]PTHrP covalently coupled to a receptor fragment extending from Tyr23, the first residue after the predicted cleavage site for the signal peptide (33), to Met46, the first methionine in the mature receptor sequence. We confirmed this assignment and refined the mapping further by using site-directed mutagenesis to introduce or remove methionines at strategic sites in the receptor. First, the rP1R-M63I mutant was generated and shown to be fully functional. When the ligand-receptor complex formed with this receptor was cleaved with CNBr, the ~9-kDa band was replaced by an ~46-kDa glycosylated band corresponding to the receptor fragment extending from the amino-terminus to Met174. This receptor segment contains three of the four potential CNBr-derived receptor fragments of similar size, such as Ala126–Met450. Two additional, fully functional receptor double mutants, rP1R-M63I/L40M and rP1R-M63I/L41M, were prepared to further refine the cross-linking site. Both mutants contained the M63I mutation to eliminate the natural CNBr cleavage site at position 63. CNBr cleavage of the complexes formed between 125I-[Bpa23]PTHrP and either of these two mutant receptors resulted in low molecular mass radiolabeled protein conjugates (Fig. 5). This result established that Bpa23 was cross-linked to a side amino-terminal to Met46 in the rP1R and clearly excluded segment 41–174 as the site of interaction. Earlier mutagenesis studies had indicated that deletion of a portion of the rP1R that included 17 residues (the E1a region) close to the amino-terminus of the mature receptor abolished binding of radiolabeled PTH or PTHrP, with only moderate effects on receptor expression (24). To further map functional binding residues in this region, we constructed four “cassette” mutants in which four or five adjacent amino acids were replaced by alanine or valine. Two of these cassette mutants, E1a-1 and E1a-2, showed normal cell-surface expression, but little or no binding of radiolabeled PTH or PTHrP. These results suggested that residues within segments 31–35 and 36–39 contribute to ligand interaction. The replacement of each of these nine residues with individual alanine substitutions confirmed this hypothesis. Two mutants, rP1R T33A and rP1R Q37A, exhibited the weakest capacity to bind the radioligand. Interestingly, the effects of these mutations on ligand binding were more pronounced with PTHrP than with PTH; this pattern might be attributable to the divergence in region 15–34 of these two ligands, a hypothesis supported by the observation that the mutations also impaired PTHrP(7–34) binding.

In summary, our physicochemical observations indicate that Bpa23 (and presumably Phe23) in the native PTHrP molecule interacts with residues at the extreme amino-terminus of the PTH/PTHrP receptor. Mutational analysis of this receptor region supported this conclusion and identified two amino acid residues, Thr34 and Gln37, as possible sites for ligand interaction. The combined use of the two techniques, photoaffinity cross-linking and receptor mutagenesis, should enable the definition of other receptor segments that comprise contact points for PTH and PTHrP.

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REFERENCES

1. Segre, G. V. (1996) in Principles in Bone Biology (Bilezikian, J. P., Raiziss, L. G., and Rodan, G. A., eds) pp. 377–403, Academic Press, New York
2. Lanske, B., Karaplis, A. C., Luz, A., Vartkamp, A., Pirro, A., Karperien, M., Defize, I. J. H. K., Ho, C., Mulligan, R. C., Abou-Samra, A. B., Jüppner, H., Segre, G. V., and Kronenberg, H. M. (1996) Science 275, 663–666
3. Jüppner, H., Abou-Samra, A. B., Freeman, M. W., Kong, X. F., Schipani, E., Urena, P., E. Bonventre, J. V., Potts, J. T. Jr., Kronenberg, H. M., and Segre, G. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2732–2736
4. Schipani, E., Karg, H., Karaplis, A. C., Potts, J. T. Jr., Kronenberg, H. M., Segre, G. V., Abou-Samra, A. B., and Jüppner, H. (1997) Endocrinology 132, 2157–2165
5. Usdin, T. B., Gruber, C., and Bonner, T. I. (1995) J. Biol. Chem. 270, 15455–15459
6. Usdin, T. B., Gruber, C., and Bonner, T. I. (1996) J. Biol. Chem. 271, 19397–19400
7. Gardella, T. J., Luck, M. D., Usdin, T. B., and Jüppner, H. (1996) J. Biol. Chem. 271, 19868–19893
8. Behar, V., Nakamoto, G., Gruber, C., Zissis, A., Al-Zein, S., Suva, J. L., Rosenblatt, M., and Chorev, M. (1996) Endocrinology 137, 4217–4224
9. Turner, P. R., Mefford, S., Bambino, T., and Nissenov, R. A. (1995) J. Biol. Chem. 270, 3836–3837
10. Jüppner, H., Schipani, E., Brighurst, F. R., McClure, I., Keutmann, H. T., Potts, J. T., Jr., Kronenberg, H. M., and Segre, G. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9732–9736
11. Bergweitz, C., Jussemaua, S. A., Luck, M. W., Potts, J. T. Jr., and Hjort, J. (1995) J. Biol. Chem. 271, 9828–9868
12. Clark, J. A., Bonner, T. I., Kim, A. S., and Usdin, T. B. (1998) Mol. Endocrinol. 12, 183–206
13. Strop, S. D., Kuestner, R. E., Serwold, T. F., Chen, L., and Moore, E. R. (1995) Biochemistry 34, 1050–1057
14. Turner, P. R., Bambino, T., and Nissenov, R. A. (1998) J. Biol. Chem. 273, 9205–9208
15. Bergweitz, C., Gardella, T. J., Flannery, M. R., Potts, J. T. Jr., Kronenberg, H. M., Goldring, S. R., and Jüppner, H. (1996) J. Biol. Chem. 271, 26469–26472
16. Boyd, N., Kage, R., Dumas, J., Krause, J., and Leeman, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 433–437
17. Suva, L., Flannery, M., Callfeld, J., Findlay, D., Jüppner, H., Goldring, S., Rosenblatt, M., and Chorev, M. (1997) J. Pharmacol. Exp. Ther. 283, 876–884
18. Zhou, A. T., Essalle, R., Bisello, A., Nakamoto, C., Rosenblatt, M., Suva, L. J., and Chorev, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3644–3649
19. Danzian, G., and Prestwich, G. D. (1994) Biochemistry 33, 5661–5673
20. Mach, G., and Prestwich, G. D. (1994) Biochemistry 33, 5661–5673
21. Bisello, A., Greenberg, Z., Behar, V., Rosenblatt, M., Suva, L. J., and Chorev, M. (1997) J. Bone Miner. Res. 12, Suppl. 1, S363
22. Gardella, T. J., Jüppner, H., Abou-Samra, A. B., Segre, G. V., Bringham, F. R., Potts, J. T. Jr., Nussbaum, S. R., and Kronenberg, H. M. (1994) Endocrinology 135, 1186–1194
23. Lee, C., Luck, M. D., Jüppner, H., Potts, J. T. Jr., Kronenberg, H. M., and Gardella, T. J. (1995) Mol. Endocrinol. 9, 1269–1278
24. Lee, C., Gardella, T. J., Abou-Samra, A. B., Nussbaum, S. R., Segre, G. V.,
Potts, J. T., Jr., Kronenberg, H. M., and Ju¨ppner, H. (1994) Endocrinology 135, 1488–1495.

25. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488–492
26. Yamamoto, I., Shigeno, C., Potts, J. T., Jr., and Segre, G. V. (1988) Endocrinology 122, 1208–1217

27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
29. Shigeno, C., Hiraki, Y., Westerberg, D. P., Potts, J. T., Jr., and Segre, G. V. (1988) J. Biol. Chem. 263, 3864–3871
30. Shigeno, C., Hiraki, Y., Westerberg, D. P., Potts, J. T., Jr., and Segre, G. V. (1988) J. Biol. Chem. 263, 3872–3878
31. Ju¨ppner, H., Abou-Samra, A. B., Uneno, S., Gu, W. X., Potts, J. T., Jr., and Segre, G. V. (1988) J. Biol. Chem. 263, 8557–8560
32. Orloff, J. J., Wu, T. L., and Stewart, A. F. (1989) Endocr. Rev. 10, 476–495
33. Nielsen, H., Egelbrecht, J., Brunak, S., and von Heijne, G. (1997) Protein Eng. 10, 1–6
Evidence for a Ligand Interaction Site at the Amino-Terminus of the Parathyroid Hormone (PTH)/PTH-related Protein Receptor from Cross-linking and Mutational Studies

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