Human PTH gene regulation in vivo using transgenic mice

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Lavi-Moshayoff V, Silver J, Naveh-Many T. Human PTH gene regulation in vivo using transgenic mice. Am J Physiol Renal Physiol 297: F713–F719, 2009. First published July 1, 2009; doi:10.1152/ajprenal.00161.2009.—To study the regulation of the human PTH (hPTH) gene in vivo, we generated transgenic mice with the hPTH gene expressed in the mouse parathyroid using a bacterial artificial chromosome (BAC) containing the hPTH gene within its 144-kb chromosomal region. The BAC construct maintains the native hPTH gene surrounding sequences and isolates it from positional effects. The transgenic mice had normal levels of serum mouse PTH (mPTH) in addition to both intact and bioactive hPTH. Despite the presence of both mPTH and hPTH, serum calcium and 1,25(OH)2 vitamin D levels were normal. The lack of response to hPTH may be due to tachyphylaxis of the mPTH receptor (PTH1R) and/or impaired recognition of the mPTH1R. In contrast, the regulation of hPTH levels in the mouse was intact. A calcium-depleted diet increased serum mPTH and both intact and bioactive hPTH. mPTH and hPTH mRNA levels were also markedly increased. The calcimimetic R-568 dramatically decreased mPTH and hPTH serum levels. Administered recombinant fibroblast growth factor (FGF)/23 decreased hPTH. Therefore, the regulation of hPTH gene expression and serum hPTH levels is intact in the transgenic mice, indicating preservation of the signal transduction of the parathyroid calcium receptor and the Klotho-FGF receptor between mouse and man.

parathyroid hormone; calcium; calcimimetic; fibroblast growth factor 23

PARATHYROID HORMONE (PTH) secretion has been studied extensively in man and laboratory animals. The major stimulus to PTH secretion is hypocalemia. In situations such as chronic kidney disease (CKD), other factors such as a low 1,25(OH)2 vitamin D [1,25(OH)2D] and a high-serum P(1) (27) participate. Both 1,25(OH)2D and FGF23 are factors that decrease serum PTH levels (2, 29). Serum PTH is actively regulated at the levels of secretion, intracellular PTH degradation, and PTH gene expression and in the longer term in parathyroid cell proliferation (27). The parathyroid has a small number of preformed secretory granules and subsequently only a limited capacity to increase serum PTH from preformed stores after appropriate stimuli. It has been estimated that bovine parathyroid cells exposed to a hypocalcemic challenge would become depleted of PTH after about 1.5 h unless a new hormone was synthesized (10). The parathyroid compensates for this by increasing the synthesis of PTH at the levels of gene expression and parathyroid cell hyperplasia. There have been numerous studies on the mechanisms of how calcium (Ca), P(1), and FGF23 regulate PTH gene expression in rats and mice (19, 24). 1,25(OH)2D decreases PTH gene transcription in vivo and in vitro (5, 12, 29, 30).

A 5-kb promoter region of the human PTH (hPTH) gene has been used effectively to target parathyroid-specific overexpression of transgenes such as cyclin D1 (11) and Gqα (23) or Cre lox deletion of Gqα (34) and the vitamin D receptor (VDR) (Naveh-Many T and Silver J, unpublished observations). The PTH gene consists of three exons and two introns (15, 16). The physiological regulation of PTH gene expression by calcium and P(1), and in CKD is posttranscriptional and mediated by the PTH mRNA 3′-untranslated region (UTR) that is coded by exon 3, which also codes for the mature hormone (18, 19). Dietary calcium and P(1) depletion as well as experimental CKD lead to changes in the binding of protective and degrading trans-acting cytosolic proteins in the parathyroid to a defined cis-acting adenine- and uridine-rich element (ARE) in the PTH mRNA 3′-UTR. The calcimimetic R-568 acts to decrease serum PTH and PTH gene expression using the same mechanism. These protein-RNA interactions determine PTH mRNA half-life and hence, PTH synthesis (13, 17, 21, 22, 26).

The studies on the regulation of PTH expression have all been performed in rodents or in bovine parathyroid primary cultures. It is of interest to understand whether human PTH gene expression is similarly regulated. To study this question, we have generated transgenic mice expressing the hPTH gene (hPTH Tg). The hPTH gene and its regulatory sequences were inserted as part of a large bacterial artificial chromosome (BAC) to isolate it from positional effects. We then studied the regulation of hPTH in the mouse by calcium, calcimimetics, and FGF23.

MATERIALS AND METHODS

Generation of hPTH Tg mice. The BAC RPCI11 15D14 was chosen by the NCBI Map Viewer tool, since it contains the whole hPTH gene and upstream and downstream sequences. The BAC was purchased from http://bacpac.chori.org, and the bacteria were grown and BAC DNA isolated according to the web site protocol (http://bacpac.chori.org/bacpacmini.htm). The BAC was linearized by digestion with PI-SceI (New England Biolabs, Ipswich, MA). DNA purification was performed as described (8), with an additional step of phenol-chloroform-isoamylalcohol precipitation to separate DNA from precipitated proteins. Isolated BAC DNA was run on a 0.7% agarose gel to view integrity of the DNA, and the corresponding DNA from precipitated proteins. Isolated BAC DNA was run on a 0.7% agarose gel to view integrity of the DNA, and the corresponding DNA band was cut and dialyzed against Tris-acetate-EDTA buffer and electroporated at 85 V for 35–40 min. DNA was ethanol precipitated and diluted to 0.2 ng/μl in injection buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA, 100 mM NaCl, 30 mM spermine, 70 mM spermidine). DNA was injected into pronuclei of fertilized oocytes of CB6F1 mice, which were transferred into pseudopregnant female mice. Eighteen offspring were born, but only one female was identified as hPTH positive by PCR. After being bred with wild-type (WT) CB6F1 males, positive hPTH second-generation offspring were crossed among themselves so that a higher ratio of hPTH-positive mice was received. All animal experiments were approved by the Hadassah Hebrew University Animal Care and Use Committee.

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**Isolation of DNA for genotyping.** The mice were ear pierced or tail clipped, and the tissue was lysed in lysis buffer (10 mM Tris, pH 7.8, 2 mM EDTA, 400 mM NaCl, 0.7% SDS, and 50 μg of proteinase K) for 2 h at 55°C and DNA ethanol precipitated.

**PCR analysis.** Genotyping of the hPTH gene was performed using the primers: forward (fw) `5′-ttatgattgtcatgttggca-3′` and reverse (rev) `5′-taaggtagaagagaggt-3′` at an annealing temperature of 52°C with a product of 700 bp. The mouse PTH (mPTH) gene was amplified by the primers fw `5′-gtctcttccaatgat-3′` and rev `5′-ttcatgatcattaaacttta-3′` at an annealing temperature of 52°C with a product of 100 bp. The hBTBD10 gene was amplified by the primers fw `5′-attgtgagctctcgcggtaa-3′` and rev `5′-ctgtagtcccccttcagtgc-3′` at an annealing temperature of 51°C, which results in a product of 200 bp. For sequencing of the hPTH cDNA, a fragment of 800 bp was amplified by the primers fw `5′-tagtttactcagcatcagctac-3′` and rev `5′-taaggtagaagagaggt-3′` at an annealing temperature of 46°C. Sequencing was performed with the forward primer.

**Low-Ca diet and injections of R-568 and FGF23.** These were performed using previously described methodology (2, 17, 20). Compounds were injected ip daily for 4 days, with the last injection 1 h before sampling.

**mPTH and hPTH biochemistry.** Blood was drawn by bleeding of the lateral tail vein or by exsanguination from abdominal vena cava. Human bioactive PTH and mouse intact PTH ELISA were performed using kits from Immutopics (San Clements, CA), and intact hPTH was performed using Immulite 2000. There is no cross-reactivity between the mPTH and hPTH assays. The Immutopics human bioactive PTH ELISA kit uses a hPTH-(39–84)-directed biotinylated capture antibody and a human PTH-(1–34)-directed horseradish peroxidase-labeled revealing antibody. The Immutopics mouse intact PTH ELISA kit uses a rat PTH-(39–84)-directed biotinylated capture antibody and a rat PTH-(1–34)-directed horseradish peroxidase-labeled revealing antibody. It uses rat PTH-(1–84) as standard, but the equimolar reactivity between rat PTH-(1–84) and mPTH-(1–84) is unknown. Serum biochemistry for calcium and phosphorus was measured using "QuantiChrom" kits (BioAssay Systems, Hayward, CA). Serum 1,25(OH)2 vitamin D3 was also measured (DiaSorin, Saluggia Italy).

**Statistical analysis.** All statistical calculations and plots were performed using Microsoft Excel (Microsoft, Redmond, WA). Statistical significance was determined using Student’s t-test. Plot bars depict means ± SE. *P < 0.05.

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**Fig. 1.** Generation of the transgenic mice expressing the human parathyroid hormone gene (hPTH Tg). A: schematic representation of human chromosome 11p15 showing the hPTH and BTBD10 genes. The 144-kb RP11-15D14 bacterial artificial chromosome (BAC) is shown below, including the 7-kb hPTH gene and part of the BTBD10 gene at the 5′-end. B: PCR product of tail DNA from wild-type (WT) and hPTH Tg (Tg) mice and a plasmid (P) expressing the hPTH gene, using primers specific for hPTH (top) and mouse PTH (mPTH; bottom). The size of PCR products was consistent with the predicted lengths of the amplified fragments. C: Northern blot analysis of mouse thyro/parathyroid tissue from WT and hPTH Tg (Tg) mice hybridized with probes for hPTH (left) or mPTH (right). The hPTH is ~150 nt longer than the mPTH. Bottom blots show ethidium bromide staining of the membranes with the 28S and 18S ribosomal RNAs. The blots were hybridized sequentially with the 2 probes.

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**Fig. 2.** Body weight and serum analysis of the hPTH Tg mice. A: body weight at different ages in male and female mice. B and C: serum calcium (Ca) and P, (n = 20) at 6 wk. D: serum 1,25(OH)2 vitamin D [1,25(OH)2D] levels (n = 6). Results for WT and hPTH Tg mice are presented as means ± SE. *P < 0.05.
standard error. A two-sided $P$ value was considered significant when $<0.05$.

RESULTS

Generation of hPTH Tg mice. We have generated mice with a human BAC containing the hPTH gene within its 144-kb chromosomal region (hPTH Tg; Fig. 1A). The BAC construct was used to maintain the native hPTH gene-surrounding sequences and to isolate it from insertional effects. Tail DNA PCR using primers specific for the hPTH gene confirmed inclusion of the hPTH sequence in the mouse genome (Fig. 1B). PCR for another gene, BTBD10, which is partially included at the 5'-end of the BAC (Fig. 1A), and sequencing of tail DNA PCR products from the 5'- and 3'-ends of the BAC confirmed that both ends of the BAC were introduced in the mouse (not shown). The mice expressed both mPTH and hPTH mRNA of the expected sizes in their parathyroids, indicating that the hPTH mRNA is correctly transcribed and processed in the mouse parathyroid (Fig. 1C). The hPTH probe did not detect any signal in the WT mice, indicating no cross-reactivity (Fig. 1C). Sequencing of PTH cDNA prepared from parathyroids of the hPTH Tg mice showed the predicted mRNA sequence. There was no expression of hPTH in other mouse tissues such as liver, kidney, and thymus (not shown).

The body weight of the hPTH Tg mice was slightly decreased at early ages but not at later ages studied (Fig. 2A). Serum calcium and 1,25(OH)2D levels were the same in hPTH Tg and WT littermates (Fig. 2B and D). Similar results were obtained at 32 wk for Ca [WT 8.3 ± 0.5 mg/dl, hPTH Tg 7.8 ± 0.06 mg/dl; $n = 6$, $P = $ not significant (NS)] and 1yr for 1,25(OH)2D (Fig. 2D). Serum phosphate was increased slightly in the hPTH Tg mice at 6 wk (Fig. 2C) but not at 32 wk (WT 8.45 ± 0.11 mg/dl, hPTH Tg 10.3 ± 0.08 mg/dl; $n = 6$, $P = $ NS). The increased serum Pi levels occurred at 6 wk, when there was a transient decrease in weight. The hPTH Tg mice expressed mPTH in the serum at the same level as the WT mice (Fig. 3). In addition, the hPTH Tg mice expressed hPTH measured as both bioactive [PTH-(1–84)] and intact [PTH-(1–84) and -(7–84)] PTH. The ratio of intact to bioactive hPTH was ~5:1. This ratio is larger than that seen in normal humans, where it is of the order of 2–3:1 (9).

Intact regulation of hPTH expression by the Ca receptor. The Ca receptor (CaR) is the major regulator of the parathyroid. Dietary Ca depletion leads to decreased serum Ca, increased serum Pi, and 1,25(OH)2D, and a marked increase in serum PTH and PTH mRNA levels in rats (18). Ca depletion had a similar effect on serum Ca and Pi in WT mice (Fig. 4A and B). In hPTH Tg mice there was a decrease in serum Ca, but serum Pi was also decreased rather than increased (Fig. 4C and D). The Ca-depleted diet increased serum-intact mPTH in both WT and hPTH Tg mice (Fig. 5A and B). In the hPTH Tg mice, both bioactive hPTH and intact hPTH were increased

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**Fig. 3.** Serum mPTH and hPTH in the hPTH Tg mice. Intact mPTH-(1–84) and mPTH-(7–84) and intact hPTH-(1–84) and hPTH-(7–84) and bioactive hPTH-(1–84) in WT and hPTH Tg mice at 6 wk of age. Results are presented as means ± SE.

**Fig. 4.** Serum Ca and Pi levels in WT and hPTH Tg mice fed a control or a Ca-depleted diet. Weanling WT (A and B) and hPTH Tg (C and D) mice were fed a control or a Ca-depleted diet for 3 wk when serum Ca and Pi, levels were measured. Results are presented as means ± SE ($n = 12$). $*P < 0.05$. 

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Bioactive hPTH was increased about threefold, and intact hPTH was increased about twofold. Intact mPTH was increased 10-fold, similar to the increase in in the WT mice (Fig. 5A and rat PTH after the same diet given to rats (18). There is no bioactive mPTH assay. The low-Ca diet also led to an increase in both mouse and human PTH mRNA levels in the hPTH Tg mice (Fig. 5, C and D). Therefore, the stimulus of chronic hypocalcemia leads to an increase in hPTH in the transgenic mice, although the effect is less dramatic than that of the endogenous mPTH.

We next studied the effect of activation of the CaR by the calcimimetic R-568. R-568 led to the expected decrease in intact mPTH in WT mice (Fig. 6A). R-568 also decreased both serum-intact mPTH and hPTH levels in the hPTH Tg mice (Fig. 6B). There was also a decrease in serum Ca and P levels after R-568 (Fig. 6, C and D). Thus both hPTH and mPTH respond to the calcimimetic in these hPTH Tg mice, but there are large differences in the extent of the decrease after R-568. Together, these experiments demonstrate that the regulation of hPTH expression by the CaR is conserved between man and mouse.

Intact regulation of hPTH expression by FGF23. We and others have recently shown that FGF23 acts on the parathyroid to decrease PTH gene expression in vivo and in vitro (2, 14). Recombinant intraperitoneal FGF23 led to a decrease in serum mPTH in WT mice and in serum hPTH in the hPTH Tg mice (Fig. 7, A and B) that was similar to the reported decrease in serum PTH in rats and mice after FGF23 (2, 35). In the hPTH Tg mice, there was a decrease in serum Ca and no change in P levels after FGF23 (Fig. 7, C and D).
serum Pi (Fig. 7, C and D). Therefore, FGF23 regulates serum hPTH levels when expressed in the mouse.

**DISCUSSION**

We have generated transgenic mice expressing the hPTH gene using a human BAC to study the response of the hPTH gene to physiological and pharmacological stimuli. The transgenic mice expressed both mPTH and hPTH. Therefore, the hPTH gene was transcribed and the pre-mRNA processed and expressed as mature prepro-PTH mRNA of the correct size. Immunoreactive hPTH was measured both by bioactive and intact PTH assays in the hPTH Tg mice, indicating that hPTH is expressed and that the translated hormone is metabolized to the mature bioactive hPTH-(1–84) and the intact hPTH-(7–84) in the mouse. The ratio of intact to bioactive hPTH was greater than the ratio in humans. In normal individuals, non-PTH-(1–84) PTH fragments [PTH-(7–84)-like fragments] normally represent ~20% of intact PTH immunoreactivity and ≤50% in renal failure by HPLC (3). In the present study we used the Immulite Intact PTH assays, which react more on a molar basis with hPTH-(1–84) than hPTH-(7–84) (32). Therefore, the differences between the intact PTH assay and the human bioactive PTH assay are probably related to a difference in hPTH-(1–84) standardization (4). The differences in these assays may affect the estimated ratio reported here. There is no kit to measure bioactive mPTH, so the ratio in mice and also in rats is not known. The intact hPTH levels are eightfold higher than the mPTH levels in the Tg mice (Fig. 3). However, it is not meaningful to compare the human and the murine assays because of different affinities of the antibodies for mPTH, hPTH, and the PTH standards utilized. Despite the large amounts of hPTH in the circulation, in addition to the normal levels of mPTH, serum calcium and 1,25(OH)2D were all normal. Serum Pi was unexpectedly slightly and transiently increased. Usatii et al. (33) showed that hPTH-(1–84) had no effect on either phosphate or 1,25(OH)2D concentrations when infused to parathyroidectomized rats. However, active rat PTH-(1–34) decreased phosphate to the same level as vehicle-treated rats and increased 1,25(OH)2D. Therefore, the recognition of hPTH by the rat renal mPTH receptor (PTH1R) may be impaired. Wilson et al. (34a) used somatic cell gene transfer of hPTH-(1–84), which resulted in hypercalcemia and osteoclastic skeletal resorption mediated by increased serum concentrations of hPTH ≤60 days. Therefore, the bone PTH1R does recognize the hPTH. In the present report the hPTH is expressed throughout the life of the transgenic mouse, which may have led to different effects, such as downregulation of the PTH1R. Alternatively, the COOH-terminal fraction of the hPTH molecule may interact with the putative COOH-terminal receptors. Slatopolsky et al. (31) administered hPTH-(1–84) and hPTH-(7–84) simultaneously in a 1:1 molar ratio; the calcemic response to hPTH-(1–84) was decreased by 94%. This observation supports the concept that there is a separate COOH-terminal PTH receptor that antagonizes the action of PTH-(1–84). In addition, in the Tg mice the hPTH may interfere with the action of mPTH. The normal calcium and

![Figure 7](http://ajprenal.physiology.org/)

Fig. 7. FGF23 decreases serum mPTH and hPTH. WT or hPTH Tg mice were treated with 4 daily ip injections of recombinant FGF23 (10 μg/mouse) or vehicle (control). A: serum mPTH in WT mice (n = 4) B: hPTH in hPTH Tg mice (n = 5). C and D: serum calcium and Pi in hPTH Tg mice (n = 5). *P < 0.05.
1,25(OH)₂D levels can be explained by these mechanisms. The increased basal serum P₃ levels in the hPTH Tg mice are not readily understandable. The high-serum P₃ may be a consequence of the interference of human COOH-terminal PTH in the action of the hPTH-(1–84). The decrease in serum P₃ in the Tg mice after the calcimimetic is compatible with the observation by Fox et al. (6) that R-568 leads to a transient decrease in serum P₃ at 1 h. They suggested that the initial hypophosphatemic response observed with the higher doses of NPS R-568 may be attributable to calcitonin-mediated inhibition of bone resorption. It is also possible that the administration of calcimimetics reduced the amount of circulating hPTH, which may facilitate the effect of the mPTH and the subsequent reduction in serum phosphate.

1,25(OH)₂D decreases PTH gene transcription in the rat in doses that do not increase serum calcium (29). In vitro in bovine parathyroid cells, 1,25(OH)₂D similarly decreases PTH gene transcription, which is also independent of serum calcium (25). In the mouse, we have found that it is not possible to separate the effect of 1,25(OH)₂D to decrease PTH expression from its hypercalcemic effects even at low 1,25(OH)₂D doses. Therefore, we were unable to study the regulation of PTH gene transpression in this hPTH Tg mouse model without introducing the posttranscriptional influence of calcium. hPTH responds to both the Caₐ and the FGF23 receptor in the hPTH Tg mice, as evidenced by the increase in hPTH by a calcium-depleted diet and the decreases in hPTH after R-568 and FGF23. Therefore, there is conserved signal transduction between man and mouse in the effects of both calcium and FGF23 on PTH expression. The effect of calcium depletion and activation of the Caₐ by calcimimetics on PTH gene expression is mainly posttranscriptional. PTH mRNA stability is dependent upon the regulated binding of trans-acting sequences in the human and bovine parathyroid hormone mRNA 3′-untranslated region. This ARE consists of a 26-nucleotide core element that is conserved among species and particularly between mouse and man (1). The observation that hPTH mRNA was increased by the low-calcium diet suggests that the regulation of the hPTH gene is in fact intact. The intact response of the hPTH expression to hypocalcemia and a calcimimetic suggests that man and mouse have a conserved signal transduction and use of trans- and cis-acting sequences in the regulation of PTH expression. It is noteworthy that although dietary-induced hypocalcemia led to an increase in both serum hPTH and hPTH mRNA levels, the effects were quantitatively different from those of mPTH. This difference in the increase in serum PTH levels after hypocalcemia may reflect the assays themselves, although there may also be a contribution of different processing of the protein or gene expression. It is of interest that the hypocalcemia produced a change in the proportion of the bioactive/intact ratio. With hypocalcemia the bioactive hPTH increased threefold, whereas intact PTH increased twofold. The stimulation of hypocalcemia in dialysis patients led to an increase in the proportion of serum bioactive PTH relative to intact PTH (25a). This may suggest that intracellular processing of hPTH is intact in the Tg mouse.

FGF23 acts on the parathyroid in the rat by activating the MAPK pathway (2) and in vitro in bovine parathyroid cells (14). It is not known whether the effect of MAPK activation by FGF23 decreases PTH transcription or affects PTH mRNA decay in rats with normal renal function. FGF23 decreased hPTH levels in the hPTH Tg mouse. FGF23 did not lead to the expected decrease in serum P₃. Therefore, FGF23 signaling is conserved from mouse to man in the parathyroid, but the effect of FGF23 on P₃ excretion is different.

In summary, we have generated transgenic mice expressing the hPTH gene in a 144-kb BAC. The hPTH transgenic mice express both mPTH and hPTH with no increase in serum calcium or 1,25(OH)₂D. Calcium depletion leads to a marked increase in mPTH and hPTH, and both the calcimimetic R-568 and FGF23 decrease hPTH, similar to their effects on endogenous PTH in rat and mouse. Therefore, the regulation of hPTH by the Caₐ and the FGF23 receptor complex, Klotho-FGFR1c, is intact in the mouse, indicating conserved signal transduction between mouse and man.

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GRANTS

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REFERENCES

12. Kilav R, Silver J, Naveh-Many T. A conserved cis-acting element in the parathyroid hormone 3′-untranslated region is sufficient for regulation of


