Parathyroid hormone/parathyroid hormone related peptide receptor gene transcripts are expressed from tissue-specific and ubiquitous promoters

Kimberly A. McCuaig, Han S. Lee, John C. Clarke, Homa Assar, Jonathan Horsford and John H. White*

Department of Physiology, McIntyre Medical Sciences Building, McGill University, 3655 Drummond Street, Montreal, Quebec H3G 1Y6, Canada

Received February 10, 1995; Revised and Accepted April 17, 1995

ABSTRACT

Parathyroid hormone (PTH) and PTH related peptide (PTHrP) stimulate diverse physiological responses in a number of tissues by binding to the same receptor. We have previously cloned the gene encoding the mouse PTH/PTHrP receptor (PTHR), and have identified a promoter region. The first exon transcribed from this promoter contains untranslated sequence and is followed by an exon encoding signal sequence and the first amino acids of the mature polypeptide. We have now identified and characterized a second promoter region, located >3 kb upstream of the original. Four partial cDNA clones, amplified from mouse kidney RNA by reverse transcription followed by the polymerase chain reaction, contain sequence corresponding to two previously unidentified exons composed of untranslated sequence. The second (3') of the two exons is spliced to the previously identified signal sequence exon. These cDNAs are highly homologous to the 5' end of a cDNA isolated from human kidney, strongly suggesting that the promoter region is conserved between mouse and humans. RNase protection and primer extension experiments have identified several transcriptional start sites extending over a region of ~100 bp. Unlike the previously identified promoter, this promoter is not (G+C)-rich. It lacks a consensus TATA element, but does contain a consensus CCAAT box. We have determined the expression patterns of both transcriptional start sites extending over a region of ~100 bp. Unlike the previously identified promoter, this promoter is not (G+C)-rich. It lacks a consensus TATA element, but does contain a consensus CCAAT box. We have determined the expression patterns of both promoters by RNase protection in a large number of tissues including heart, vascular smooth muscle, bladder, breast, skin, pancreas, adrenal cortex and medulla, bone, kidney, in developing fetal organs and other tissues (9,12–15). Its role in the adult remains largely unknown, although it is unlikely that it plays any role in regulation of extracellular fluid calcium under normal physiological conditions.

INTRODUCTION

The parathyroid hormone/parathyroid hormone-related peptide receptor (PTHR) is bound specifically by parathyroid hormone (PTH) and PTH related peptide (PTHrP) through a 34 amino acid region conserved in both hormones. PTH is secreted by the parathyroid gland and acts principally to regulate calcium and phosphate metabolism by binding to receptors expressed in kidney and bone (1–5). PTHrP was first identified as a major cause of malignancy-associated hypercalcemia (6–9). Gene ablation experiments in mice have shown that PTHrP is essential for normal skeletal development (10,11). PTHrP is required for normal proliferation and differentiation of chondrocytes that occurs in the developing skeleton prior to endochondral bone formation (10,11). PTHrP has been shown to act in a paracrine or autocrine manner. Unlike PTH, PTHrP is secreted by a large number of normal tissues including heart, vascular smooth muscle, bladder, breast, skin, pancreas, adrenal cortex and medulla, bone, kidney, in developing fetal organs and other tissues (9,12–15). Its role in the adult remains largely unknown, although it is unlikely that it plays any role in regulation of extracellular fluid calcium under normal physiological conditions.

The receptor is also widely expressed. In bone it is found in cells of the osteoblast lineage (2,5,16,17), and in kidney it is expressed in the glomerulus and at several sites along the nephron (18,19). Northern analyses have also detected PTHR transcripts in a large number of tissues in addition to kidney and bone (20). Its wide tissue distribution, along with its high affinity for PTHrP, strongly suggest that the PTHR serves as a receptor for PTHrP in many tissues that do not regulate extracellular calcium levels. Indeed, gene ablation experiments have recently shown that pthr null mice display a very similar phenotype to mice lacking PTHrP (21). It is therefore likely that the PTHR mediates the variety of physiological responses controlled by the two hormones. The PTHR is a G-protein-coupled receptor containing seven predicted trans-membrane domains (1–3 and references therein).
of ligand to the PTHR stimulates cAMP production, raises intracellular calcium and increases levels of inositol 1,4,5-trisphosphate (2).

Given its potentially numerous physiological functions and its broad pattern of expression, it is of interest to understand the mechanisms controlling PTHR expression. We (22), and others (23), have cloned the mouse PTHR gene and have identified 15 exons. There are four exons encoding extracellular domain(s) and eight encoding the seven predicted transmembrane domains, a structure which is highly conserved in the rat and human genes (23). This arrangement is very similar to that of the growth hormone releasing factor receptor, calcitonin receptor and glucagon receptor genes (22,24–26), particularly in the transmembrane region where these receptors are most similar. This suggests that these receptor genes, and probably those of other members of their subfamily, including receptors for glucagon-like peptide, vasoactive intestinal peptide, secretin, gastric inhibitory peptide, corticotropin releasing factor, pituitary adenylate cyclase stimulating peptide, and insect diuretic hormone, have evolved from a common precursor.

In most tissues in the rat a major PTHR transcript of 2.3 kb is observed, although both larger and smaller transcripts have been seen, particularly in testes where a 1.5 kb mRNA predominates (20). The 2.3 kb transcript expressed in the rat osteoblast-like osteosarcoma cell line ROS 17/2.8 is essentially identical in size to the predominant mouse kidney transcript (22). A promoter region has been identified in the mouse PTHR gene (22), which is highly (G+C)-rich and lacks apparent TATA box or initiator element homologies, but does contain a number of potential Sp-1 sites.

Here, we have amplified the 5' end of mouse kidney PTHR transcripts by reverse transcriptase/PCR. A second promoter region and two previously uncharacterized exons containing untranslated sequence have been identified. We find that activity of the newly characterized promoter is restricted to strong expression in kidney and weak expression in liver. In contrast, the previously characterized (G+C)-rich promoter is widely expressed. These results indicate that transcription of the PTHR gene is under control of multiple regulatory signals, some of which act in a tissue-specific manner.

**MATERIALS AND METHODS**

**Plasmids**

Plasmids used to make probes for RNase protection were pU3A/X, containing an Apal–Xhol fragment of genomic DNA inserted in Bluescript SK+ (Stratagene), described previously (22), and pU1IX/X, constructed by inserting in Bluescript SK+ digested with Xhol and Xhol a fragment generated by PCR of genomic DNA containing the U1 exon and the 5' proximal promoter region using the 5' primer (TGAACCTTCTAGACAGGTGCTTAAACCTTG) and the 3' primer (AGAGATCTCGAG-TCTTACTTACC). The plasmids pP1-2300/luc, pP2-656 and pP2-1473/luc were constructed by inserting 2.3 kb BamHI–PvuII, 0.8 kb PvuII–BamHI and 1.6 kb KpnI–BamHI fragments, respectively, into the polylinker region of the promoterless luciferase expression vector pXP2 (27). All partial PTHR cDNA and genomic DNA fragments were inserted into the polylinker of Bluescript SK+ (Stratagene) for sequencing.

**RNA purification**

Total RNA was isolated from mouse tissues and purified on cesium chloride gradients as described (28), and polyA+ RNA was isolated using an Oligotex kit (Qiagen).

**Reverse transcriptase/polymerase chain reaction amplification of PTHR cDNAs**

Mouse kidney total RNA was prepared as previously described (22). Ten μg was reverse transcribed by AMV reverse transcriptase (Pharmacia) in 50 mM Tris–HCl (pH 8.15), 6 mM MgCl2, 40 mM KCl, 1 mM DTT, 1 mM each dATP, dCTP, dGTP and dTTP, and 10 pmol primer 5'(GACATCGTCCCAGTCCAC-CAGCGC) at 42°C for 1 h. The primer is complementary to sequence in the previously characterized E1 exon of the PTHR gene (Figs 1a and 8; 22). The reaction was purified on a Centricon-100 column (Amicon) and dialysed down to 11 μl. A poly A+ tail was added by incubating for 10 min at 37°C with 100 μM potassium cacodylate pH 7.2, 2 mM CoCl2, 0.2 mM DTT, 20 mM dATP and 7.5 U terminal deoxynucleotidyl transferase (Gibco).

One tenth of this reaction was used as a template for PCR amplification between 5'(CGCGGTACCTCGAG(T)i and the phosphorylated oligonucleotide 5'(CTGAGCAGTGGAGC-AGAG); complementary to sequences in the SS exon of the PTHR gene (see Fig. 1) under the following conditions: 10 mM KCl, 10 mM (NH4)2SO4, 20 mM Tris–HCl pH 8.8, 2 mM MgSO4, 0.1% Triton X-100, 0.2 mM each dATP, dCTP, dGTP and dTTP, 40 pmol each primer and 2 U Ventg DNA polymerase (NEB). PCR was performed for 30 cycles of 55°C for 1 min, 72°C for 45 s and 95°C for 1 min. The products were digested with Xhol and subcloned into SK+ (Stratagene) for sequencing. Positive clones were identified by colony hybridization (28) using a probe containing sequence from the signal sequence exon of the rat PTHR cDNA. Sequencing was performed by the dideoxy chain termination method (Pharmacia).

**Ribonuclease protection assays**

32P-labelled anti-sense RNA probes were generated by in vitro transcription under conditions previously described (22). The 3' promoter-specific (U3 exon) probe was synthesized by restriction of plasmid SK-U3A/X (see Fig. 3) with Xhol and transcription with T7 RNA polymerase. The 5' promoter-specific (U1 exon) probe was prepared by linearization of plasmid SK-U1X/X with Xhol (see Fig. 3) and transcription also with T7 RNA polymerase. GAPDH probe was derived from plasmid pTri-GAPDH mouse (Ambion) by restriction with HindIII and transcription with T7 RNA polymerase. From 5 × 105 to 1 × 106 c.p.m. of each probe was incubated overnight with the specified amounts of tissue RNA at 55°C in hybridization buffer (40 mM PIPES, 80% formamide, 0.4 M sodium acetate, 1 mM EDTA). Hybridization mixes were subsequently digested with 1–3 U of RNase A according to the manufacturer's directions (Promega), precipitated, and resolved on 6% polyacrylamide denaturing gel.

**Primer extension**

Five ng of 32P-labelled primer was annealed to 10 μg of total mouse kidney RNA for 16 h at 50°C in 300 mM KCl, 20 mM Tris–HCl (pH 8.0), 2 mM EDTA. The primer was extended with AMV reverse transcriptase (Pharmacia) after adding 25 mM Tris–HCl (pH 8.3), 1 mM DTT, 6 mM MgCl2, 0.5 mM each of...
Isolation of partial PTHR cDNA clones containing novel 5' untranslated sequence

In previous work, a promoter region of the mouse PTHR gene was characterized using a combination of primer extension and S1 nuclease analyses (22). The first exon transcribed from this promoter contains untranslated sequence which is homologous to the corresponding region of a previously characterized rat PTHR cDNA (2,5). Here, we have used reverse transcriptase/PCR to further characterize the 5' ends of PTHR transcripts in the kidney. Reverse transcription was performed with a primer complementary to sequences in the E1 exon of the PTHR gene, and PCR was performed with an oligonucleotide complementary to sequences in the SS exon and an oligo-dT primer complementary to the poly A+ tail of the reverse transcript (see Experiment Procedures for details). Three of the partial cDNA clones isolated using this protocol, numbered 20, 32 and 34 (Fig. 1b), contain sequence from the signal sequence exon fused to previously uncharacterized 5' untranslated sequence. Clones 20 and 34 are identical and clone 32 contains 24 additional 5' base pairs. A fourth cDNA was also identified which differed from clones 20 and 34 by only an additional 1 nt at the 5' end which did not correspond to either genomic or primer DNA (data not shown).

The newly identified untranslated sequence is fused to the SS exon using the previously described splice acceptor site (Fig. 1; 22). Fifty-eight base pairs of sequence (exon U2) fused to the SS exon are 100% homologous to sequences lying between 1051 and 1109 bp upstream of the previously characterized transcriptional start site (Fig. 1; 22). The 5′-most sequence of cDNAs 20, 32 and 34 (exon U1) was mapped by Southern analysis to a 2.5 kb BamHI–XhoI fragment upstream of exon U2, and the 3′ end of exon U1 identified 10 bp upstream of the XhoI site by DNA sequencing (data not shown). This places exon U1 –3.2 kb upstream of the 5′-most exon (now called U3) identified in our previous study (22). The newly identified putative splice donor and acceptor sites contain GT and AG dinucleotides, respectively, immediately adjacent to exonic sequences (Fig. 4; 22; data not shown). The above results are summarized in Figure 1a.

Multiple ATGs in the 5′ untranslated regions of PTHR transcripts

A recent survey of the 5′ untranslated regions of 699 transcripts showed that only 10% of all mRNAs contain upstream AUGs (33). It is noteworthy that there are four AUGs upstream of the putative translational start site in the 5′ untranslated sequence formed by exons U1 and U2 (Fig. 1b). The three most 5′ AUGs are in weak translational contexts with pyrimidines at position –3, but the fourth AUG is in a context, GUC AUG G, which corresponds closely to the consensus sequence A/GCC AUG G described by Kozak (33). The fourth AUG is not in frame with the putative translational start site (boxed in Fig. 1b). The first (5′-most) and third AUGs are part of potential open reading frames which terminate upstream of the putative translational start site (Fig. 1B, underlined). Exon U3, transcribed from the previously characterized promoter, contains two upstream AUGs in weak translational contexts and a single potential upstream open reading frame (data not shown).
Figure 2. Homology between the 5' end of mouse cDNAs 20, 32 and 34 and the 5' end of a cDNA amplified from human kidney RNA (34) The translational start site and Kozak consensus sequences are boxed. The boundaries between U1 and U2, and U2 and SS exons in the mouse gene are indicated.

The U1 and U2 exons are homologous to the 5' end of a human kidney PTHR cDNA

There is a strong homology between the 5' end of a PTHR cDNA amplified from a human kidney RNA (34) and exons U1 and U2 of the mouse gene (Fig. 2). Exons U1 and U2 are 75 and 72%, similar, respectively, to the available sequences of the human cDNA, whereas the 5' untranslated sequence of the SS exons are 100% conserved except for a block of 3 bp unique to the human clone. Given the high level of conservation between other portions of the mouse and human genes (23) this strongly suggests that the splicing pattern observed between U1, U2 and SS exons in the mouse also exists in humans.

Mapping transcriptional start sites

Transcriptional initiation sites of the newly identified promoter have been mapped by RNase protection assay of total mouse kidney RNA using the probe diagrammed in Figure 3A (see Experimental Procedures for details). Several start sites were identified extending over a region of -100 bp (Fig. 3B). This pattern of expression is stable following 30 or 60 min of RNase digestion (Fig. 3B, lanes 2 and 3), strongly suggesting that the observed products do not represent digestion intermediates. The two upstream minor start sites (asterisks) are upstream of the 5' end of the longest cDNA clone (clone 32). The 5'-most major start site (arrowhead) is one base pair upstream of the 5' end of cDNA clones 20 and 34 (and the clone containing a single aberrant 5' nucleotide).

For comparison, an RNase protection assay specific for the previously identified downstream promoter is provided (Fig. 3B, lane 4). The start site is identical to that determined by S1 nuclease analysis (22). Given the relative intensity of the protection products, the results indicate that transcripts synthesized from the upstream promoter should represent the predominant form of PTHR mRNA in the kidney.

The position of the first major transcriptional start site was also analysed by primer extension analysis using primer p1 (see Figs 3 and 4). A parallel sequencing reactions were run using the same primer. Two major extension products differing in length by 3 nt were identified in several experiments (Fig. 3C, lane 5, arrowheads; data not shown). The 5' end of the shorter of the two products lies one base upstream of that of cDNA clones 20 and 34. Extension products corresponding to the two weaker upstream start sites identified by RNase protection (Fig. 3B) were not detected.

Taken together these results (summarized in Fig. 4) indicate there are several transcriptional start sites in the newly characterized promoter. The site identified by RNase protection and primer extension lying one base upstream of the 5' ends of cDNA clone 20 and 34, and the additional clone with one aberrant nucleotide (see above), has been designated +1 (Fig. 4). The newly characterized promoter has been designated PI and the previously identified downstream promoter has been renamed P2. Unlike P2, promoter P1 is not highly (G+C)-rich. For example, the (G+C) content of sequence from -100 to +30 is only 48% (Fig. 4), compared with 85% for P2 (22). While promoter P2 is rich in Sp-1 consensus sequences, no such sites are found in the proximal promoter region of PI. Promoter P1 lacks a consensus TATA element which is usually located >25 bp upstream of transcriptional start sites. However, a 25 bp sequence which is 96% (A+T)-rich lies immediately upstream of the two start-sites
extracellular calcium concentrations (1-5). PTHrP acts on a large number of tissues, and has been implicated in a number of processes including among others, control of normal skeletal development, smooth muscle relaxation and differentiation of keratinocytes (8-10,13,14,36). Responses to these hormones can be modulated by controlling levels of expression of the PTHR. Given that the combined actions of PTH and PTHrP control a broad range of physiological responses, it is likely that there exist tissue-specific mechanisms for controlling PTHR expression. We have therefore analysed the tissue specificity of P1 and P2 promoter activity in a number of tissues expressing the PTHR by RNase protection using the promoter-specific probes shown in Figure 3A.

PTHR expression patterns were first compared in bone and kidney cells. Previous studies have shown that the PTHR is expressed in bone cells of the osteoblast lineage (2,5,15). RNase protection experiments were performed on total RNA isolated from the mouse osteoblast-like cell line MC3T3 (37), and from primary cultures of mouse osteoblasts (MOB). RNase protection products comigrating with those observed with kidney RNA are detected readily with RNA from MC3T3 cells as well as with a weaker signal with RNA from mouse osteoblasts (Fig. 6, lanes 6-8). However, no protection products are seen with RNA from either MC3T3 or MOB cells with the P1-specific probe in this and in other experiments, although a strong signal is obtained with kidney RNA (Fig. 6, lanes 2-4; data not shown).

Expression patterns of PTHR transcripts in other tissues

Northern analysis with RNA from rat tissues has shown that, in addition to kidney and bone, a 2.3 kb PTHR transcript is expressed at low to moderate levels in a number of tissues (20). We prepared RNA from mouse heart, bladder, lung, spleen, liver and brain and performed a number of RNase protection experiments as above with P1- and P2-specific probes. Expression of the P2 promoter is readily detected in bladder and heart using 5-10 µg of mouse poly A+ RNA (Fig. 7A, lanes 7 and 8). However no expression from P1 is seen under these conditions (Fig. 7A, lanes 3 and 4), after prolonged exposure of the autoradiogram, or in other experiments (data not shown).
Figure 6. RNase protection experiments with RNA from mouse kidney and bone cells. RNase protection experiments were performed with P1 and P2 probes shown in Figure 3A. RNA-probe hybrids were incubated with RNase I for 60 min at 37°C. Thirty μg of total RNA from mouse kidney (lanes 2 and 6), mouse osteoblast-like MC3T3 cells (lanes 4 and 8) or from cultures of mouse osteoblasts (MOB, lanes 3 and 7) were incubated with P1 and P2 probes as indicated. The products protected with the P2 probe are indicated by the arrowhead. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts were determined using 0.3 μg of poly A+ RNA from each tissue.

Expression of P2 is detected with RNA from brain, spleen, liver and lung (Fig. 7B, lanes 9–12), whereas no expression is detected by the P1-specific probe with RNA from brain, lung and spleen (Fig. 7B, lanes 3, 4 and 6). In contrast, weak expression of P1 is reproducibly seen in liver (note that RNase protection assays were performed with total kidney and poly A+ liver RNA; Fig. 7B, compare lanes 1 and 5).

Taken together, the results of Figures 6 and 7 show that the P2 promoter is active in all tissues tested; kidney, osteoblasts and osteoblast-like cells, as well as bladder, brain, heart, liver, lung and spleen. In contrast, activity of the P1 promoter is restricted primarily to kidney, and to liver.

DISCUSSION

The structure of the mouse PTHR gene, based on previous results (22,23) and those presented here, is summarized in Figure 8, with splicing patterns identified to date shown up to exon E1. The gene is composed of a total of 17 exons of which the first three, U1–U3, contain untranslated sequence. Our results indicate that the two promoters identified give rise to transcripts with largely different 5' untranslated regions (Fig. 1). RNase protection experiments with transcript-specific probes demonstrate that the newly identified promoter P1 is specifically active in the kidney whereas P2-specific transcripts are widely expressed. Significantly, the sequences of the U1 and U2 exons transcribed from the P1 promoter are ~75% similar to the 5′ end of cDNA amplified from human kidney RNA (Fig 2; 34), suggesting that P1 and the splicing pattern of its transcript are conserved between mice and humans.

Kidney and bone are the major sites of action of PTH. PTHR expression may be regulated by specific signals in these tissues in order to fine tune their response to PTH. The selective expression of P1 in the kidney supports this hypothesis. It remains to be seen if expression in bone is regulated by bone-specific transcription factors acting on P2 or on an unidentified promoter expressed selectively in bone.

In the kidney, receptors have been identified by a combination of PTH binding studies and PTH-dependent stimulation of adenylate cyclase activity in the glomerulus and at several sites along the nephron, including the proximal and distal convoluted tubules (PCT and DCT) and the thick ascending limb of the loop of Henle (19). Various techniques have been used to show that PTH acts to inhibit phosphate and bicarbonate reabsorption by acting at sites in the PCT (19,38). The proximal convoluted tubule is also the major site of PTH stimulation of production of 1α,25-dihydroxyvitamin D3 production through activation of the 1α-hydroxylase enzyme. In contrast, PTH-mediated stimulation of calcium reabsorption occurs through interaction with receptors.
expressed in the thick ascending limb and the DCT. The distinct effects of PTH on the PCT and DCT raise the possibility that PTHR expression may be differentially regulated in the PCT and DCT. This suggests that P1- and P2-specific transcripts may be expressed at different locations in the kidney. It will be important to map transcript expression patterns in the kidney to aid in determining the potential regulatory signals controlling P1 and P2 promoter activities. These studies will also be important for determining which cell lines can serve as appropriate models for determination of key regulatory elements, particularly in P1.

The kidney-specific expression of P1 is intriguing because it may provide insights into certain defects in PTH signalling seen in humans. Pseudohyoparathyroidism describes a broad range of defects in PTH signalling that are not caused by disruption of PTH synthesis and secretion. Of these, pseudohyoparathyroidism type 1b may involve a disruption of PTH signalling in the kidney because of a defect in the PTHR itself (39). Our results raise the possibility that a kidney-specific defect in PTHR action could arise because of lack of production of mature transcripts generated from the P1 promoter, due to defects at either the transcriptional or posttranscriptional levels (eg. splicing).

From the major start sites identified (Fig. 3), the combined length of exons U1 and U2 ranges from 169 to 219 bp, very close to the 220 bp length of U3, the previously characterized exon containing untranslated sequence (22). Assuming common splicing patterns downstream of the SS exon, this gives rise to mature transcripts of 2156–2206 and 2207 nt in length from the P1 and P2 promoters, respectively, based on the previously defined 3’ end of the PTHR message (22). This is consistent with Northern analyses of RNA from mouse kidney (22) and several rat tissues showing a single predominant band of ~2.3 kb in length (20). Our results indicate that in kidney, but not in the other tissues examined, the 2.3 kb mRNA band is a combination of mRNA species with differing 5’ untranslated sequences. This also indicates that one cannot account for mRNA species of other lengths observed in the kidney and testes (20,40) simply based on selection of either the P1 or P2 promoters. Moreover, the longer transcripts observed in the kidney (20,40) must contain as yet unidentified exonic sequence.

There is no evidence that the two promoters arose by a gene duplication event within the PTHR genomic DNA, given that there is no significant homology between P1 and P2 sequences. Whereas P2 is highly (G+C)-rich and contains numerous potential binding sites for Sp-1, P1 is ~50% (G+C)-rich and lacks Sp-1 motifs in the promoter proximal region. In addition, the extended CCAAT box homology AGCCAAT, located at ~41, is not present in P2 (data not shown). Neither promoter contains a sequence resembling a TATA box located 25–30 bp upstream from major transcriptional start sites, although there is an (A+T)-rich sequence upstream of one of the major start sites identified for P1.

Several criteria indicate that the stimulation of luciferase expression by P1 promoter fragments represents basal activity. Activity of P2 is very similar to, and activity of P1 slightly lower than, that seen with a recombinant containing a truncated herpes simplex virus promoter (~109 to +51), and both promoters several-fold less active than recombinants containing enhancers from either SV40 or human cytomegalovirus (data not shown). Several P1 and P2 recombinants, containing up to 7 kb of promoter sequence, display similar levels of activity in cell lines derived from different tissues. Thus, there exist tissue-specific enhancer(s) in far upstream sequences controlling expression of P1, or tissue specificity of P1 activity is not reproduced in transient transfection experiments.

Finally, it is noteworthy that the 5’ untranslated regions of PTHR transcripts synthesized from either promoter P1 or P2 are unusual by virtue of both their length (>200 nt), and the fact that they contain multiple upstream AUGs. Both of these parameters are characteristic of <10% of vertebrate mRNAs (33), and multiple upstream AUGs are concentrated in mRNAs encoding protooncogenes and growth factor receptors (33). There are four upstream AUGs in transcripts containing U1 and U2, of which one is in a strong translational context and out of frame with the putative translational start site. Transcripts containing U3 have
two upstream AUGs in weak translational contexts (Fig. 1). Previous studies have suggested that translation can be inhibited by upstream AUGs that are out of frame and in strong translational contexts (41,42). Thus, it is possible that the mRNAs transcribed from promoters P1 and P2 may be translated with different efficiencies.

In summary, we have characterized a second promoter region, upstream of the original promoter, in the mouse PTHR gene. This promoter is expressed in kidney but not bone or any of the other tissues examined, thus indicating that PTHR gene expression is regulated in a tissue-specific manner.

ACKNOWLEDGEMENTS

We are grateful to Drs David Goltzman and John Orlowski for a critical reading of the manuscript. We thank Dr Janet Rubin (Emory University, Atlanta) for the generous gift of mouse osteoblast RNA. This work was supported by operating grants (MT-11704 and U1-12004) from the Medical Research Council of Canada to JHW. JHW is a chercheur-boursier of the Fonds de la Recherche en Santé du Québec.

REFERENCES


