Mechanism of Increased Parathyroid Hormone mRNA in Experimental Uremia: Roles of Protein RNA Binding and RNA Degradation

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Abstract. Patients with chronic renal failure develop secondary hyperparathyroidism with increased synthesis and secretion of parathyroid hormone (PTH) resulting in severe skeletal complications. In rats with secondary hyperparathyroidism due to 5/6 nephrectomy, there are increased PTH mRNA levels, and this mechanism was studied. Parathyroid glands were micro-dissected from control and 5/6 nephrectomy rats and analyzed for PTH mRNA and control genes, and the nuclei were used for nuclear run-on experiments. The cytosolic proteins of the parathyroids were used to study PTH mRNA protein binding by ultraviolet cross-linking and the degradation of the PTH transcript in vitro. Nuclear run-ons showed that the increase in PTH mRNA levels was posttranscriptional. Protein binding to the PTH mRNA 3'UTR determines PTH mRNA stability and levels. Parathyroid proteins from uremic rats bound PTH mRNA similar to control rats by ultraviolet cross-linking. To determine the effect of uremia on PTH mRNA stability, an in vitro RNA degradation assay was performed with parathyroid proteins from uremic rats. When parathyroid proteins from control rats were incubated with PTH mRNA, there was transcript degradation already at 30 min, reaching 50% at 60 min and 90% at 180 min. With uremic parathyroid proteins, the PTH mRNA was not degraded at all at 120 min and was moderately decreased at 180 min. This decrease in degradation by uremic parathyroid proteins suggests a decrease in parathyroid cytosolic endonuclease activity in uremia resulting in a more stable PTH transcript. The increased PTH mRNA levels would translate into increased PTH synthesis and serum PTH levels, which would lead to metabolic bone disease in many patients with chronic renal failure.

The secondary hyperparathyroidism of chronic renal failure is characterized by increased levels of parathyroid hormone (PTH) mRNA, PTH secretion, and parathyroid cell hyperplasia (1). The increased PTH gene expression and parathyroid cell number lead to an increased synthesis of PTH, which is then secreted in excess due to the chronic stimuli of hypocalcemia and hyperphosphatemia. In addition, there is a decrease in the parathyroid's response to calcium and 1,25(OH)2 vitamin D3 (125(OH)2D3), because of a downregulation in the parathyroid's Ca-sensing receptor (2–4) and vitamin D receptor (5) and possibly a direct effect of uremic serum to decrease the binding of the vitamin D receptor to its response element (6). The mechanism of the increase in PTH gene expression is not known. The systemic factors that are known to be involved in the pathogenesis of secondary hyperparathyroidism are hypocalcemia, hypophosphatemia, and decreased levels of serum 1,25(OH)2D.

Administered 1,25(OH)2D3 decreases PTH gene expression at a transcriptional level (7,8) by binding of the 1,25(OH)2D3 receptor to a vitamin D-responsive element in the PTH gene's promoter (9,10). Hypocalcemia increases PTH mRNA levels (11), and hypophosphatemia decreases PTH mRNA levels (12). We have recently shown that the effects of calcium and phosphate on the PTH gene are independent of any changes in serum 1,25(OH)2D (12) and are posttranscriptional (13). Post-transcriptional regulation of gene expression usually involves changes in protein binding to the 3'UTR of mRNA (14,15). Protein-RNA binding studies showed that hypocalcemia increased the binding of parathyroid cytosolic proteins to the PTH mRNA 3'UTR, and this binding was decreased with proteins from hypophosphatemic rats (13).

There is no parathyroid cell line and hence no cellular assay for PTH mRNA stability. However, the effect of parathyroid cytosolic proteins on PTH mRNA stability was studied by an in vitro degradation assay (13). This assay has been used to define the posttranscriptional regulation of other mRNA, such as vascular endothelial growth factor by hypoxia (16,17). After addition of the proteins from rats on a control diet to the full-length PTH mRNA probe, the transcript was still intact at 40 min. After proteins from hypophosphatemic rats were added, the decrease was dramatic and already evident at 5 min, reaching 20% of the initial transcript. Parathyroid proteins from hypocalcemic rats led to an intact transcript up to 180 min (13). The changes in PTH mRNA degradation in rats with hypocalcemia or hypophosphatemia were specific to the para-
thyroid, and not found with cytosolic proteins from other organs, such as liver and brain, in these rats (13). The degradation was dependent on the presence of the 3′-UTR, and a probe without the 3′-UTR was not degraded. Therefore, there are instability regions in the PTH mRNA 3′-UTR that are degraded by parathyroid cytosolic proteins. In addition to proteins that degrade RNA, the cytosolic protein preparation includes proteins that stabilize the PTH mRNA by binding to the 3′-UTR and prevent degradation. The mRNA half-life in vivo and in vitro is determined by both these degrading and stabilizing proteins.

We have now studied rats with experimental uremia due to 5/6 nephrectomy, to determine the mechanism of the increase in PTH mRNA levels in chronic renal failure. PTH gene transcript run-ons showed that the effect of uremia was posttranscriptional, and additional studies were then performed on posttranscriptional cytosolic proteins binding to the PTH transcript as well as their effect on PTH mRNA degradation in vitro. The results show that uremia did not lead to a change in protein-RNA binding, but there was a marked decrease in in vitro degradation, the net result being an increase in PTH mRNA levels. Therefore, we propose that in chronic renal failure there is a decrease in parathyroid cytosolic endonuclease activity, leading to increased levels of PTH mRNA.

Materials and Methods

Animals

Male Hebrew University strain rats (180 to 200 g) underwent 5/6 nephrectomy under ether anesthesia as reported previously (18) and were fed a standard rodent chow containing 1.0% calcium and 0.6% phosphorus. All animal experiments were carried out according to institutional guidelines. Groups of rats were studied at 2, 3, or 4 wk after the 5/6 nephrectomy. The rats were anesthetized with pentobarbital, a blood sample was collected by aortic puncture, and the parathyroids were microdissected from the surrounding thyroid tissue. The parathyroid tissue for RNA extraction was frozen in liquid nitrogen and stored at −70°C until analysis. To confirm the accuracy of the microdissection, Northern blots were performed and showed that the microdissected parathyroids had PTH mRNA and no thyroglobulin mRNA, and the isolated thyroid had no PTH mRNA even after a prolonged exposure. Tissues for nuclear run-on assays and protein extracts were used immediately as described below.

Serum Measurements

Serum creatinine, Ca²⁺, and phosphate were measured in a Roche autoanalyzer (Roche Products, Hertfordshire, United Kingdom). Serum 1,25(OH)₂D₃ levels were measured by a radioreceptor assay (Incstar Corp., Stillwater, OK).

Measurement of Cellular mRNA Levels

RNA was extracted from rat parathyroid tissue, and the levels of PTH mRNA were measured by Northern blots after extraction with TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH) as described previously (19). The integrity of the RNA and the uniformity of RNA transfer to the membrane were determined by ultraviolet (UV) visualization of the ribosomal RNA bands of the gels and the filters. Hybridization was performed with a random-primed rat PTH cDNA probe and 18S as control.

Nuclear Run-On Transcription Assay

Nuclei were prepared from pooled thyroparathyroid tissue of 10 rats, and nuclear run on transcription assays were performed as described previously (12). RNA was extracted and resuspended in 300 µl of hybridization buffer (7% sodium dodecyl sulfate [SDS], 10% polyethylene glycol [8000], 1.5% saline-sodium phosphate-ethylene-diaminetetra-acetic acid). Aliquots of RNA from treated and untreated samples were counted in a scintillation counter, and an equal number of counts from each condition (1 to 2 × 10⁶ cpm) was hybridized to linearized cDNA (5 µg) for PTH, actin, 18S, and Bluescript-KS (Stratagene, La Jolla, CA) DNA (12), which were immobilized to Hybond filters using a slot blot apparatus. Hybridization was performed at 65°C for 72 h. The filters were washed 3 times at room temperature in 2× SSC, 0.2% SDS for 5 min, then washed once at 55°C for 15 min. The blots were exposed to Agfa CURIX-RP2 film at −70°C with intensifying screens for 14 d. In addition, the filters were exposed for 24 h to a Bio Imaging Plate and quantified by a Bioimaging analyzer BAS2000 (Fuji Photo Film, Tokyo, Japan).

Plasmid Constructs and Labeling of RNA

Rat PTH cDNA in Bluescript II KS+ plasmid (Stratagene) included the fragment spanning the region of the PTH cDNA from 32 to 817 bp and includes most of the 5′-UTR and all of the 3′-UTR including the long poly(A) tail (13). The transcript for the 3′-UTR (239 nucleotides) was prepared as before (13).

Radiolabeled RNA probes for UV cross-linking and in vitro degradation assays were prepared from templates using the appropriate RNA Polymerase (13) in a transcription kit from Promega (Madison, WI). The specific activity of the RNA probe was 0.5 to 1.0 × 10⁶ cpm/ng.

Cytoplasmic Protein Purification

Cytoplasmic proteins (S100) were extracted by the method of Dignam et al. (20). Different tissues were removed from the rats and immediately washed in cold phosphate-buffered saline. Parathyroid proteins were prepared from microdissected parathyroids or from thyroparathyroid tissue. The tissue was cut with a scalpel, suspended in 5 vol of buffer A containing 10 mM Hepes, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride and incubated on ice for 10 min. After centrifugation at 600 × g for 10 min (4°C), the pellet was resuspended in 2 vol of buffer A and homogenized by a polytron. The homogenate was centrifuged at 600 × g for 10 min, and the supernatant was carefully decanted, mixed with 0.1 vol of buffer B containing 0.3 M Hepes, 1.4 M KCl, and 0.03 M MgCl₂, and centrifuged (4°C) at 100,000 × g for 1 h (Beckman type TL-100, Fullerton, CA). The high-speed supernatant (S-100) was dialyzed for 20 h at 4°C against 50 vol of buffer D containing 20 mM Hepes, 20% glycerol, 0.1 M KCl, 0.2 mM ethylendiaminetetra-acetic acid, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM DTT. For RNA degradation assays, the S100 fraction was prepared by homogenizing the tissue with a polytron in 2 vol of 10 mM Tris/HCl, pH 7.4, 0.5 mM DTT, 10 mM KCl, and 1.5 mM MgCl₂. A total of 0.1 vol of the extraction buffer (1.5 mM KCl, 15 mM MgCl₂, 100 mM Tris-HCl, pH 7.4, and 5 mM DTT) was added, and the homogenate was centrifuged at 14,000 × g for 2 min to pellet the nuclei. The supernatant was centrifuged at 100,000 × g for 1 h at 4°C. Cytoplasmic extracts were immediately frozen at −80°C in aliquots. Protein concentration was determined by optical density spectrophotometry (595-µm wavelength), using a Bradford reagent (Bio-Rad, Hercules, CA).
degradation in a total volume of 40 ml cytoplasmic extract and 80 U/ml RNasin to prevent nonspecific RNA.

MgCl₂, 40 mM KCl, and 5% glycerol (binding buffer). After 30 min at room temperature, heparin was added to a final concentration of 5 mg/ml to eliminate nonspecific binding, and the samples were irradiated at 2.5 J/cm² with a UV light source of 312 nm. RNase A-XII (Sigma Chemical Co., St. Louis, MO) was then added for 15 min at 37°C to a final concentration of 1 mg/ml to digest unprotected RNA. The samples were heated for 5 min at 65°C after addition of 5 μl of Laemmli sample buffer containing 50% glycerol, 10% SDS, 0.4 M Tris, pH 6.8, and 0.5 M DTT, and a pinch of bromphenol blue. The samples were then loaded on an SDS 10% polyacrylamide electrophoresis gel. RNA-protein binding was visualized by autoradiography. A molecular weight marker (Bio-Rad) was also run on the gel for size estimation of the protein-RNA bands.

In Vitro Cell-Free Degradation Assay

In vitro cell-free degradation was performed essentially as described (13,16,21,22). Radiolabeled RNA transcripts for the full-length PTH RNA (0.3 × 10⁶ cpm) were incubated with 40 μg of cytoplasmic extract and 80 U/ml RNAsin to prevent nonspecific RNA degradation in a total volume of 40 μl at room temperature. At each time point, 6 μl was transferred to a tube containing 300 μl of TRI-Reagent (Molecular Research Center, Inc.) and RNA was extracted. Samples were run on formaldehyde-agarose gels, transferred to Hybond membranes (Amersham), and autoradiographed. The remaining undegraded transcripts at the different time points were quantified by densitometry.

Table 1. Serum chemistry in partially nephrectomized rats at 3 wk

<table>
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<th>Group</th>
<th>Weight (g)</th>
<th>Creatinine (mg/dl)</th>
<th>Calcium (mg/dl)</th>
<th>Phosphorus (mg/dl)</th>
<th>1,25(OH)₂D₃</th>
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<tbody>
<tr>
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<td>4 Weeks</td>
<td>2 Weeks</td>
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<tr>
<td>235 ± 4.6</td>
<td>245 ± 7.4</td>
<td>0.4 ± 0.7</td>
<td>0.4 ± 0.9</td>
<td>11.0 ± 0.1</td>
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<td>4.7 ± 0.1</td>
<td>7.1 ± 0.3</td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 1.7</td>
<td>25.1 ± 3.1</td>
<td>7.5 ± 0.9</td>
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<tr>
<td>6</td>
<td>5/6 NX rats</td>
<td>245 ± 4.8</td>
<td>248 ± 16</td>
<td>1.2 ± 0.2</td>
<td>10.8 ± 0.1</td>
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<td>0.2 ± 1.7</td>
<td>10.7 ± 0.1</td>
<td>0.1 ± 0.4</td>
<td>0.3 ± 10.8</td>
<td>22.9 ± 4.3</td>
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a Values are mean ± SEM. n = 4 rats per group. 1,25(OH)₂D₃, 1,25 dihydroxyvitamin D₃; NX, nephrectomized.

b Significant difference between 5/6 NX and control rats, P < 0.05.

Statistical Analyses

Results were analyzed by one-way ANOVA with the post hoc Bonferroni multiple comparisons test to determine the significance of differences between means. P values <0.05 were considered statistically significant. Results are presented as mean ± SEM.

Results

The 5/6 nephrectomized rats had increased levels of creatinine at 2, 3, and 4 wk with no differences in body weight, serum calcium, phosphate, or 1,25(OH)₂D levels (Table 1). PTH mRNA levels in microdissected parathyroids were increased in the uremic rats after 3 and 4 wk of uremia (Figure 1). PTH mRNA levels in uremic rats at 4 wk were increased twofold compared with controls (chronic renal failure [CRF] 2.6 ± 0.1, control 1.3 ± 0.1; P < 0.01), and when the PTH mRNA levels were corrected for different control genes, 18S, 28S, and actin mRNA, the same twofold increase in uremic rats compared with controls was found (P < 0.01 for each control gene). To determine whether the increase in PTH mRNA levels was transcriptional or posttranscriptional, we performed nuclear transcript run-on experiments on nuclei from parathyroids of control and uremic rats. There was no difference in the PTH gene transcription rate between control and uremic rats after 2 wk (not shown) and 4 wk of uremia (Figure 2). Therefore, in these rats with experimental uremia, the increase in PTH mRNA levels was posttranscriptional, similar to the increase in PTH mRNA levels in hypocalcemic rats (13). However, because the increase in PTH mRNA was only twofold, the nuclear run-on results do not exclude a transcriptional component as well. The

Figure 1. Rats with experimental uremia for 4 wk have an increase in parathyroid hormone (PTH) mRNA levels. Agarose gel electrophoresis of RNA from the microdissected parathyroids of single rats hybridized for PTH mRNA and 18S RNA. Lanes 1 through 5, control rats; lanes 6 through 11, 5/6 nephrectomized rats (5/6 NX).

Figure 2. Nuclear transcript run-on for PTH and control genes of parathyroids from control and 5/6 nephrectomized rats at 4 wk. There was no difference in the transcription rates for PTH and control genes, glucose-6-phosphate dehydrogenase, and actin. The plasmid Blue-script KS was used as a negative control.
present studies were performed to determine the mechanism of the posttranscriptional increase.

Posttranscriptional regulation of RNA stability may involve changes in protein-RNA interactions at the 3'-UTR of an mRNA, and we have recently shown that hypocalcemia increases, and hypophosphatemia decreases, the binding of parathyroid cytosolic proteins to the PTH mRNA as measured by UV cross-linking (13). Therefore, we performed UV cross-linking studies to the PTH mRNA, using parathyroid cytosolic proteins from rats with experimental uremia for different time periods. Incubation of proteins with a probe for the full-length (not shown) or the 3'-UTR of the PTH mRNA resulted in three bands of approximately 110, 60, and 50 kD (Figure 3). Competition experiments with excess unlabeled RNA showed specificity of the binding, the addition of proteinase K abolished binding, and when no protein was added there were no protected RNA bands (not shown), indicating that the binding was a result of protein-RNA interaction. Parathyroid cytosolic proteins from control and uremic rats at 4 wk of uremia showed no difference in the binding to the PTH mRNA (Figure 3). Similar

**Figure 3.** Parathyroid cytosolic proteins from rats with experimental uremia show the same binding as control rats to PTH mRNA. Parathyroid and hepatic protein extracts, from control (C) and 5/6 nephrectomized (NX) rats at 4 wk, were ultraviolet (UV) cross-linked to 32P-labeled PTH mRNA 3'-UTR, run on a sodium dodecyl sulfate-polyacrylamide gel, and visualized by radioautography. The size of molecular mass markers is shown on the right.

**Figure 4.** In vitro degradation of PTH mRNA by cytosolic proteins is decreased with parathyroid proteins from 5/6 nephrectomized (5/6 NX) rats and not by their hepatic proteins. (A) Gel electrophoresis of full-length PTH mRNA riboprobe incubated with cytosolic proteins from control rats’ parathyroids and 5/6 NX (for 3 wk) rats’ parathyroids for different time periods, or incubated without protein. (B) Time–response curves of intact full-length PTH mRNA after incubation with parathyroid cytosolic proteins as in A. Each point represents the mean ± SEM of three different experiments. At some points, the mean is less than the size of the graphic symbols. The PTH transcript is degraded by proteins from control rats and remains intact for a longer time period with proteins from 5/6 NX rats.
results were found with rats after 2 wk of uremia (not shown). This result was found in four of five repeat experiments using different pools of parathyroid proteins (not shown). Binding experiments with increasing amounts of protein and RNA probe also showed similar results for control and uremic parathyroid proteins (not shown). Proteins from the liver of uremic and control rats also showed no difference in binding to the PTH mRNA 3'-UTR (Figure 3).

To understand the increase in PTH mRNA levels in uremia, we performed an in vitro degradation assay. In this assay, a probe for the full-length PTH mRNA was incubated with cytosolic proteins from control and 5/6 NX rats for different time periods. The degradation of the PTH transcript is the same with hepatic proteins from control and 5/6 NX rats at 3 wk, indicating that the effect of uremia is specific to the parathyroid.

Figure 5. Gel electrophoresis of full-length PTH mRNA riboprobe incubated with cytosolic hepatic proteins from control and 5/6 NX rats for different time periods. The degradation of the PTH transcript is the same with hepatic proteins from control and 5/6 NX rats at 3 wk, indicating that the effect of uremia is specific to the parathyroid.

Figure 6. Model of PTH mRNA including the 5' untranslated region (5'-UTR), the coding region, and the 3'-UTR and the parathyroid cytosolic proteins that interact with the 3'-UTR. The parathyroid proteins contain both protective factors ( ), measured by UV cross-linking, and degrading factors (endonucleases) ( ), measured by an in vitro degradation assay. In normal rats, the basal levels of PTH mRNA is determined by balance between the protective and degrading factors in the cytoplasm. In hypocalcemia, there is an increase in PTH mRNA associated with an increase in the binding of protective factors, which leads to a more stable transcript. In hypophosphatemia, there is a decrease in protective factors, which leads to a less stable transcript and a decrease in PTH mRNA levels. In rats with chronic renal failure due to 5/6 nephrectomy, there is no change in the protective factors together with a decrease in endonuclease activity, resulting in increased PTH mRNA levels.
mRNA levels in uremia is posttranscriptional and is due to the effect of a decrease in cytosolic endonuclease activity. A model depicting how hypocalemia and hypophosphatemia, as well as chronic renal failure, regulate PTH mRNA stability by determining the amount of functional protective and degrading (endonuclease activity) factors is shown in Figure 6.

Discussion

mRNA stability plays a crucial role in the regulation of gene expression. This is a result of the balance between stabilizing and degrading forces on the transcript. In the PTH mRNA, there are specific sequences in its 3′-UTR to which parathyroid cytosolic proteins bind to stabilize the transcript and which may also be involved in its degradation by cytosolic endonucleases. As a result of these interactions, the PTH transcript is more stable in hypocalcemic rats and less stable in hypophosphatemic rats.

The increase in PTH mRNA shown here in experimental uremia confirms our own data using thyroparathyroid tissue (18) and published data using microdissected parathyroids (23,24), where increases of two- to fourfold were reported. The increase in PTH mRNA levels only at the longer time intervals confirms previous findings, where increases were reported after 4 (23) and 5 wk (24) of renal failure.

We have now shown that in experimental uremia, the increase in PTH mRNA was posttranscriptional. There was no change in binding of parathyroid proteins to the PTH RNA 3′-UTR, but there was a marked decrease in RNA degradation by parathyroid proteins. When parathyroid proteins from control rats were incubated with PTH mRNA, there was partial transcript degradation already at 30 min, reaching 90% at 180 min. With uremic parathyroid proteins, the PTH mRNA was only degraded after 120 min. Our previous study showed that hypocalemia was associated with increased protein-PTH mRNA binding and less degradation (13). That study indicated that the increased protein binding represented an increase in protective factors which bound to instability regions in the PTH 3′-UTR and prevented its degradation. In uremia, the PTH mRNA is not degraded in an in vitro degradation assay. This indicates either a decrease in factors responsible for RNA degradation in the cytosol or an RNase inhibitory factor induced by uremia. The binding of the uremic parathyroid proteins, which represents the stabilizing proteins, was the same as controls. The net result is an increase in PTH mRNA levels in uremia. The degradation assay is an in vitro assay, which is not quantitative in determining the extent of the increase in PTH mRNA levels in vivo. It does, however, demonstrate a mechanism whereby uremia increases PTH mRNA stability. The degradatory factors in the parathyroid that are selectively decreased in uremia are probably mainly cytosolic endonucleases that have as yet not been characterized. The effect of experimental uremia on the cytosolic proteins was specific to the parathyroid and not found with hepatic proteins.

The parathyroid is especially sensitive to changes in circulating calcium and phosphate, which raises the question of whether they may have a role in the pathogenesis of the posttranscriptional increase in PTH mRNA in uremia. In the present experiments, serum calcium and phosphate did not change in the uremic rats up to 4 wk. However, it is possible that there were changes in serum phosphate, such as postdial increases, that were not detected. A further consideration is that experimental chronic renal failure may be complicated by systemic acidosis that may also have a role in the increased PTH mRNA levels. The role of acidosis was not determined in the present study; however, most studies have shown that chronic metabolic acidosis has no effect on serum PTH levels (25,26).

There are well-characterized paradigms of protein-RNA interactions determining mRNA stability (27). An example is the effect of iron on the transferrin receptor mRNA stability and ferritin translation (14,28,29). Hypoxia increases the stability of the mRNA for vascular endothelial growth factor and erythropoietin by regulating their degradation (16,17).

In other systems, there is also a combination of protein-RNA binding and active mRNA degradation determining mRNA stability (15). An example of this is in Xenopus oocytes, in which the RNA Xlhbbox2b contains endonuclease recognition sites that are bound and protected by an endonuclease inhibitor (30). A cytosolic endonuclease cleaves the recognition sites when the protective factor is not present in excess. When there is an excess of substrate RNA, compared to the protective factor, then the endonuclease rapidly cleaves the exposed sites, leading to the rapid degradation of the substrate RNA molecules. The Xenopus endonuclease and protective factor have slightly different recognition sites within a 17-mer sequence, with the protective factor binding site being a subset of the endonuclease recognition site. This interplay of endonucleases and endonuclease inhibitors is similar to the role of the parathyroid cytosolic proteins regulating PTH mRNA stability. In chloroplast precursor mRNA, a stable stem-loop-protein complex is formed between three chloroplast proteins and the 3′-UTR of petD pre-mRNA (31). One of these chloroplast stem-loop binding proteins, CSP41, has substantial endonuclease activity, with certain conditions favoring RNA binding over ribonuclease activities, suggesting a mode of in vivo regulation. A similar bifunctional role for the same cytosolic protein determining both stabilization and decay of an mRNA has been proposed for the A+U-rich binding protein, AUFI. This protein is associated with destabilization of the c-fos mRNA and with stabilization of the human α-globin mRNA’s 3′-UTR as part of a complex of three proteins (32).

In the present report, we have shown in an in vivo model of uremic secondary hyperparathyroidism that the increase in PTH mRNA levels was independent of changes in serum calcium and phosphate and was posttranscriptional. Studies with parathyroid cytosolic proteins binding to the PTH mRNA demonstrated that there was no change in binding to the PTH mRNA 3′-UTR in uremic rats. The degradation of PTH mRNA in an in vitro degradation assay was dramatically decreased by the parathyroid proteins of uremic rats. This decrease in degradation leads to the increase in PTH mRNA levels in these uremic rats. The identification of the parathyroid proteins that bind and degrade PTH mRNA, as well as the identification of the target sequences in the PTH mRNA 3′-UTR, will help
clarify the mechanisms that determine how uremia affects the parathyroid cell and in particular the stability of the PTH transcript. It may then allow the design of novel therapies for secondary hyperparathyroidism.

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References