Identification of AUFP1 as a Parathyroid Hormone mRNA 3′-Untranslated Region-binding Protein That Determines Parathyroid Hormone mRNA Stability

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Parathyroid hormone (PTH) mRNA levels are post-transcriptionally increased by hypocalcemia and decreased by hypophosphatemia, and this is mediated by cytosolic proteins binding to the PTH mRNA 3′-untranslated region (UTR). The same proteins are also present in other tissues, such as brain, but only in the parathyroid is their binding regulated by calcium and phosphate. The function of the PTH mRNA 3′-UTR-binding proteins was studied using an in vitro degradation assay. The competition for the parathyroid-binding proteins by excess unlabeled 3′-UTR destabilized the full-length PTH transcript in this assay, indicating that these proteins protect the RNA from RNase activity. The PTH mRNA 3′-UTR-binding proteins were purified by RNA affinity chromatography of rat brain S-100 extracts. The eluate from the column was enriched in PTH mRNA 3′-UTR binding activity. Addition of eluate to the in vitro degradation assay with parathyroid protein extracts stabilized the PTH transcript. A major band from the eluate at 50 kDa was sequenced and was identical to AU-rich binding protein (AUFP1). Recombinant AUFP1 bound the full-length PTH mRNA and the 3′-UTR. Added recombinant AUFP1 also stabilized the PTH transcript in the in vitro degradation assay. Our results show that AUFP1 is a protein that binds to the PTH mRNA 3′-UTR and stabilizes the PTH transcript.

Serum calcium is maintained within a narrow physiological range mainly due to the action of PTH, 1 which acts together with the biologically active metabolite of vitamin D, 1α,25-dihydroxyvitamin D (1). A seven-transmembranous calcium-sensing receptor on the parathyroid (PT) cell recognizes small changes in serum ionized calcium to regulate PTH secretion (2). Low serum calcium increases not only PTH secretion, but also PTH mRNA levels (3) and, if prolonged, PT cell proliferation (4). PTH then acts to correct serum calcium by mobilizing calcium from bone and renal reabsorption of calcium. Phosphate also regulates the PT, with low serum phosphate decreasing serum PTH, PTH mRNA levels, and parathyroid cell proliferation (5–8). Patients with chronic renal failure are unable to excrete the large amounts of phosphorus in the diet. They develop severe complications due to high serum phosphate, including hyperparathyroidism with overactivity of the PT gland, bone pain, and increased mortality (9). There is therefore great interest in understanding the regulation of the PT by calcium and phosphate.

PTH gene expression is markedly increased by hypocalcemia and decreased by hypophosphatemia, and these effects in vivo are post-transcriptional (5, 10). The PTH cDNA consists of three exons coding for the 5′-UTR (exon I), the prepro region of PTH (exon II), and the structural hormone together with the 3′-UTR (exon III) (11, 12). The rat 3′-UTR is 239 nt long out of the 712 nt of the full-length PTH RNA (12). We have shown that cytosolic proteins from parathyroid bind to the 3′-UTR of the rat PTH mRNA and regulate mRNA stability (10). PT proteins from hypocalcemic rats show increased binding to the PTH mRNA 3′-UTR by mobility shift and UV cross-linking assays, and this protein-RNA binding is decreased with hypophosphatemic PT proteins. Thus, the level of protein-RNA binding directly correlates with PTH mRNA levels. Since there is no PT cell line, an in vitro PTH RNA stability assay was utilized. This assay showed stabilization of the transcript by hypocalcemic proteins and marked instability with hypophosphatemic proteins (10). These studies indicate that there are instability regions in the PTH mRNA 3′-UTR that are protected by RNA-binding proteins. This protein-RNA interaction determines PTH mRNA stability.

In the present study, we have isolated one of the proteins that bind to the PTH mRNA 3′-UTR by affinity chromatography and characterized its functional role in stabilizing the PTH mRNA. Addition of recombinant AUFP1 stabilized the PTH transcript in an in vitro degradation assay with PT proteins. These results suggest an important function for AUFP1 in the transduction of changes in serum calcium and phosphate to the stability of the PTH mRNA.

EXPERIMENTAL PROCEDURES

Animals—Weaning male Sabra rats were fed a normal calcium (0.6%), normal phosphate (0.3%) diet; a low calcium (0.02%), normal phosphate (0.3%) diet; or a low phosphate (0.02%), normal calcium (0.6%) diet (Teklad) for 3 weeks. At 3 weeks, the microdissected parathyroids and other tissues were removed under pentobarbital anesthesia and blood samples were taken for serum calcium and phosphate. The low calcium diet resulted in a serum calcium of 4.8 ± 0.5 mg/dl (control = 10.7 ± 0.5 mg/dl). The low phosphate diet resulted in a serum phosphate of 4.2 ± 0.3 mg/dl (control = 9.7 ± 0.9 mg/dl) and serum calcium of 12.5 ± 0.6 mg/dl (control = 10.7 ± 0.5 mg/dl).
Isolation and Identification of the 50-kDa Protein—S100 extracts were prepared from rat brain tissue. The tissue was removed from the rat under pentobarbital anesthesia and immediately washed in phosphate-buffered saline buffer at 4 °C. The tissue was homogenized with a Polytron in one volume of S100 buffer (50 mM Tris, pH 7.5, 25% glycerol, 7 mM MgCl2, 5 mM DTT, 0.1 M NaCl, 0.1 mM phenylmethylsulfonyl fluoride). The homogenate was centrifuged at 12,000 × g for 15 min at 4 °C and the supernatant was centrifuged again at 100,000 × g for 1 h (Beckman type TL-100) at 4 °C. The high speed supernatant (S100) was stored at −80 °C until it was used for protein purification and binding assays.

Heparin-Sepharose (6 g) (Amersham Pharmacia Biotech) was used to prepare a 1.5-mL bed volume column. The heparin-Sepharose column was washed with 250 ml of buffer B (50 mM Tris, pH 7.8, 2 mM EDTA, 5% glycerol, 7 mM β-mercaptoethanol) containing 0.1 mM NaCl. S100 brain tissue extract was from 2 rats (300 mg) was applied to the column (twice). The column was washed with 550 ml of buffer B containing NaCl (0.1 M), and the bound proteins were eluted from the column by a step gradient of buffer B containing increasing NaCl concentrations (0.1–1 M).

The fractions were assayed for binding to the PTH 3′-UTR by UV cross-linking. Fractions that showed maximal binding to the PTH 3′-UTR eluted at 230–550 mM NaCl and were pooled. The pooled fractions were then loaded on a CNBr-activated Sepharose column bound to 200 μg of PTH mRNA 3′-UTR that had been synthesized in vitro. The column was washed with buffer B containing 0.1 mM NaCl and the fraction of proteins that bound was then eluted with increasing NaCl concentrations and assayed by a UV cross-linking assay. Fractions that showed maximal binding were pooled and concentrated using a Centricon 30 filter (Amicon, Beverly, MA). A sample was used to identify the RNA-binding proteins by Northwestern analysis with PTH 3′-UTR as a labeled probe. The pooled fractions were run on a preparative polyacrylamide gradient gel (7–12%) and stained with Coomassie Blue. A 50-kDa band was excised from the gel, degraded with the endoprotease LysC, and the peptide products were analyzed by high performance liquid chromatography. Five peptides were microsequenced by Edman degradation.

UV Cross-linking Assay—UV cross-linking assay was performed using 10 μg of either brain S100 extracts or microdissected PT extracts as described previously. To measure binding activity of eluates from the RNA column, salt concentrations were normalized (as measured by conductivity). Equivalent aliquots of each fraction were tested. In some experiments, recombinant AUFP protein was used. The proteins were incubated with 32P-labeled RNA for the full-length or the 3′-UTR of the PTH cDNA. After UV cross-linking, the samples were digested by RNase A, fractionated by SDS-polyacrylamide gel electrophoresis, and autoradiographed as described previously (10).

Protein Gel Electrophoresis and Northwestern Analysis—Protein extracts were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels and electrotransferred onto nitrocellulose membrane (0.2 μm, Schleicher & Schuell).

For Northwestern assays, the membranes were pre-soaked in Tris-buffered saline plus Tween (10 mM Tris, pH 8, 150 mM NaCl, 0.05% Tween) and then incubated in a buffer containing 10 mM HEPES, pH 7.6, 40 mM KCl, 5% glycerol, 1 mM EDTA, 0.3 mM phenylmethanesulfonyl fluoride, 0.2% Nonidet P-40, 0.5 mM NaCl, 3 mM MgCl2, 0.1 mM EDTA, and 5 mg/ml BSA for 15 min at room temperature. The membranes were washed twice in TNE buffer (10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM DTT), and then incubation was performed in binding buffer (10 mM HEPES, pH 7.6, 150 mM KCl, 5 mM MgCl2, 0.2 mM DTT, 8% glycerol) supplemented with 100 μg/ml RNase-free tRNA (Roche Molecular Biochemicals) and the RNA probe (1 × 106 cpm/ml) for 20 min at 95 °C and then for 2 h at room temp. The membranes were washed at low temperature for 3 times with TNE buffer, and RNA binding to the proteins was visualized by autoradiography.

RNA Electrophoretic Mobility Shift Assays (REMSA)—The PTH 3′-UTR RNA probe (5000 cpm) was incubated with microdissected PT or brain extracts (10 μg) or AUFP as indicated in a final volume of 10 μl containing 10 mM Tris, pH 7.5, 0.1 mM potassium acetate, 5 mM magnesium acetate, 2 mM EDTA, 8 units of RNase, 2 μg of tRNA, 50 μg of heparin, and 0.5 μl of PTH RNA in 10 μl with 50 mM Tris, pH 7.5, 1 mM MgCl2, supplemented with 100 μg/ml RNase-free tRNA, 5 mM MgCl2, and 0.2 mM DTT, 8% glycerol) supplemented with 100 μg/ml RNase-free tRNA (Roche Molecular Biochemicals) and the RNA probe (1 × 106 cpm/ml) for 20 min at 95 °C and then for 2 h at room temp. The membranes were washed at low temperature for 3 times with TNE buffer, and RNA binding to the proteins was visualized by autoradiography.

**RESULTS**

Excess PTH RNA 3′-UTR Leads to Rapid Decay of the Full-length PTH RNA in Vitro—PTH mRNA levels are determined by RNA-binding proteins that interact with sequences in the 3′-UTR of the PTH mRNA. When there is less binding, then the RNA is more accessible to RNase activity (10). Competition for the binding proteins with excess PTH mRNA 3′-UTR should lead to more rapid decay in an in vitro degradation assay of PTH mRNA transcript and PT cytosolic extract. Excess PTH RNA 3′-UTR led to a dramatic decrease in PTH RNA stability (Fig. 1). Addition of unlabeled PTH transcript that did not include the 3′-UTR as a control did not lead to a decrease in PTH RNA stability (Fig. 1). In fact, there was a slight increase in PTH RNA stability, which is not readily explainable. These experiments suggest that the cloning region pucHea<sup>Ψ</sup>-UTR competed for stability factors in the PT protein extract, resulting in their depletion from the extract. This resulted in rapid degradation of the PTH transcript. We therefore performed experiments to characterize the binding proteins.

**Purification of the PTH mRNA 3′-UTR-binding Proteins**—To identify the proteins that bind to the PTH mRNA 3′-UTR, we performed affinity chromatography. The proteins that bind the
PTH mRNA 3′-UTR are present in all tissues examined (10). We therefore used rat brain protein extracts and not the minute parathyroids as the source for the RNA-binding proteins. Rat brain S-100 extracts were chromatographed first on a heparin-Sepharose column to enrich for proteins that bind RNA. The fractions that showed maximum binding to the PTH 3′-UTR on UV cross-linking were then chromatographed on a PTH RNA affinity column. The affinity column consisted of cyanogen bromide-activated Sepharose linked to in vitro transcribed PTH RNA 3′-UTR. The proteins that bound the 3′-UTR column were eluted with increasing salt concentrations and studied by UV cross-linking to the PTH 3′-UTR RNA probe (Fig. 2A). There were three protein-RNA bands, at about 50, 60, and 110 kDa, for brain and parathyroid, consistent with our earlier studies (10). The proteins that eluted between 220 and 500 mM NaCl exhibited maximum binding (Fig. 2A) and were combined and concentrated. A sample was run on an SDS-polyacrylamide gel and transferred to a nitrocellulose membrane, which was stained for protein by Ponceau. The staining revealed several bands (Fig. 2B). To identify the RNA-binding proteins, the membrane was then incubated with a riboprobe for the PTH 3′-UTR for Northwestern analysis (Fig. 2B). The PTH 3′-UTR showed the most intense binding to three of the proteins. There was one protein at approximately 60 kDa, two at about 50 kDa, and other less intense bands.

**Fig. 1. Competitor PTH RNA 3′-UTR accelerates PTH RNA degradation in vitro.** The full-length, radiolabeled PTH mRNA transcript was incubated with cytosolic parathyroid protein extracts (10 μg) from rats fed a normal diet. At timed intervals samples were extracted, run on agarose gels, and autoradiographed to measure the intact transcript remaining. The degradation assay was performed in the presence of PT extracts without competitor, with 25 ng of unlabeled PTH RNA 3′-UTR, with unlabeled PTH RNA excluding the 3′-UTR (without 3′-UTR), or with a 63-nt unlabeled transcript comprising the sequences necessary for binding of proteins to the PTH RNA 3′-UTR. Excess 3′-UTR or the 63-nt transcripts led to accelerated decay of the full-length PTH transcript by the PT proteins. The competitor transcript that did not contain the 3′-UTR had no effect.

**Fig. 2A.** Polyacrylamide gel of rabbit brain cytosolic proteins. The PTH transcript was labeled with [32P]CTP and UV cross-linked to the PTH RNA probe (Fig. 1). The proteins that bound to the RNA probe were eluted with decreasing amounts of NaCl and run on a gel. The bands were visualized by autoradiography. **Fig. 2B.** Northwestern analysis of rabbit brain cytosolic proteins after UV cross-linking of the PTH RNA probe (Fig. 1). The RNA-binding proteins were eluted with decreasing amounts of NaCl and run on a gel. The proteins were then transferred to a nitrocellulose membrane and stained for protein by Ponceau. The PTH transcript was then added to the protein-RNA complexes and UV cross-linked. The complexes were then digested with RNase A. The RNase-resistant complexes were visualized by autoradiography.

**Fig. 3.** The full-length, radiolabeled PTH mRNA transcript was incubated with cytosolic parathyroid protein extracts (10 μg) from rats fed a normal diet. At timed intervals samples were extracted, run on agarose gels, and autoradiographed to measure the intact transcript remaining. The degradation assay was performed in the presence of PT extracts without competitor, with 25 ng of unlabeled PTH RNA 3′-UTR, with unlabeled PTH RNA excluding the 3′-UTR (without 3′-UTR), or with a 63-nt unlabeled transcript comprising the sequences necessary for binding of proteins to the PTH RNA 3′-UTR. Excess 3′-UTR or the 63-nt transcripts led to accelerated decay of the full-length PTH transcript by the PT proteins. The competitor transcript that did not contain the 3′-UTR had no effect. (Fig. 3). The same stabilizing effect was also found when the eluate was added to PT cytosolic extracts from control rats (data not shown). These results show that proteins eluted from the RNA column stabilize the PTH transcript in vitro and that the eluate can overcome the degrading effect of the PT proteins from low phosphate rats, whose PT proteins show decreased binding to the PTH 3′-UTR.

**Fig. 4A.** The full-length, radiolabeled PTH mRNA transcript was incubated with cytosolic parathyroid protein extracts (10 μg) from rats fed a normal diet. At timed intervals samples were extracted, run on agarose gels, and autoradiographed to measure the intact transcript remaining. The degradation assay was performed in the presence of PT extracts without competitor, with 25 ng of unlabeled PTH RNA 3′-UTR, with unlabeled PTH RNA excluding the 3′-UTR (without 3′-UTR), or with a 63-nt unlabeled transcript comprising the sequences necessary for binding of proteins to the PTH RNA 3′-UTR. Excess 3′-UTR or the 63-nt transcripts led to accelerated decay of the full-length PTH transcript by the PT proteins. The competitor transcript that did not contain the 3′-UTR had no effect. (Fig. 3). The same stabilizing effect was also found when the eluate was added to PT cytosolic extracts from control rats (data not shown). These results show that proteins eluted from the RNA column stabilize the PTH transcript in vitro and that the eluate can overcome the degrading effect of the PT proteins from low phosphate rats, whose PT proteins show decreased binding to the PTH 3′-UTR.

**One of the PTH mRNA 3′-UTR-binding Proteins Is AUF1—** The eluate from the RNA column contained several proteins. One of the proteins at 50 kDa was present in the highest concentration (Fig. 2B). For this reason the 50-kDa protein was gel-purified and microsequenced generating five peptide sequences of 10–17 residues each. Data base search identified the polypeptide as being identical to AU-rich binding protein (AUF1), which is known to be important to the half-life of other mRNAs (reviewed in Ref. 15). The peptide sequences did not identify which of the AUF1 isoforms had been isolated. However, the binding assays suggest that the PTH RNA 3′-UTR bound all isoforms. One of these isoforms, p40AU1, was further studied.

**Fig. 4B.** The full-length, radiolabeled PTH mRNA transcript was incubated with cytosolic parathyroid protein extracts (10 μg) from rats fed a normal diet. At timed intervals samples were extracted, run on agarose gels, and autoradiographed to measure the intact transcript remaining. The degradation assay was performed in the presence of PT extracts without competitor, with 25 ng of unlabeled PTH RNA 3′-UTR, with unlabeled PTH RNA excluding the 3′-UTR (without 3′-UTR), or with a 63-nt unlabeled transcript comprising the sequences necessary for binding of proteins to the PTH RNA 3′-UTR. Excess 3′-UTR or the 63-nt transcripts led to accelerated decay of the full-length PTH transcript by the PT proteins. The competitor transcript that did not contain the 3′-UTR had no effect. (Fig. 3). The same stabilizing effect was also found when the eluate was added to PT cytosolic extracts from control rats (data not shown). These results show that proteins eluted from the RNA column stabilize the PTH transcript in vitro and that the eluate can overcome the degrading effect of the PT proteins from low phosphate rats, whose PT proteins show decreased binding to the PTH 3′-UTR.

**AUF1 Stabilizes the PTH RNA Transcript in an in Vitro Degradation Assay with Parathyroid Proteins—** To demonstrate the function of AUF1 in PTH mRNA stability, we performed in vitro degradation assays. In the presence of cytosol, there is gradual degradation of the transcript as seen previously in Fig. 1. We measured the effect of added eluate from the RNA column on the ability of PT protein extracts from hypophosphatemic rats to degrade PTH RNA in vitro. Hypophosphatemic PT proteins showed more rapid degradation of PTH RNA in an in vitro degradation assay compared with PT proteins of control rats and also less binding to the PTH mRNA 3′-UTR (10). Proteins from hypophosphatemic PTs are therefore depleted in stabilizing factors. Complete depletion of these factors from PT cytosolic proteins by 3′-UTR affinity chromatography is not practicable because of the small size of the rat PT gland, which would require the use of >150 rats for each experiment. The degradation assay was therefore performed with PT proteins from hypophosphatemic rats and increasing amounts of eluate (~200 and 400 ng of protein) from the RNA column. The added eluate had no effect upon transcript stability at lower concentrations; however, higher concentrations of added eluate stabilized the PTH transcript throughout the experiment (t1/2 80 min, compared to 30 min with no eluant) (Fig. 3). The same stabilizing effect was also found when the eluate was added to PT cytosolic extracts from control rats (data not shown). These results show that proteins eluted from the RNA column stabilize the PTH transcript in vitro and that the eluate can overcome the degrading effect of the PT proteins from low phosphate rats, whose PT proteins show decreased binding to the PTH 3′-UTR.

**Fig. 5A.** The full-length, radiolabeled PTH mRNA transcript was incubated with cytosolic parathyroid protein extracts (10 μg) from rats fed a normal diet. At timed intervals samples were extracted, run on agarose gels, and autoradiographed to measure the intact transcript remaining. The degradation assay was performed in the presence of PT extracts without competitor, with 25 ng of unlabeled PTH RNA 3′-UTR, with unlabeled PTH RNA excluding the 3′-UTR (without 3′-UTR), or with a 63-nt unlabeled transcript comprising the sequences necessary for binding of proteins to the PTH RNA 3′-UTR. Excess 3′-UTR or the 63-nt transcripts led to accelerated decay of the full-length PTH transcript by the PT proteins. The competitor transcript that did not contain the 3′-UTR had no effect. (Fig. 3). The same stabilizing effect was also found when the eluate was added to PT cytosolic extracts from control rats (data not shown). These results show that proteins eluted from the RNA column stabilize the PTH transcript in vitro and that the eluate can overcome the degrading effect of the PT proteins from low phosphate rats, whose PT proteins show decreased binding to the PTH 3′-UTR.

**Fig. 5B.** The full-length, radiolabeled PTH mRNA transcript was incubated with cytosolic parathyroid protein extracts (10 μg) from rats fed a normal diet. At timed intervals samples were extracted, run on agarose gels, and autoradiographed to measure the intact transcript remaining. The degradation assay was performed in the presence of PT extracts without competitor, with 25 ng of unlabeled PTH RNA 3′-UTR, with unlabeled PTH RNA excluding the 3′-UTR (without 3′-UTR), or with a 63-nt unlabeled transcript comprising the sequences necessary for binding of proteins to the PTH RNA 3′-UTR. Excess 3′-UTR or the 63-nt transcripts led to accelerated decay of the full-length PTH transcript by the PT proteins. The competitor transcript that did not contain the 3′-UTR had no effect. (Fig. 3). The same stabilizing effect was also found when the eluate was added to PT cytosolic extracts from control rats (data not shown). These results show that proteins eluted from the RNA column stabilize the PTH transcript in vitro and that the eluate can overcome the degrading effect of the PT proteins from low phosphate rats, whose PT proteins show decreased binding to the PTH 3′-UTR.
hypophosphatemic rats, where there is more rapid degradation with hypophosphatemic parathyroid proteins (t1/2 of 30 min with hypophosphatemic PT proteins, and 60 min with normal PT proteins). Addition of p40 AUF1 to the hypophosphatemic proteins stabilized the transcript (t1/2). Even more than when the degradation assay was performed in the presence of proteins from normal rats. Control proteins had no effect (Fig. 5B). The control proteins used were BSA and LC8. LC8 also binds to the PTH mRNA 3′-UTR (16).

To understand the effect of AUF1 and the other proteins in the eluate on the degradation reaction, we added recombinant p40AUFI and the eluate to the reaction with hypophosphatemic proteins in concentrations where alone they had no effect on PTH RNA degradation (Figs. 3 and 5A). The PTH RNA was now markedly stabilized (Fig. 5A). Therefore, there is an additive effect of p40AUFI and the RNA-binding proteins eluted from the RNA column.

**DISCUSSION**

The post-transcriptional regulation of PTH gene expression by calcium and phosphate is mediated by the binding of cytosolic proteins to the PTH mRNA 3′-UTR (10). We have now identified one of the proteins that bind to the PTH mRNA 3′-UTR by RNA affinity chromatography and microsequencing as AUF1. Recombinant p40AUFI bound the PTH mRNA 3′-UTR by REMSA. There is no PT cell line; therefore, to demonstrate that AUF1 has a functional role in determining PTH mRNA stability, we performed in vitro degradation assays. Addition of p40AUFI with PT cytosolic extracts, stabilized the PTH transcript. Surprisingly, immunodepletion of rAUF1 from PT cytosolic extracts had no little or no effect on degradation of the transcript (data not shown). This result may reflect functional
full-length radiolabeled PTH mRNA was incubated with cytosolic parathyroid protein extracts (10 μg) from hypophosphatemic rats and at timed intervals samples were extracted, run on agarose gels and autoradiographed to measure the intact transcript remaining. In the presence of increasing doses of recombinant p40AUF1, p40AUF1 stabilized the PTH transcript dose-dependently. Addition of eluate (200 ng), prepared as in Fig. 3, together with 10 ng of p40AUF1 stabilized the PTH transcript at doses that alone had no effect. On the contrary, degradation with PT proteins from normal and hypophosphatemic (−P) rats, without and with added recombinant p40AUF1 (200 ng), or BSA (6 μg), or LC8 (6 μg). Recombinant p40AUF1, but not BSA or dynein light chain (LC8) stabilized the PTH transcript.

redundancy in the PT cytosolic extracts, in that only AUF1 was depleted and not the other PTH mRNA-binding proteins. In hypophosphatemia there are decreased binding in three parathyroid-protein-PTH RNA species, and a less stable transcript. This suggests that all three protein-RNA species seen by UV cross-linking are involved in determining the RNA stability. To deplete all the PTH RNA 3′-UTR-binding proteins from the PT cytosolic extract, we added excess PTH RNA 3′-UTR, or a smaller transcript of 63 nt that is sufficient for binding, to the degradation assay of PTH RNA with PT proteins (Fig. 1). This resulted in a rapid degradation of the PTH RNA, suggesting competition for the stabilizing proteins. Polysomal associated in vitro degradation of granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA was similarly enhanced by AUF1-containing competitor RNA (17, 18). The addition of the competitor RNA depleted the polysome associated AUUUA motif, which stabilized the GM-CSF mRNA by masking AUUUA motifs.

The short half-life of many proto-oncogenes and cytokines is mediated in part by the rapid turnover of their mRNAs (15). A+U-rich elements in the 3′-untranslated regions of these mRNAs serve as one recognition signal targeting the mRNAs for rapid degradation. AUF1 is a cytosolic protein that both binds to the proto-oncogene c-myc A+U-rich element (ARE) and specifically destabilizes c-myc mRNA in a cell-free mRNA decay system, which reconstituted mRNA decay processes found in cells (19). Moreover, the ARE binding affinities of AUF1 correlated with the potency of an ARE to direct degradation of a heterologous mRNA (20). These studies established a role for AUF1 in ARE-directed mRNA decay that is based upon its affinity for different AREs.

The role of AUF1 in mRNA decay is not restricted to proto-oncogenes. The developmental immaturity of neonatal phagocytic function is associated with a shorter half-life of GM-CSF mRNA. In vitro the decay of the GM-CSF in mononuclear cells is also unstable, and this instability was accelerated by protein fractions enriched for AUF1 (21). Moreover, this accelerated ARE-dependent decay of the GM-CSF 3′-UTR was attenuated by immunodepletion of AUF1, thereby demonstrating that the in vitro RNA decay is ARE- and AUF1-dependent (21). Anti-AUF1 immunoblotting showed significantly higher levels of two AUF1 protein isoforms and lower levels of one in cord than in adult mononuclear cell extracts. The results suggested that increased levels of specific AUF1 isoforms in cord mononuclear cells destabilized the GM-CSF mRNA by targeting it for increased degradation (22). The ARE destabilizing function in K562 cells was dramatically impeded during hemin-induced erythroid differentiation (23). Ectopic expression of hnRNP D/AUF1 in hemin-treated K562 cells restored the rapid decay directed by the ARE. The extent of destabilizing effect varied among the four isoforms of AUF1, with p37 and p42 displaying the most profound effect. These results demonstrated a specific cytoplasmic function for AUF1 as an RNA-destabilizing protein in ARE-mediated decay pathway.

In contrast to the role of AUF1 in the rapid degradation of mRNAs, it may have a role in the stabilization of other mRNAs, such as α-globin mRNA. Kiledjian et al. (24) identified AUF1 as one of the proteins that, together with two other proteins, α CP1 and α CP2, bind to the 3′-UTR of the α-globin mRNA and regulate the erythrocyte-specific accumulation of α-globin mRNA. Alone, none of these proteins can bind the α-globin 3′-UTR, and they only bind when they are complexed with the other proteins of the α-complex. However, Chkhaidze et al. (25) showed that in α-globin 3′-UTR the poly(C)-binding proteins, α CPs, were quantitatively incorporated into the α-complex in the absence of AUF1, suggesting that AUF1 may not be essential for the protein-RNA complex formation.

We have now shown that AUF1 binds PTH mRNA 3′-UTR...
and determines PTH mRNA stability. The PTH mRNA 3′-UTR has a region that is rich in A and U but does not have the classical ARE configuration. The PTH mRNA ARE is an example of a regulatory element, which is stabilized by AUF1 and other parathyroid cytosolic RNA-binding proteins. RNA-protein binding regulates PTH mRNA levels in response to changes in serum calcium and phosphate. The role of AUF1 in the regulation of PTH mRNA stability in response to changes in serum calcium and phosphate remains to be determined.

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