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Pin1 regulates parathyroid hormone mRNA stability
Rajiv Kumar

Secondary hyperparathyroidism often occurs in chronic kidney disease (CKD) and vitamin D deficiency, resulting in increased fractures and mortality. Understanding factors that stimulate parathyroid hormone (PTH) synthesis is important for devising methods to treat this condition. Previous work has demonstrated that murine Pth mRNA levels are regulated by proteins that bind AU-rich elements (AREs) within the 3′ UTR region of Pth mRNA and influence Pth mRNA stability. In this issue of the JCI, Nechama et al. demonstrate that in murine secondary hyperparathyroidism associated with CKD or Ca deficiency, the activity of Pin1, a peptidyl-prolyl isomerase, is reduced (see the related article beginning on page 3102). Reduced Pin1 activity resulted in the phosphorylation and degradation of an ARE-binding protein, K-homology splicing regulator protein (KSRP), which normally enhances the degradation of Pth mRNA. The activity of other ARE-binding proteins, such as AU-rich binding factor 1 (AUF1), that increase Pth mRNA stability, was increased, thereby increasing PTH synthesis. This work suggests new ways by which to regulate PTH synthesis in secondary hyperparathyroidism.

RNA processing in the cytoplasm regulates RNA concentrations
Following transcription, nascent RNA is processed by 5′ methyl capping, splicing, cleavage, and polyadenylation in the nucleus (1–4) (Figure 1). RNA is exported from the nucleus and associates with various cellular structures prior to association with the ribosome. In the cytoplasm, RNA transcripts interact with RNA-binding proteins that influence RNA half-life and stability within the cell (5–8) (Figure 1). RNA-binding proteins (Table 1) associate with sequence-specific elements (adenine- and uridine-rich elements [AREs]) either within the coding or, more usually, within the 3′ UTRs of RNA. AREs regulate the rate at which mRNAs are degraded in cells and were first described as important elements involved in the regulation of the stability and half-life of proteoxygen and cytokine mRNAs (1, 9–12). AREs often contain overlapping adenine- and uridine-containing AUUUA pentamers that are found in U-rich regions within the 3′ UTRs of various genes (13). Three classes of AREs have been described: class I AREs contain...
several copies of the AUUUA motif dispersed within U-rich regions; class II AREs possess at least two overlapping UAUUUUA(U/A) nonamers; and class III AREs are less well defined and generally do not contain an AUUUA sequence (1, 10, 13). Whether an mRNA species containing an ARE bound to ARE-binding proteins (ARE-BPs) is degraded or stabilized is partly dependent upon the cellular milieu, physiological circumstances, and the relative amounts of different bound stabilizing or destabilizing ARE-BPs. Following binding of ARE-BPs to an ARE, RNAs are targeted for translation or degradation. RNAs targeted for degradation undergo deadenylation, decapping, and degradation in a large multiprotein complex, the exosome, or in cytoplasmic compartments known as GW bodies or P-bodies (14–16).

**Pathogenesis of secondary hyperparathyroidism**

Secondary hyperparathyroidism occurs in the clinical context of vitamin D deficiency, Ca deficiency, and chronic kidney disease (CKD) (26, 27). The pathogenesis of secondary hyperparathyroidism in CKD is multifactorial and includes phosphate retention and hyperphosphatemia, hypocalcemia, 1α, 25-dihydroxyvitamin D deficiency, intestinal Ca malabsorption, the reduction in vitamin D receptor concentrations within the PT gland, and reduced Ca\(^{2+}\)-sensing receptor amounts in PT tissue (28–30). Not only is PTH synthesis increased with concomitant increases in serum PTH concentrations, but PT hyperplasia often occurs as well (29, 30). In CKD and dialysis patients, uncontrolled secondary hyperparathyroidism is associated with an increased incidence of fractures and increased mortality (31–34). Numerous methods, including the control of serum phosphate concentrations, the administration of vitamin D analogs, Ca supplementation, and the administration of calcimimetics, have been developed to control PTH levels in CKD and dialysis patients (35–38), but secondary hyperparathyroidism in CKD remains a significant problem. Additional methods for the treatment of this condition would therefore be of value.

**Pth mRNA amounts are regulated by Ca\(^{2+}\) and Pi by posttranscriptional mechanisms**

In murine PT glands, changes in serum Ca\(^{2+}\) concentrations are sensed by PT chief cells via a cell-surface, G protein–coupled receptor, the Ca\(^{2+}\)-sensing receptor, and result in rapid (within minutes) alterations in parathyroid hormone (PTH) secretion (22, 23). More long-term changes in serum Ca\(^{2+}\) concentrations (over several hours) result in increases or decreases in PTH synthesis and PTH mRNA concentrations in the PT gland (24, 25).
portion of the 3′ UTR of Pth mRNA (Figure 2). A 63-nt ARE in the 3′ UTR regulates Pth mRNA stability in response to changes in Ca\(^{2+}\) and Pi concentrations (39, 41, 42). The 63-nt element consists of a core 26-nt minimal binding sequence and adjacent flanking regions. The Pth RNA ARE does not contain AUUUA sequences and falls into the class III category of AREs (42, 43). Two proteins, AU-rich binding factor 1 (AUF1) and K-homology splicing regulator protein (KSRP), bind the ARE in the 3′ UTR of Pth mRNA (43, 44). AUF1 increases Pth mRNA half-life, whereas KSRP has the opposite effect (43, 44). Both proteins are regulated by changes in serum Ca\(^{2+}\) and Pi concentrations and are altered by CKD (43, 45).

**Table 1**

<table>
<thead>
<tr>
<th>ARE-BP</th>
<th>Effect on stability of mRNA of various genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUF1 (HNRNPD, heterogenous nuclear ribonucleoprotein D0)</td>
<td><strong>Increase</strong> c-myc, c-fos, PTH, GMCSF, TNFA, GMCSF, IL3</td>
</tr>
<tr>
<td>HuR (Hu-antigen R, ELAV-like protein 1)</td>
<td>c-myc, c-fos, MynO, cyclin A, cyclin B1, cyclin D1, NOS2, GMCSF, TNFA, COX2, IL3, VEGF, myogenin</td>
</tr>
<tr>
<td>Hel-N1 (Hu-antigen B, ELAV-like protein 2)</td>
<td>TNFA, GLUT1</td>
</tr>
<tr>
<td>HuD (Hu-antigen D, ELAV-like protein 4)</td>
<td>GAP43</td>
</tr>
<tr>
<td>KSRP (far upstream element-binding protein 2, FUSE-binding protein 2)</td>
<td>–</td>
</tr>
<tr>
<td>TTP (tristetraproline)</td>
<td>–</td>
</tr>
<tr>
<td>BRF-1 (B-related factor 1, transcription factor IIIB 90 kDa subunit)</td>
<td>–</td>
</tr>
<tr>
<td>Nucleolin</td>
<td>Bcl-2</td>
</tr>
<tr>
<td>TINO (RNA-binding protein MEX3D, RING finger- and KH domain-containing protein 1, RING finger protein 193)</td>
<td>–</td>
</tr>
<tr>
<td>PAIP2 (Polyadenylate-binding protein–interacting protein 2)</td>
<td>VEGF</td>
</tr>
</tbody>
</table>

Modified with permission from Nucleic Acids Research (9).
Pin1, a peptidyl-prolyl isomerase, alters KSRP phosphorylation and binding of KSRP to the Pth ARE

In the accompanying article, Nechama et al. have elegantly dissected the manner in which Pth mRNA is degraded via AREs and cognate ARE-BPs in secondary hyperparathyroidism in rodents and have shown a role for the enzyme peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (Pin1) in this process (46, Figure 2). The authors demonstrate that the secondary hyperparathyroidism associated with CKD or Ca deficiency is due in part to reduced Pin1 activity in the PT glands. The reduction in Pin1 activity reduced the ratio of the ARE-BPs, KSRP, and AUF1, which normally exert opposite effects on Pth mRNA stability. As a result, Pth mRNA half-life and stability were increased due to unopposed AUF1 activity. The data suggest that it is possible to modulate Pth mRNA half-life and stability by altering the activity of Pin1 and by changing KSRP concentrations within the PT cell.

Pin1 is a peptidyl-prolyl isomerase that specifically binds serine/threonine-protein motifs and catalyzes the cis-trans isomerization of peptide bonds, thereby changing the activity of proteins (47, 48). Previous work from other laboratories has shown that Pin1 interacts with AUF1 and stabilizes GMCSF and TGFβ mRNAs (49, 50). Nechama et al. (46) hypothesized that Pin1 might also alter Pth mRNA stability and play a role in the pathogenesis of secondary hyperparathyroidism seen in CKD. They showed the presence of Pin1 epitopes and Pin1 enzymatic activity in PT glands and PT extracts. Induction of secondary hyperparathyroidism by feeding rats a diet low in Ca or by inducing CKD with adenine reduced Pin1 activity. Reduced Pin1 activity correlated with increased Pth mRNA levels in the PT glands of such animals. Inhibition of Pin1 activity with the inhibitor juglone increased Pth mRNA levels. The increase in Pth mRNA levels in juglone-treated PT was posttranscriptional, since nuclear run-on experiments revealed no changes in the transcription rate of Pth. In a surrogate cell line, HEK293 (a PT cell line is not available for transfection experiments), Pin1 overexpression accelerated Pth mRNA decay, whereas Pin1 knockdown with siRNA decreased Pth mRNA decay. Also, Pin1 overexpression was without effect on the half-life of a Pth transcript lacking the ARE-containing Pth 3′ UTR.

Both the protein-interaction domain and the peptidyl-prolyl cis-trans isomerization domains of Pin1 were necessary for the effect of this protein on Pth mRNA stability. Interestingly, when the 63-nt Pth ARE was introduced into the 3′ UTR of a growth hormone reporter gene (GH63nt), Pin1 overexpression decreased chimeric GH mRNA levels. Treatment with juglone of cells transfected with such a construct decreased Pin1 activity and increased GH63-nt mRNA levels. Pin1 bound KSRP, an ARE-BP that increases the degradation of Pth mRNA. In cells transfected with a chimeric GH63-nt reporter containing the Pth 63-nt ARE, KSRP overexpression decreased GH63-nt mRNA levels. Conversely, KSRP depletion increased GH63-nt mRNA levels in the presence of Pin1 expression. In such cells, Pin1 inhibition prevented KSRP-mediated decreases in GH63-nt mRNA. The authors also demonstrated that inhibition of Pin1 by juglone in PT glands in vivo reduced KSRP binding to Pth mRNA, thus increasing Pth mRNA half-life. Nechama et al. showed that Pin1 prevents the phosphorylation of KSRP at serine residue 181 and that a mutant KSRP (S181A) that is incapable of being phosphorylated had increased activity. Pin1-knockout mice had increased PT gland PTH levels and circulating serum PTH concentrations without changes in serum Ca and Pi levels.

In summary, the data reported in this issue by Nechama et al. (46) are consistent with a biological role for Pin1 in the pathogenesis of secondary hyperparathyroidism in rat PT via regulation of the amount of active, nonphosphorylated KSRP in PT cells. Several additional areas of investigation remain to be explored. For example, it is unclear what triggers the reduction in Pin1 activity in the parathyroids in chronic renal failure and Ca deficiency. Future studies might be directed at identifying factors that regulate Pin1 activity and expression in the PT gland. Precise quantification of KSRP/AUF1 ratios in the PT gland in different conditions would also be of value. The development of PT gland-specific modulators of ARE-BPs might result in drugs that are effective for the control of secondary hyperparathyroidism and PT hyperplasia. Stay tuned for developments in this area.

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Inflammation-associated lung injury is a major cause of morbidity and mortality for patients in intensive care units. Although the cellular and molecular events that initiate lung inflammation are now well understood, the mechanisms that promote its resolution remain poorly defined. In this issue of the JCI, D’Alessio et al. show in a mouse model that recovery from acute lung injury is not simply a passive process, but involves Tregs in an active resolution program (see the related article beginning on page 2898).

Overview of lung injury
Acute lung injury (ALI) is a syndrome defined by bilateral pulmonary infiltrates on chest radiography and arterial hypoxemia in the absence of left atrial hypertension. This syndrome has a rapidly progressive clinical presentation characterized by acute onset of dyspnea and respiratory fail-

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Resolving lung injury: a new role for Tregs in controlling the innate immune response

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