Phosphate and the parathyroid

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The phosphate (Pi) retention in patients with chronic kidney disease leads to secondary hyperparathyroidism (2HPT). 2HPT is the physiological response of the parathyroid not only to Pi retention but also to decreased synthesis of $1,25(OH)_2$ vitamin D, and the attendant hypocalcemia. 2HPT is characterized by increased PTH synthesis, secretion, and parathyroid cell proliferation. Extracellular fluid (ECF) Ca^{2+} is recognized by the parathyroid calcium receptor and a small decrease in the ECF Ca^{2+} results in relaxation of the calcium receptor and allows the unrestrained secretion and synthesis of PTH and in the longer term, parathyroid cell proliferation. Both $1,25(OH)_2$ vitamin D and fibroblast growth factor 23 inhibit PTH gene expression and secretion. Secondary hyperparathyroidism can initially be controlled by a single therapeutic intervention, such as a Pi-restricted diet, a calcimimetic, or an active vitamin D analog. In this review we discuss the mechanisms whereby Pi regulates the parathyroid. Pi has a direct effect on the parathyroid which requires intact parathyroid tissue architecture. The effect of Pi, as of Ca^{2+}, on PTH gene expression is post-transcriptional and involves the regulated interaction of parathyroid cytosolic proteins to a defined *cis* acting sequence in the PTH mRNA. Changes in serum Ca^{2+} or Pi regulate the activity of *trans* acting interacting proteins in the parathyroid, which alters their binding to a defined 26 nucleotide *cis* acting instability sequence in the PTH mRNA 3′-untranslated region. The *trans* factors are either stabilizing or destabilizing factors and their regulated binding to the PTH *cis* acting element determines the PTH mRNA half-life. The responses of the parathyroid to changes in serum Pi are now being revealed but the sensing mechanisms remain a mystery.

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Pi HOMEOSTASIS

Extracellular fluid (ECF) phosphate (Pi) concentration is regulated by a combination of local and humoral factors. The major humoral factors are the phosphatonins, fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH).[1] Both FGF23 and PTH are phosphaturic hormones and act to independently inhibit the activity of the renal sodium phosphate cotransporter, Na/Pi-2a, with resultant phosphaturia.[2] The local factors act as sensing mechanisms in different tissues, which determine the activity of the Na^+/Pi cotransporters.[3] The local tissue response to ECF Pi has been best characterized in the kidney, although it occurs also in both the duodenum and the parathyroid.[4–6] The kidney has the innate ability to detect changes in serum Pi and regulate the active reabsorption of Pi, an effect that is maintained in proximal tubule cell lines, and is independent of any hormonal affect.[7] The change in intracellular Pi then activates signal transduction and has local tissue effects, which may be physiological or pathological, depending on the concentration and duration of the Pi stimulus. Recently, it has been shown that the intestine detects the presence of increased dietary phosphate and rapidly increases renal phosphate excretion.[4] An increase in ECF Pi leads to an increase in FGF23 and PTH secretion. These effects are independent of any changes in $[Ca^{2+}]_o$ or $1,25(OH)_2$ vitamin D, which themselves regulate both PTH and FGF23 secretion, although in opposite directions.[8–10] A high $[Ca^{2+}]_o$ or $1,25(OH)_2$ vitamin D suppresses PTH secretion and stimulates FGF23 secretion.[9,11] The response to changes in Pi concentration implies a sensitive Pi-sensing system, the nature of which is a mystery.[12] Pi homeostatic mechanisms are well developed not only in mammals but also in unicellular organisms, both prokaryotes and eukaryotes. Growth in a nutrient medium without Pi results in the induction of genes that code for proteins responsible for Pi transport and secreted enzymes that would increase the supply of Pi in the medium and for those regulating the cell cycle.[13] The power of yeast genetics has provided an unparalleled strength to dissect out the regulatory pathways used by yeast in response to Pi depletion.[14] However, even in yeast, the Pi sensor remains to be identified.

Pi AND FGF23 EXPRESSION

Fibroblast growth factor 23 is predominantly secreted by osteocytes and is a major factor in the regulation of Pi homeostasis.[10,15] Dietary Pi loading leads to an increase in

bone FGF23 mRNA and serum FGF23. This effect is not a rapid response like that of the parathyroid calcium receptor (CaR) in response to changes in Ca^{2+} concentration or a hormone ligand and its receptor, but is rather only evident after longer time periods. It is not known how bone cells sense the increase in serum Pi. $1,25(OH)_2$ vitamin D also increases FGF23 synthesis and secretion by bone cells and it was shown that the vitamin D receptor expression in chondrocytes was necessary for this regulation.[10,16] Both *in vivo*, using mice with chondrocyte-specific inactivation of vitamin D receptor, and *in vitro* analysis showed that normal FGF23 production by osteoblasts or osteocytes is dependent on vitamin D receptor genomic action in chondrocytes.[16] Therefore, the vitamin D receptor signaling in chondrocytes regulates FGF23 synthesis.

An increase in serum calcium is another factor that increases FGF23 secretion.[9] In turn, FGF23 acts on the kidney to cause phosphaturia and a decreased synthesis of $1,25(OH)_2$ vitamin D and potentially corrects the high levels of Pi and $1,25(OH)_2$ vitamin D. FGF23 also regulates PTH gene expression and secretion. It acts on its receptor, Klotho-FGF receptor1c in the parathyroid, to cause a decrease in PTH mRNA levels and PTH secretion, an effect mediated by the mitogen-activated protein kinase pathway.[17] However, in chronic kidney disease (CKD), there are increased levels of serum FGF23 and PTH, indicating a resistance to the effect of FGF23 in the parathyroid in CKD.[18–21] The mechanism of the resistance of parathyroid to FGF23 remains to be explained.

Pi AND THE PARATHYROID

The parathyroid is geared to respond to a low serum Ca^{2+} by secreting PTH, which then acts on its target tissues to correct the serum Ca^{2+}.[22] The parathyroid senses serum Ca^{2+} through a membrane receptor, the G-protein-coupled receptor (GPCR), the CaR.[23] A high ECF Ca^{2+} activates the CaR to initiate signal transduction that inhibits PTH synthesis and secretion and parathyroid cell proliferation.[24] When the serum Ca^{2+} is decreased, more PTH is secreted, which then acts on its cognate G-protein-coupled receptor, the PTH1R, at its target tissues, bone and the renal tubule, and corrects the serum Ca^{2+}. PTH also causes phosphaturia and hence decreases serum Pi. A major hormone regulating Pi homeostasis is FGF23, which acts on the kidney to cause Pi loss and inhibits $1,25(OH)_2$ vitamin D secretion. However, FGF23 and PTH also share a direct interaction, in which FGF23 acts on the parathyroid to decrease PTH gene expression and secretion.[17,25] The trio is maintained in tempo by the action of $1,25(OH)_2$ vitamin D, which fine tunes PTH and FGF23 by increasing FGF23 and decreasing PTH. Pi in turn regulates parathyroid gland activity and PTH secretion independently of secondary changes in ECF Ca^{2+}, $1,25(OH)_2$ vitamin D, or FGF23, thus completing a network of endocrinological feedback loops (Figure 1).[26] These endocrinological feedback loops have been studied in animals with a normal renal function.

Pi REGULATES THE PARATHYROID INDEPENDENTLY OF CALCIUM AND $1,25(OH)_2$ VITAMIN D

The demonstration of a direct effect of high Pi on the parathyroid *in vivo* has been difficult. One of the reasons is that the various maneuvers used to increase or decrease serum Pi invariably lead to a change in the ionized Ca^{2+} concentration. In moderate renal failure, Pi clearance decreases and serum Pi increases; this increase becomes an important problem in severe renal failure. Hyperphosphatemia has always been considered central to the pathogenesis of secondary hyperparathyroidism (2HPT), but it has been difficult to separate the effects of hyperphosphatemia from those of the attendant hypocalcemia and decrease in serum $1,25(OH)_2$ vitamin D levels. However, it was shown by careful studies in dogs with experimental CKD that dietary Pi restriction prevented 2HPT.[27,28] Pi restriction corrected the 2HPT of CKD independent of changes in serum calcium and $1,25(OH)_2$ vitamin D levels.[28] Dietary restriction of both calcium and Pi led to lower levels of serum Pi and Ca^{2+}, with no increase in the low levels of serum $1,25(OH)_2$ vitamin D. Despite this, there was a 70% decrease in PTH levels. This study suggested that, at least in CKD, Pi affected the parathyroid cell by a mechanism independent of its effect on serum $1,25(OH)_2$ vitamin D and Ca^{2+} levels.[28] Therefore, Pi plays a central role in the pathogenesis of 2HPT, both by its effect on serum $1,25(OH)_2$ vitamin D and Ca^{2+} levels and, possibly, independently. These results were later substantiated in clinical studies that demonstrated that Pi restriction in patients with CKD prevented the increase in serum PTH levels.[29–33] The mechanism of this effect was not clear, although at least part of it was considered to be due to changes in serum $1,25(OH)_2$ vitamin D concentrations. In a study of patients with early CKD, Levin *et al.*[34] showed that low levels of $1,25(OH)_2$ vitamin D occur earlier in the course of estimated glomerular filtration rate (GFR) decline than do elevations in serum PTH levels. The increased serum PTH preceded the changes in serum Ca^{2+} or Pi. The low $1,25(OH)_2$ vitamin D levels might then lead to a secondary increase in PTH[11,35] that would lead to increased phosphaturia. As long as there are adequate renal reserves the augmented phosphaturia would prevent hyperphosphatemia. The time sequence of serum FGF23 levels during the induction of CKD and the subsequent progression of CKD remains to be studied. Serum FGF23 increases in patients with CKD stage. FGF23 was elevated at CKD stage 4 and 5 compared with CKD 1–2 in parallel with hyperphosphatemia.[21] It was suggested that high levels of FGF23 predict the development of hyperparathyroidism in dialysis patients.[36] The low levels of serum $1,25(OH)_2$ vitamin D may reflect a response to a direct effect of Pi on the renal synthesis of $1,25(OH)_2$ vitamin D. Pi directly regulated the production of $1,25(OH)_2$ vitamin D by kidney cells in culture[37,38] and *in vivo*.[30,39]

The effects of serum Pi on PTH gene expression and serum PTH levels are also independent of any changes in serum Ca^{2+} or $1,25(OH)_2$ vitamin D in rats with a normal

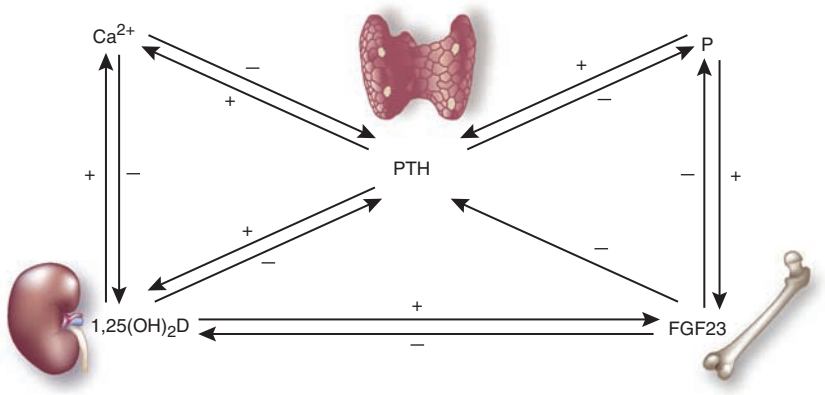

Figure 1 | Inter-relationships between Ca^{2+} and Pi and their hormones, PTH, FGF23, and $1,25(OH)_2$ vitamin D. There is a network of endocrinological feedback loops that govern mineral homeostasis. The effect of calcium to increase serum FGF23 levels, a low Pi level to increase serum $1,25(OH)_2D$, and a high Pi level to decrease serum $1,25(OH)_2D$ is not shown. FGF23, fibroblast growth factor 23; PTH, parathyroid hormone

renal function.[26] This was shown using vitamin D-deficient rats fed a diet with no vitamin D, low calcium, and low Pi. After one night of this diet, serum Pi had decreased markedly with no changes in serum Ca^{2+} or $1,25(OH)_2$ vitamin D. These rats with isolated hypophosphatemia had marked decreases in PTH mRNA levels and serum PTH. *In vitro* studies have shown that the effect of serum Pi on the parathyroid was direct. A high Pi concentration stimulated PTH secretion. Interestingly, the direct effect required maintenance of tissue architecture.[5,40,41] The requirement for intact tissue suggests either that the sensing mechanism for Pi is damaged during the preparation of isolated cells or that the intact gland structure is important for the Pi response. In addition, Estepa *et al.*[42] showed, in careful short-term studies in dogs, that up to 120 min, increasing serum Pi increased serum PTH at 4 mM Pi only and not at 3 mM.

The parathyroid responds to changes in serum Pi at the level of secretion, gene expression, and cell proliferation, although the mechanism of these effects is unknown.[43] The effect of high Pi in increased PTH secretion may be mediated by phospholipase A_2-activated signal transduction. Arachidonic acid and its metabolites inhibit PTH secretion.[44–46] It was suggested that Pi decreases the production of arachidonic acid in the parathyroid and that arachidonic acid decreases PTH secretion, but it is less clear to what extent the effect of Pi on PTH secretion is dependent upon this pathway. The effect of Pi and Ca^{2+} on PTH gene expression is currently being investigated in our laboratory.

FIBROBLAST GROWTH FACTOR 23 AND THE PARATHYROID

Fibroblast growth factor 23 signals through fibroblast growth factor receptors (FGFRs) bound by the transmembrane protein Klotho.[47] As most tissues express FGFRs, the expression of Klotho determines FGF23 target organs. Klotho protein is expressed not only in the kidney but also in the parathyroid, pituitary, and sino-atrial node.[48] We have shown that the administration of recombinant FGF23 suppresses PTH gene expression and secretion *in vivo* in rats and *in vitro* in organ cultures of rat parathyroids[17] (Figure 2). These studies were performed on rats with normal renal function. FGF23 also decreases PTH expression in primary cultures of bovine parathyroid cells.[25] In addition, a patient with a homozygous missense mutation in the *klotho* gene presented with severe tumoral calcinosis and defects in mineral ion homeostasis including marked hyperphosphatemia and hypercalcemia as well as elevated serum levels of PTH and FGF23.[49] The increased FGF23 may reflect that in the absence of a functional Klotho protein, neither FGF23 nor the hypercalcemia is effective in decreasing PTH secretion. Alternatively, the high PTH levels may represent an appropriate response to hyperphosphatemia. However, the *in vivo* and *in vitro* experimental data show conclusively that FGF23 acts directly on the parathyroid to decrease serum PTH.[17] This novel bone–parathyroid endocrine axis adds a new dimension to the understanding of mineral homeostasis (Figure 2). The paradox of the high PTH and FGF23 levels in CKD remains to be explained. FGF23 levels increase early in CKD before the development of serum mineral abnormalities and are independently associated with serum Pi, Pi excretion, and $1,25(OH)_2D$ deficiency.[50] It was suggested that increased FGF23 may contribute to maintaining normal serum Pi levels in the face of advancing CKD but may worsen $1,25(OH)_2D$ deficiency and thus contribute to the development of 2HPT.[50] FGF23 did not increase postprandially in patients with CKD or in health.[51]

PROTEIN–PTH mRNA INTERACTIONS DETERMINE THE REGULATION OF PTH GENE EXPRESSION BY SERUM CALCIUM AND Pi

The clearest rat *in vivo* models for effects of Ca^{2+} and Pi on PTH gene expression are diet-induced hypocalcemia with a large increase in PTH mRNA levels and diet-induced hypophosphatemia with a large decrease in PTH mRNA levels. In both instances, the effect was post-transcriptional, as shown by nuclear transcript run-on experiments.[52,53]

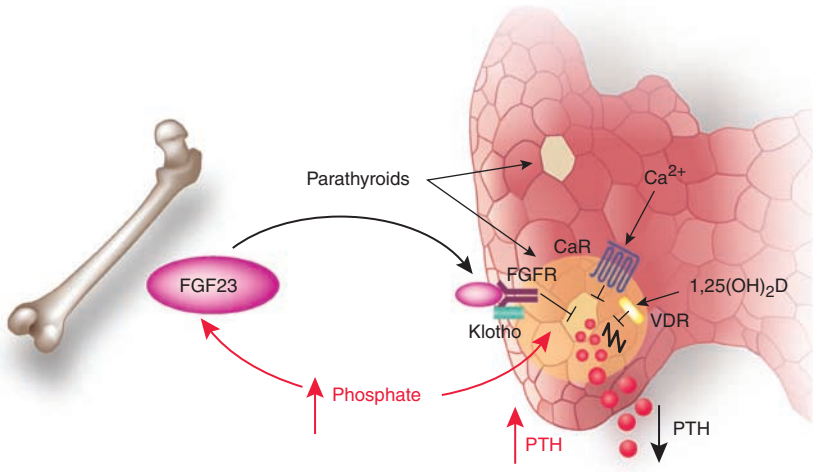

Figure 2 | FGF23 acts on the parathyroid to decrease PTH synthesis and secretion—a novel bone–parathyroid endocrine axis. FGF23 is secreted by bone after the stimulus of a high Pi level. In addition to the action of FGF23 on the kidney to cause Pi excretion and decrease the synthesis of $1,25(OH)_2$ vitamin D, it is now shown to act on the parathyroid to decrease PTH synthesis and secretion. This new endocrine axis contributes to our understanding of how the metabolism of bone Pi and Ca^{2+} is so tightly regulated. PTH is the major regulator of Ca^{2+} and FGF23 of Pi, and together with vitamin D they contribute to normal mineral and bone metabolism. This new endocrine axis may be useful for the discovery of new drugs to regulate PTH secretion. FGF23, fibroblast growth factor 23; FGFR, fibroblast growth factor receptor; CaR, calcium receptor; PTH, parathyroid hormone; VDR, vitamin D receptor. The red arrows indicate stimulatory pathways.

Parathyroid cytosolic proteins bound *in vitro*-transcribed PTH mRNA. Interestingly, this binding was increased with parathyroid extracts from hypocalcemic rats (with increased PTH mRNA levels) and decreased with parathyroid extracts from hypophosphatemic rats (with decreased PTH mRNA levels). Proteins from other tissues bound to PTH mRNA, but this binding is regulated by Ca^{2+} and Pi only with parathyroid proteins. Intriguingly, binding requires the presence of the terminal 60 nucleotides of the PTH transcript.[53]

In the absence of a parathyroid cell line, we have utilized an *in vitro* degradation assay to study the effects of hypocalcemic and hypophosphatemic parathyroid proteins on PTH mRNA stability.[53] In this assay, parathyroid cytosolic extracts from rats fed a control, low-calcium, or low-Pi diet are incubated with a radiolabeled PTH transcript and the decay of the PTH transcript by the parathyroid extracts is measured. Hypocalcemic parathyroid extracts stabilized the transcript, whereas hypophosphatemic parathyroid extracts led to a rapid degradation of the transcript. Moreover, the rapid degradation of PTH mRNA by hypophosphatemic extracts was totally dependent on an intact 3-untranslated region (UTR) and, in particular, on the terminal 60 nucleotides.

A CONSERVED SEQUENCE IN PTH mRNA 3-UTR BINDS PARATHYROID CYTOSOLIC EXTRACTS AND DETERMINES mRNA STABILITY IN RESPONSE TO CHANGES IN CALCIUM AND Pi

We have identified the minimal sequence for protein binding in the PTH mRNA 3′-UTR and determined its functionality.[54] A minimum sequence of 26 nucleotides was sufficient for PTH RNA–protein binding and competition (Figure 3). Significantly, this sequence was preserved among species.[55] The PTH mRNA 3′-UTR-binding element is an adenosine uradine (AU)-rich element. Sequence analysis of the PTH mRNA 3′-UTR of different species revealed a preservation of the 26-nucleotide protein-binding element among different species.[55,56] In contrast to protein-coding sequences that are highly conserved, UTRs are less conserved.[57] The conservation of the protein-binding element in the PTH mRNA 3′-UTR suggests that this element represents a functional unit that has been evolutionarily conserved. The *cis*-acting element is at the 3′-distal end in all the species in which it is expressed.[55]

To study the functionality of the sequence in the context of another RNA, a 63-bp cDNA PTH sequence consisting of the 26 nucleotide and flanking regions was fused with growth hormone cDNA. The conserved PTH RNA protein-binding region was necessary and sufficient for responsiveness to Ca^{2+} and Pi in *in vitro* degradation assay and therefore determines PTH mRNA stability and levels[52,54] (Figure 3). Therefore, Ca^{2+} and Pi change the properties of parathyroid cytosolic proteins, which bind specifically to the PTH mRNA 3′-UTR element and determine its stability. What are these proteins?

IDENTIFICATION OF THE PTH mRNA 3′-UTR-BINDING PROTEINS THAT DETERMINE PTH mRNA STABILITY

AU-rich binding factor 1 and upstream of N-*Ras*

Two of the PTH mRNA-binding proteins were identified as AU-rich binding factor (AUF1)[58] and Upstream of N-*ras*

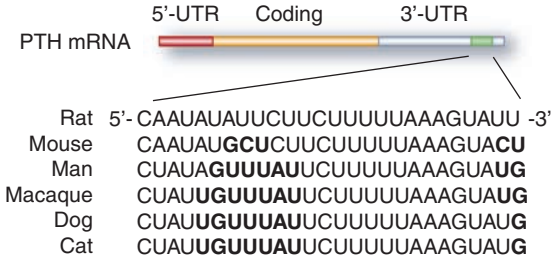

Figure 3 | Schematic representation of the PTH mRNA and the preserved *cis*-acting element in the PTH mRNA 3′-UTR. PTH mRNA including the 5′-UTR (red), coding region (yellow), and the 3′-UTR (white) is shown with the 26-nucleotide *cis*-acting element (green). The nucleotide sequence of the element in different species is shown. Nucleotides that differ from the rat sequence are in bold. PTH, parathyroid hormone; UTR, untranslated region.

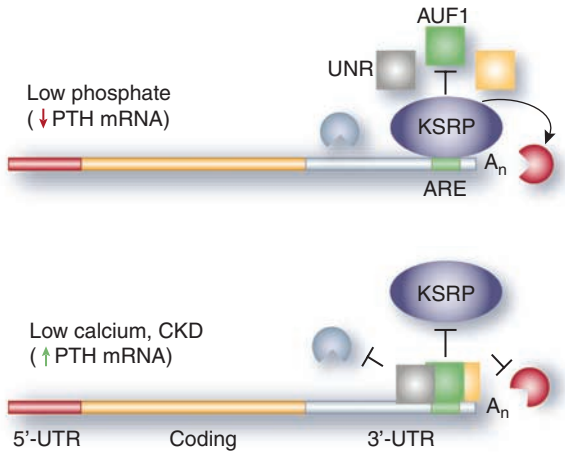

Figure 4 | Model for the regulation of PTH mRNA stability by changes in calcium and Pi levels and experimental chronic kidney disease, the role of PTH mRNA-interacting proteins. Low serum Pi increases the association of PTH mRNA with KSRP through the PTH mRNA 3′-UTR ARE (green box). KSRP may then recruit the exosome to PTH mRNA leading to decreased PTH mRNA stability and levels. A calcium-restricted diet induces the binding of a stabilizing complex consisting of AUF1 and Unr to the PTH mRNA ARE. This complex competes for the binding of KSRP to PTH mRNA and thereby inhibits PTH mRNA degradation leading to increased PTH mRNA stability and levels. Similar to calcium depletion, kidney failure increases *PTH* gene expression and this is associated with decreased PTH mRNA–KSRP interaction compared with control rats. ARE, AU-rich element; KSRP, KH-splicing regulatory protein; PTH, parathyroid hormone.

(Unr).[59] Recombinant AUF1 or Unr bound the full-length PTH mRNA and the 3′-UTR. Added recombinant AUF1 stabilized the PTH transcript in the *in vitro* degradation assay. Unr and AUF1 PTH stabilized full-length PTH mRNA in cotransfection experiments in HEK293 cells, but not a PTH mRNA lacking the terminal 60-nucleotide *cis*-acting protein-binding region. Depletion of AUF1 or Unr by siRNA decreased CMV promoter-driven PTH gene expression in HEK293 cells. Our results show that both AUF1 and Unr are PTH mRNA 3′-UTR-binding proteins that stabilize the PTH mRNA.

Post-translational regulation of AUF1

The regulation of protein–PTH mRNA binding involves post-translational modification of AUF1.[60,61] AUF1 levels are not regulated in parathyroid extracts from rats fed calcium- and Pi-depleted diets. However, two-dimensional gels showed post-translational modification of AUF1 that included phosphorylation.[60] Cyclosporine A, the calcineurin inhibitor, regulated AUF1 post-translationally and increased transfected growth hormone-PTH 63-nucleotide mRNA levels but not control growth hormone mRNA in HEK293 cells. Mice with a genetic deletion of the *calcineurin-Aβ* gene had markedly increased PTH mRNA levels that were still regulated by low-calcium and low-Pi diets. Therefore, calcineurin regulates AUF1 post-translationally *in vitro* and PTH gene expression *in vivo*, but still allows its physiological regulation by calcium and Pi.[60]

Post-translational modifications of AUF1 in experimental CKD

Most patients with CKD develop 2HPT with disabling systemic complications. Calcimimetic agents are effective tools in the management of 2HPT, acting through allosteric modification of the CaR on the parathyroid gland to decrease PTH secretion and parathyroid cell proliferation. R-568 decreased both PTH mRNA and serum PTH levels in adenine high-Pi-induced CKD.[61] The effect of the calcimimetic on PTH gene expression was post-transcriptional and correlated with differences in protein–RNA binding and post-translational modifications of the *trans*-acting factor AUF1 in the parathyroid. The AUF1 modifications as a result of CKD were reversed to those of normal rats by treatment with R-568. Therefore, CKD and activation of the CaR mediated by calcimimetics modify AUF1 post-translationally. These modifications in AUF1 correlate with changes in protein–PTH mRNA binding and PTH mRNA levels.[61]

THE mRNA DECAY-PROMOTING PROTEIN KH-SPLICING REGULATORY PROTEIN IS A PTH mRNA-REGULATING PROTEIN

We have recently shown that the mRNA decay-promoting protein KH-splicing regulatory protein (KSRP) binds to PTH mRNA in intact parathyroid glands and in transfected cells.[62] RNA immunoprecipitation analysis demonstrated that KSRP specifically interacts with PTH mRNA. This binding is decreased in glands from calcium-depleted or experimental chronic kidney failure rats in which PTH mRNA is more stable, compared with parathyroid glands from control and Pi-depleted rats in which PTH mRNA is less stable. These interactions were performed by cross-linking of parathyroid glands and therefore represent the protein–RNA interactions *in vivo*. KSRP recruits the RNA-degrading complex, the exosome, to AU-rich element-containing mRNAs resulting in their degradation. We showed that PTH mRNA decay depends on the KSRP-recruited exosome in parathyroid extracts. In transfected cells, KSRP overexpression and knockdown experiments show that KSRP decreases PTH

mRNA stability and steady-state levels through the PTH mRNA AU-rich element. Overexpression of one of the four AUF1 isoforms, p45, blocked KSRP–PTH mRNA binding and partially prevented the KSRP-mediated decrease in PTH mRNA levels. AUF1 also interacts with PTH mRNA in the RNA immunoprecipitation assay. In contrast to KSRP, AUF1–PTH mRNA interactions are increased in parathyroid glands from calcium-depleted or experimental CKD rats and decreased in parathyroid glands from control and Pi-depleted rats (Figure 4). Therefore, calcium or Pi depletion, as well as CKD, regulates the interaction of both KSRP and AUF1 with PTH mRNA and its half-life. These data indicate a novel role for KSRP in PTH gene expression.

Another PTH mRNA-binding protein is dynein light chain (M_r 8000) (LC8).[63] LC8 is part of the cytoplasmic dynein complexes that function as molecular motors that translocate along microtubules. PTH mRNA colocalized with polymerized microtubules in the parathyroid gland, as well as with a purified microtubule preparation from calf brain, and this was mediated by LC8. The dynein complex may be the motor for the transport and localization of mRNAs in the cytoplasm and is not involved in the regulation of PTH mRNA stability.

Pi AND PARATHYROID CELL PROLIFERATION

Patients with CKD often develop large nodular glands that in the past have necessitated subtotal or total parathyroidectomy. Pi accelerates the development of parathyroid hyperplasia and 2HPT in rats with renal failure, and Pi restriction prevents these abnormalities independent of changes in serum Ca^{2+} and $1,25(OH)_2$ vitamin D.[64] In experimental CKD, there is proliferation of parathyroid cells as measured by immunostaining, and this can be controlled by Pi restriction.[64,65] Many patients with CKD have low serum Ca^{2+} levels and hypocalcemia itself stimulates the proliferation of the parathyroid cells.[65] How Pi regulates parathyroid cell proliferation is not known. Interestingly, parathyroid gland growth in CKD rats fed a high-Pi diet was apparent within 2 days of uremia and increased nearly twofold by 2 weeks.[64] So the combination of a high serum Pi, low serum Ca^{2+}, and low serum $1,25(OH)_2$ vitamin D acts together to cause the hyperparathyroidism of CKD. However, despite this, control of just one of these factors is often adequate to control the hyperparathyroidism, whether it be Pi restriction, $1,25(OH)_2$ vitamin D, or activation of the CaR. For instance, the activation of the CaR by calcimimetics is able to potently decrease parathyroid cell proliferation despite CKD and hyperphosphatemia.[66] Inhibition of signaling by endothelin[67,68] or EGFR antagonists[69] was also able to inhibit parathyroid cell proliferation. Therefore, there is a common downstream signaling node that may be a shared target for the effects of Pi depletion, high extracellular Ca^{2+}, calcimimetics, or $1,25(OH)_2$ vitamin D to decrease PTH gene expression, parathyroid cell proliferation, and PTH secretion. EGFR and endothelin antagonists may also converge on this common pathway. Much remains to be discovered.

SUMMARY

Pi regulates PTH gene expression, serum PTH, and parathyroid cell proliferation, and this effect appears to be independent of the effect of Pi on serum Ca^{2+} and $1,25(OH)_2$ vitamin D and certainly of FGF23. The effects of Pi and Ca^{2+} on PTH gene expression are post-transcriptional. *Trans*-acting parathyroid cytosolic proteins bind to a defined *cis* element in the PTH mRNA 3′-UTR. This binding determines the degradation of PTH mRNA and thereby PTH mRNA half-life. The post-transcriptional effects of Ca^{2+} and Pi are the result of changes in the balance of stabilizing and degrading factors on PTH mRNA. These interactions also regulate PTH mRNA levels in experimental CKD (Figure 4). In diseases such as CKD, 2HPT involves abnormalities in PTH secretion, synthesis, and parathyroid cell proliferation. FGF23 acts on its receptor, Klotho-FGFR1c, to decrease PTH mRNA levels and secretion (Figure 2). An understanding of how the parathyroid is regulated at each level will help devise rational therapy for the management of conditions, such as CKD, in which 2HPT is associated with so much morbidity and contributes to the high mortality.

DISCLOSURE

All the authors declared no competing interests.

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REFERENCES

1. Berndt T, Kumar R. Phosphatonins and the regulation of phosphate homeostasis. *Annu Rev Physiol* 2007; **69**: 341–359.
2. Fukumoto S, Yamashita T. Fibroblast growth factor-23 is the phosphaturic factor in tumor-induced osteomalacia and may be phosphatonin. *Curr Opin Nephrol Hypertens* 2002; **11**: 385–389.
3. Werner A, Moore ML, Mantei N *et al.* Cloning and expression of cDNA for a Na/Pi-cotransport system of kidney cortex. *Proc Natl Acad Sci USA* 1991; **88**: 9608–9612.
4. Berndt T, Thomas LF, Craig TA *et al.* Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. *Proc Natl Acad Sci USA* 2007; **104**: 11085–11090.
5. Almaden Y, Canalejo A, Hernandez A *et al.* Direct effect of phosphorus on parathyroid hormone secretion from whole rat parathyroid glands *in vitro*. *J Bone Miner Res* 1996; **11**: 970–976.
6. Norbis F, Boll M, Stange G *et al.* Identification of a cDNA/protein leading to an increased Pi-uptake in *Xenopus laevis* oocytes. *J Membr Biol* 1997; **156**: 19–24.
7. Tenenhouse HS, Murer H. Disorders of renal tubular phosphate transport. *J Am Soc Nephrol* 2003; **14**: 240–248.
8. Kolek OI, Hines ER, Jones MD *et al.* 1alpha,25-Dihydroxyvitamin D3 upregulates FGF23 gene expression in bone: the final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G1036–G1042.
9. Shimada T, Yamazaki Y, Takahashi M *et al.* Vitamin D receptor-independent FGF23 actions in regulating phosphate and vitamin D metabolism. *Am J Physiol Renal Physiol* 2005; **289**: F1088–F1095.
10. Liu S, Tang W, Zhou J *et al.* Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. *J Am Soc Nephrol* 2006; **17**: 1305–1315.
11. Silver J, Naveh-Many T, Mayer H *et al.* Regulation by vitamin D metabolites of parathyroid hormone gene transcription *in vivo* in the rat. *J Clin Invest* 1986; **78**: 1296–1301.
12. Silver J, Dranitzki-Elhalel M. Sensing phosphate across the kingdoms. *Curr Opin Nephrol Hypertens* 2003; **12**: 357–361.
13. Lenburg ME, O'Shea EK. Signaling phosphate starvation. *Trends Biochem Sci* 1996; **21**: 383–387.

14. Lee YS, Huang K, Quiocho FA *et al.* Molecular basis of cyclin-CDK-CKI regulation by reversible binding of an inositol pyrophosphate. *Nat Chem Biol* 2008; **4**: 25–32.
15. Liu S, Gupta A, Quarles LD. Emerging role of fibroblast growth factor 23 in a bone-kidney axis regulating systemic phosphate homeostasis and extracellular matrix mineralization. *Curr Opin Nephrol Hypertens* 2007; **16**: 329–335.
16. Masuyama R, Stockmans I, Torrekens S *et al.* Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest* 2006; **116**: 3150–3159.
17. Ben Dov IZ, Galitzer H, Lavi-Moshayoff V *et al.* The parathyroid is a target organ for FGF23 in rats. *J Clin Invest* 2007; **117**: 4003–4008.
18. Imanishi Y, Inaba M, Nakatsuka K *et al.* FGF-23 in patients with end-stage renal disease on hemodialysis. *Kidney Int* 2004; **65**: 1943–1946.
19. Nakanishi S, Kazama JJ, Nii-Kono T *et al.* Serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. *Kidney Int* 2005; **67**: 1171–1178.
20. Fukagawa M, Nii-Kono T, Kazama JJ. Role of fibroblast growth factor 23 in health and in chronic kidney disease. *Curr Opin Nephrol Hypertens* 2005; **14**: 325–329.
21. Westerberg PA, Linde T, Wikstrom B *et al.* Regulation of fibroblast growth factor-23 in chronic kidney disease. *Nephrol Dial Transplant* 2007; **22**: 3202–3207.
22. Silver J, Kilav R, Naveh-Many T. Mechanisms of secondary hyperparathyroidism. *Am J Physiol Renal Physiol* 2002; **283**: F367–F376.
23. Brown EM, Gamba G, Riccardi D *et al.* Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. *Nature* 1993; **366**: 575–580.
24. Kifor O, MacLeod RJ, Diaz R *et al.* Regulation of MAP kinase by calcium-sensing receptor in bovine parathyroid and CaR-transfected HEK293 cells. *Am J Physiol Renal Physiol* 2001; **280**: F291–F302.
25. Krajisnik T, Bjorklund P, Marsell R *et al.* Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. *J Endocrinol* 2007; **195**: 125–131.
26. Kilav R, Silver J, Naveh-Many T. Parathyroid hormone gene expression in hypophosphatemic rats. *J Clin Invest* 1995; **96**: 327–333.
27. Slatopolsky E, Bricker NS. The role of phosphorus restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. *Kidney Int* 1973; **4**: 141–145.
28. Lopez-Hilker S, Dusso AS, Rapp NS *et al.* Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. *Am J Physiol* 1990; **259**: F432–F437.
29. Lucas PA, Brown RC, Woodhead JS *et al.* 1,25-dihydroxycholecalciferol and parathyroid hormone in advanced chronic renal failure: effects of simultaneous protein and phosphorus restriction. *Clin Nephrol* 1986; **25**: 7–10.
30. Portale AA, Booth BE, Halloran BP *et al.* Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J Clin Invest* 1984; **73**: 1580–1589.
31. Lafage MH, Combe C, Fournier A *et al.* Ketodiet, physiological calcium intake and native vitamin D improve renal osteodystrophy. *Kidney Int* 1992; **42**: 1217–1225.
32. Aparicio M, Combe C, Lafage MH *et al.* In advanced renal failure, dietary phosphorus restriction reverses hyperparathyroidism independent of the levels of calcitriol. *Nephron* 1994; **63**: 122–123.
33. Combe C, Aparicio M. Phosphorus and protein restriction and parathyroid function in chronic renal failure. *Kidney Int* 1994; **46**: 1381–1386.
34. Levin A, Bakris GL, Molitch M *et al.* Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. *Kidney Int* 2007; **71**: 31–38.
35. Naveh-Many T, Silver J. Regulation of parathyroid hormone gene expression by hypocalcemia, hypercalcemia, and vitamin D in the rat. *J Clin Invest* 1990; **86**: 1313–1319.
36. Nakanishi S, Kazama JJ, Nii-Kono T *et al.* Serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. *Kidney Int* 2005; **67**: 1171–1178.
37. Tanaka Y, DeLuca HF. The control of vitamin D by inorganic phosphorus. *Arch Biochem Biophys* 1973; **154**: 566–570.
38. Condamine L, Vztovsnik F, Friedlander G *et al.* Local action of phosphate depletion and insulin-like growth factor 1 on *in vitro* production of 1,25-dihydroxyvitamin D by cultured mammalian kidney cells. *J Clin Invest* 1994; **94**: 1673–1679.
39. Portale AA, Halloran BP, Curtis Morris J. Physiologic regulation of the serum concentration of l,25-dihydroxyvitamin D by phosphorus in normal men. *J Clin Invest* 1989; **83**: 1494–1499.
40. Slatopolsky E, Finch J, Denda M *et al.* Phosphate restriction prevents parathyroid cell growth in uremic rats. High phosphate directly stimulates PTH secretion *in vitro*. *J Clin Invest* 1996; **97**: 2534–2540.
41. Nielsen PK, Feldt-Rasmusen U, Olgaard K. A direct effect of phosphate on PTH release from bovine parathyroid tissue slices but not from dispersed parathyroid cells. *Nephrol Dial Transplant* 1996; **11**: 1762–1768.
42. Estepa JC, Aguilera-Tejero E, Lopez I *et al.* Effect of phosphate on parathyroid hormone secretion *in vivo*. *J Bone Miner Res* 1999; **14**: 1848–1854.
43. Silver J, Naveh-Many T, Kronenberg HM. Parathyroid hormone: molecular biology, chapter 25. In: Bilezikian JB, Raisz LG, Rodan GA (eds). *Principles of Bone Biology*, vol. 1, 2nd edn, Academic Press: San Diego, 2002, pp 407–422.
44. Bourdeau A, Souberbielle J-C, Bonnet P *et al.* Phospholipase-A_2 action and arachidonic acid in calcium-mediated parathyroid hormone secretion. *Endocrinology* 1992; **130**: 1339–1344.
45. Bourdeau A, Moutahir M, Souberbielle JC *et al.* Effects of lipoxygenase products of arachidonate metabolism on parathyroid hormone secretion. *Endocrinology* 1994; **135**: 1109–1112.
46. Almaden Y, Canalejo A, Ballesteros E *et al.* Effect of high extracellular phosphate concentration on arachidonic acid production by parathyroid tissue *in vitro*. *J Am Soc Nephrol* 2000; **11**: 1712–1718.
47. Kurosu H, Ogawa Y, Miyoshi M *et al.* Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem* 2006; **281**: 6120–6123.
48. Takeshita K, Fujimori T, Kurotaki Y *et al.* Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. *Circulation* 2004; **109**: 1776–1782.
49. Ichikawa S, Imel EA, Kreiter ML *et al.* A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest* 2007; **117**: 2684–2691.
50. Gutierrez O, Isakova T, Rhee E *et al.* Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol* 2005; **16**: 2205–2215.
51. Isakova T, Gutierrez O, Shah A *et al.* Postprandial mineral metabolism and secondary hyperparathyroidism in early CKD. *J Am Soc Nephrol* 2008; **19**: 615–623.
52. Kilav R, Silver J, Naveh-Many T. Regulation of parathyroid hormone mRNA stability by calcium and phosphate, chap. 5. In: Naveh-Many T (ed). *Molecular Biology of the Parathyroid*, 1st edn, Landes Bioscience and Kluwer Academic/Plenum Publishers: New York, 2005, pp 57–67.
53. Moallem E, Silver J, Kilav R *et al.* RNA protein binding and post-transcriptional regulation of PTH gene expression by calcium and phosphate. *J Biol Chem* 1998; **273**: 5253–5259.
54. Kilav R, Silver J, Naveh-Many T. A conserved cis-acting element in the parathyroid hormone 3′-untranslated region is sufficient for regulation of RNA stability by calcium and phosphate. *J Biol Chem* 2001; **276**: 8727–8733.
55. Bell O, Silver J, Naveh-Many T. Parathyroid hormone, from gene to protein, chap. 2. In: Naveh-Many T (ed). *Molecular Biology of the Parathyroid*, 1st ed. Landes Bioscience and Kluwer Academic/Plenum Publishers: New York, 2005, pp 8–28.
56. Bell O, Silver J, Naveh-Many T. Identification and characterization of *cis*-acting elements in the human and bovine parathyroid hormone mRNA 3′-untranslated region. *J Bone Miner Res* 2005; **20**: 858–866.
57. Naveh-Many T, Nechama M. Regulation of parathyroid hormone mRNA stability by calcium, phosphate and uremia. *Curr Opin Nephrol Hypertens* 2007; **16**: 305–310.
58. Sela-Brown A, Silver J, Brewer G *et al.* Identification of AUF1 as a parathyroid hormone mRNA 3′-untranslated region binding protein that determines parathyroid hormone mRNA stability. *J Biol Chem* 2000; **275**: 7424–7429.
59. Dinur M, Kilav R, Sela-Brown A *et al. In vitro* evidence that upstream of N-ras participates in the regulation of parathyroid hormone messenger ribonucleic acid stability. *Mol Endocrinol* 2006; **20**: 1652–1660.
60. Bell O, Gaberman E, Kilav R *et al.* The protein phosphatase calcineurin determines basal parathyroid hormone gene expression. *Mol Endocrinol* 2005; **19**: 516–526.
61. Levi R, Ben Dov IZ, Lavi-Moshayoff V *et al.* Increased parathyroid hormone gene expression in secondary hyperparathyroidism of experimental uremia is reversed by calcimimetics. correlation with posttranslational modification of the trans acting factor AUF1. *J Am Soc Nephrol* 2006; **17**: 107–112.

62. Nechama M, Ben Dov IZ, Briata P *et al.* The mRNA decay promoting factor K-homology splicing regulator protein post-transcriptionally determines parathyroid hormone mRNA levels. *FASEB J* 2008; **22**: 3458–3468.
63. Epstein E, Sela-Brown A, Ringel I *et al.* Dynein light chain (*M*$_r$ *8000*) binds the parathyroid hormone mRNA 3′-untranslated region and mediates its association with microtubules. *J Clin Invest* 2000; **105**: 505–512.
64. Denda M, Finch J, Slatopolsky E. Phosphorus accelerates the development of parathyroid hyperplasia and secondary hyperparathyroidism in rats with renal failure. *Am J Kidney Dis* 1996; **28**: 596–602.
65. Naveh-Many T, Rahamimov R, Livni N *et al.* Parathyroid cell proliferation in normal and chronic renal failure rats: the effects of calcium, phosphate and vitamin D. *J Clin Invest* 1995; **96**: 1786–1793.
66. Colloton M, Shatzen E, Miller G *et al.* Cinacalcet HCl attenuates parathyroid hyperplasia in a rat model of secondary hyperparathyroidism. *Kidney Int* 2005; **67**: 467–476.
67. Fujii Y, Moreira JE, Orlando C *et al.* Endothelin as an autocrine factor in the regulation of parathyroid cells. *Proc Natl Acad Sci USA* 1991; **88**: 4235–4239.
68. Kanesaka Y, Tokunaga H, Iwashita K *et al.* Endothelin receptor antagonist prevents parathyroid cell proliferation of low calcium diet-induced hyperparathyroidism in rats. *Endocrinology* 2001; **142**: 407–413.
69. Cozzolino M, Lu Y, Sato T *et al.* A critical role for enhanced TGF-alpha and EGFR expression in the initiation of parathyroid hyperplasia in experimental kidney disease. *Am J Physiol Renal Physiol* 2005; **289**: F1096–F1102.