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MOLECULAR CONTROL OF PHOSPHORUS HOMEOSTASIS (B VAN DER EERDEN, SECTION EDITOR)

Interplay Between FGF23, Phosphate, and Molecules Involved in Phosphate Sensing

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Abstract

Purpose of Review Despite the important progress made in understanding the regulation of phosphate (Pi) homeostasis over the past 20 years, the mechanisms underlying the very early step leading to the regulating cascade involving multiple hormones (PTH, vitamin D, FGF23) and organs (kidney, intestine, bone, parathyroid glands) are not deciphered. Particularly, knowledge on the Pi-sensing mechanism present within or on the surface of the cell that is able to detect changes in serum or local Pi concentrations and trigger an appropriate FGF23 synthesis/secretion is limited or absent.

Recent Findings Several molecular actors have recently been involved as potential key players in Pi sensing and Pi-dependent control of FGF23 secretion. Among them, the PiT1/Slc20a1 and PiT2/Slc20a2 proteins are standing out.

Summary We are just beginning to accumulate in vitro and in vivo data that will provide invaluable molecular tools to explore and understand the integrated response of the body to variations of Pi concentration.

Keywords Phosphate sensing · FGF23 · Slc20a1 · Slc20a2

Introduction

Discovered in 2000, Fibroblast growth factor 23 (FGF23) is an endocrine factor that plays a central role in Pi homeostasis in mammals [1, 2]. It is expressed mainly but not exclusively by osteoblasts and osteocytes in bone [3•] and targets the kidney to inhibit the renal reabsorption of phosphate (Pi) and the production of the active form of vitamin D, $1,25(OH)_2D$ [4–6]. Accordingly, increased serum levels of active FGF23 are observed in inherited or acquired disorders of Pi homeostasis leading to renal Pi wasting and abnormal vitamin D metabolism [7–9]. FGF23 depends on its co-receptor αKlotho and FGFR1c to mediate this action [10]. High serum FGF23 levels are also observed during chronic kidney disease (CKD) where unregulated prolonged hyperphosphatemia resulting from renal excretion defects leads to life-threatening situations due to inappropriate deposition of calcium-Pi crystals in vessels [11, 12]. These observations illustrate the need for a tight regulation of FGF23 secretion in maintaining normal serum Pi levels.

The $1,25(OH)_2$ vitamin D was shown to enhance FGF23 production and FGF23 serum levels [13, 14], but the low $1,25(OH)_2D$ serum levels during CKD [15] argues for the existence of other determinants of FGF23 secretion. Recently, FGF23 expression and/or secretion was shown to be regulated by various factors, including FGFR1 signaling [7, 16], iron deficiency [17], and pro-inflammatory stimuli [18, 19], but it remains to be determined whether these regulations have a role in Pi homeostasis regulation. Interestingly enough, while an important progress has been made in elucidating the role of FGF23 in Pi homeostasis regulation over the past 20 years, the mechanisms underlying the very early step of Pi homeostasis are still not deciphered. This first step is the ability of a cell or an organism to detect the variations of intra- and/or extracellular concentrations of Pi that will trigger the regulating cascade aimed at normalizing the Pi serum levels.

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Therefore, this review will explore the possible mechanisms by which Pi could directly regulate FGF23 production and/or secretion by summarizing the interplay between FGF23 and molecules involved in Pi sensing.

Phosphate-Dependent Regulation of FGF23

Studies conducted in humans demonstrated that serum FGF23 levels are associated with dietary Pi [20–22], and that intravenous infusion of Pi or acute duodenal Pi load increased FGF23 levels [23]. A relationship between dietary Pi load and circulating Fgf23 levels was also illustrated in mice [24, 25]. Nevertheless, it is important to differentiate short-term (direct) changes in FGF23 levels following dietary Pi loads from long-term (indirect) changes through endocrine loops that may involve other organs than bone. For instance, we have recently showed that changes in Pi diet can influence Klotho protein expression in vivo in the kidney [26••], while another group has shown that FGFR1 mRNA expression can be regulated by extracellular Pi concentration in vitro [27], underscoring the role of these factors in the Pi-dependent regulation of FGF23. In addition, it is unclear by which mechanisms FGF23 is regulated by Pi (transcription, post-transcription, secretion, activity, and/or cleavage).

In vitro studies have been conducted in order to decipher the Pi-dependent FGF23 regulation mechanisms, but results are conflicting. Extracellular Pi concentration directly stimulates the transcription of FGF23 in some studies [28, 29] but not in others [26••, 27, 30, 31•, 32]. Undoubtedly, in vitro studies are limited by the absence of appropriate models for FGF23 secretion, since only fully differentiated osteocytes secrete significant levels of FGF23, maybe due to the importance of extracellular matrix and 3D environment that is lacking in established cell lines. Based on this observation, we recently set up an ex vivo bone shaft model in which we were able to illustrate significant Pi-dependent FGF23 secretion, together with no effect of Pi on FGF23 transcription [26••].

FGF23 biological action can also be regulated by intracellular cleavage of the protein, a phenomenon that is regulated by the balanced action of two molecules. Polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) protects FGF23 activity by its O-glycosylation activity at the Thr^{178} cleavage site providing protection of furin-mediated cleavage [33]. Another post-translational modification has more recently been involved, which is a phosphorylation of Ser^{180} by family with sequence similarity 20, member C (FAM20C) kinase [34•]. This phosphorylation inhibits the O-glycosylation, thus promoting FGF23 cleavage. However, GALNT3 and FAM20C regulators remain poorly described for now, except for one study showing Pi-dependent regulation of GALNT3 expression [35].

Phosphate as a Signaling Molecule

In addition to its well-known role as a component of apatite crystals, evidence of a role of Pi as a signaling molecule has slowly emerged in the scientific community. The first evidence of such a role was shown approximately 15–20 years ago owing to the work of Beck GR et al. that demonstrated the regulation of secreted phosphoprotein 1 (Spp1) expression by Pi [36, 37]. Since then, the direct effect of Pi on bone cell function and on extra-skeletal organs has been illustrated multiple times through the Pi-dependent regulation of many genes [31•, 38, 39••].

Being a "signaling" molecule implies Pi to activate a signaling pathway. Consistently, the early works of Beck GR and collaborators have described that the induction of the Spp1 gene by Pi was mediated by the activation of the ERK1/2 MAPK pathway [37]. The role of the ERK pathway in mediating the signaling function of Pi has now been confirmed by many other teams including ours [38, 40–45, 46•]. In addition to the ERK pathway, the Akt pathway has been found to be activated by Pi in adipose [4] and human bronchial cells [47], but not in HEK293 cells [44] or the chondrogenic cell line ATDC5 [43]. The PKC and proteasome were also found to be activated [37]. In contrast, several pathways have been shown not to be activated by Pi such as the P38 or JNK MAPK pathways [37, 40]. While knowledge on the signaling pathways and genes being regulated by Pi is accumulating, one key unanswered question is how Pi activates these pathways, and which pathway is relevant to the Pi-dependent FGF23 secretion.

Phosphate Sensing vs Phosphate Sensors

The mechanism by which Pi signals to the cell and activates genes or pathways is referred as Pi sensing. Although the existence of such a mechanism is now largely accepted [38, 39••, 48•], it is not known whether in mammalian organisms this involves a cell surface receptor that binds to Pi, or intracellular proteins that respond to changes in intracellular Pi concentrations (that may result from changes in the extracellular Pi). Moreover, since changes in Pi concentration occur in either compartment, both extracellular and intracellular Pi-sensing mechanisms are likely to co-exist.

Central to the sensing mechanism, the Pi sensor is thought to be a single molecule or a complex of molecules that is able to bind Pi and trigger the secondary events leading to the cellular response. A Pi sensor that detects changes in extracellular Pi concentrations would necessarily be present at the external cell surface. As such, membrane-associated proteins such as high-affinity Pi transporters or receptors represent good candidates. On the other hand, an intracellular Pi sensor

could either be an intracytoplasmic protein or a membrane-associated protein with exposed intracellular regions.

Identifying a Pi sensor in higher eukaryotes is a very delicate task. An elementary difficulty relates to the definition of a Pi sensor, which, even more than the Pi-sensing mechanism, can be questionable. In addition, many processes have been proposed as part of a Pi detection mechanism, despite the lack of information on the identity of the Pi sensor itself. For instance, in yeasts, in condition of Pi starvation, the 5′-diphosphoinositol pentakisphosphate (IP7) accumulates in the cytoplasm. IP7 then binds to the SPX domain of the cyclin-dependent kinase inhibitor phosphate system positive regulatory protein (Pho81) and the Pi exporter XPR1, triggering the PHO pathway that will eventually activate Pi transporters to correct for intracellular Pi [49]. In this mechanism, in which IP7 serves as a second messenger to respond to a decrease in intracellular Pi concentration, the identity of the molecules that actually detect the intracellular Pi decrease is unknown. In mammals, IP7 is generated from IP6 by a family of IP6 kinases (IP6K1–3) [50, 51]. Early studies have identified IP6K2 as PiUS (standing for phosphate uptake stimulator), a protein characterized by its ability to enhance Pi uptake [52]. More recently, IP6K2 and IP6K3 have been identified as genetic determinants of serum Pi level regulation in humans [53], whereas PPIP5K, another enzyme responsible for the synthesis of inositol pyrophosphates, is strongly inhibited by extracellular Pi in human epithelial cells [54]. It is therefore important to design experiments to study the role of these kinases as potential intracellular Pi sensors.

Although it is expected that a Pi sensor will bind the Pi, the opposite is not necessarily true since a molecule that binds Pi does not necessarily represent a Pi sensor. This may be particularly difficult to conceptualize when considering Pi transporters at the plasma membrane. The binding of Pi on the transporter leads to a conformational change and ultimately to the transport of the Pi ion into the intracellular space, which will change the intracellular Pi concentration, triggering an adaptive cellular response. It is therefore true that a transporter participates in the sensing mechanism, but the molecule that informs the cell of a change in intracellular Pi is not necessarily the transporter itself [48•, 49]. It should be demonstrated that, independently of Pi transport, the transporter can signal to the cell (using transport-deficient transporters or non-metabolizable Pi analogues). Otherwise, it is the change in intracellular Pi itself that triggers the cellular response, via a still unknown intracellular Pi sensor.

Finally, while PHEX and DMP1 are involved in the regulation of Pi homeostasis through complex regulatory loops involving FGF23 secretion [9], it has recently been shown that mutations in these genes alter the responsiveness to extracellular Pi [55, 56] that may involve FGFR1 [7].

Candidate Molecules as Pi Sensors Involved in the Control of FGF23 Secretion

When considering the above definitions, candidate intracellular or membrane-bound proteins capable of binding Pi and acting as mediator of the Pi-dependent regulation of FGF23 are rare. The best candidates for mediating the Pi-dependent FGF23 regulation are presently the PiT/Slc20 proteins. PiT1/Slc20a1 and PiT2/Slc20a2 are high-affinity Na^+-dependent Pi transporters [57, 58] that were originally identified as retrovirus receptors [59–61]. Unlike the other high-affinity Na^+-Pi co-transporters described in mammals and which belong to the Slc34 family [62], PiT proteins are widely expressed, including in the main organs involved in the regulation of Pi homeostasis [63]. PiT1 expression was shown to be regulated by extracellular Pi concentration [32, 64–66]. Notably, PiT1 is involved in the Pi-dependent activation of the ERK1/2 pathway in numerous cell types [43–45, 46•]. The PiT1 paralog PiT2, although less studied, has also been suggested to be involved in Pi signaling.

We recently demonstrated that the Pi-dependent activation of ERK1/2 pathway requires both PiT1 and PiT2, and that Pi-dependent/PiT-mediated ERK1/2 phosphorylation does not require Pi transport through the PiT proteins [46•]. Although this was the first time that PiT2 was demonstrated to play a role in Pi signaling, Pi-independent function of PiT1 on ERK signaling has also been shown earlier [45, 67]. We also showed that PiT1 and PiT2 were able to form low-abundant heterodimers in addition to high-abundant homodimers and that the heterodimerization was enhanced by increased extracellular Pi. Importantly, we showed that the heterodimerization was dependent upon the binding of Pi to the PiTs, but not the transport of Pi. Early studies have also suggested a homodimerization of PiT2 in response to extracellular Pi variations that was independent of its transport function [68, 69]. These results reinforce the idea that PiT protein dimers are strong Pi sensor candidates mediating extracellular Pi variations. However, the cell-specificity of the redundancy or non-redundancy of PiT1 and PiT2 in mediating Pi effects and the physiological relevance of these findings are both unknown. As a first step to illustrate a physiological role for PiTs in Pi sensing, we recently illustrated in vitro, ex vivo in bone shafts culture, and in vivo in mice that lack of PiT2 blunted the Pi-dependent regulation of FGF23 secretion [26••]. Importantly, we also showed in this model that the ERK1/2 pathway was not involved in the Pi-dependent FGF23 secretion, despite a normal expression of FGFR1, suggesting that mechanistic links other than the ERK1/2 pathway may be at work between PiT2 and FGF23 secretion. Despite this observation, a role for FGFR1 in mediating the effect of Pi and PiTs on FGF23 secretion should still be considered. Indeed, the osteocyte-specific deletion of Fgfr1 partially restored the overproduction of FGF23 seen in Hyp mice [16].

Moreover, exogenous over-expression of FGFR1 in HEK cells rescued the decrease in the Pi-induced phosphorylation of ERK1/2 in PiT1-deficient cells [70], suggesting that FGFR1 may be a molecular link between PiT1 and the Raf/MEK/ERK pathway. Clearly, further studies are therefore necessary to decipher the link between PiT1/PiT2, FGFR1, and the ERK pathway in regulating FGF23 secretion.

A contribution in the sensing of extracellular Pi variations may involve the calcium-sensing receptor (CaSR). Not surprisingly, the CaSR was identified in a genome-wide association study as a genetic determinant of serum Pi concentration [53]. More intriguingly, the recent crystal structure of the receptor revealed multiple binding sites for PO_4^{3-} ions in the extracellular domain of CaSR [71••]. Although no functional studies have been performed and the role of CaSR in the detection of Pi has not been tested, the authors suggest that binding Pi may modify the conformation of CaSR, which could modify the binding properties of Ca^{2+} to the receptor and have indirect consequences on the regulation of Pi homeostasis through Pi sensing.

Since the deletion of the intestinal high-affinity Na^+-Pi cotransporter Npt2b/Slc34a2 leads to a decrease in Fgf23 serum levels [72] and that serum Pi levels have been shown to be regulated through an intestine-kidney axis [73, 74], the involvement of Npt2b in Pi sensing was suggested [75]. Although a role of Npt2b in regulating Pi concentration is consistent with the current observations, Npt2b expression is not regulated by Pi [76] and no study has yet been conducted to determine whether Npt2b could represent a Pi sensor. Particularly, it remains to be established whether its possible involvement in a Pi-sensing mechanism relates to its sole transport function, whereby Npt2b-mediated Pi entry in the cell would be detected by a yet unknown intracellular Pi sensor triggering the cell's response.

The retrovirus receptor XPR1 [77] has recently been described as the only known Pi exporter in mammalian cells [78••]. XPR1 contains an N-terminal SPX (Syg1/Pho81/Xpr1) domain that is not required for Pi export function [78••], suggesting a potential role in another function. Consistently, the SPX domain is described in yeasts and plants as a domain that could mediate signal transmission from variations of extracellular Pi concentration [79, 80]. Recently, as outlined above, it was reported that the SPX domain binds inositol polyphosphates (IP), particularly IP7 [81••], the

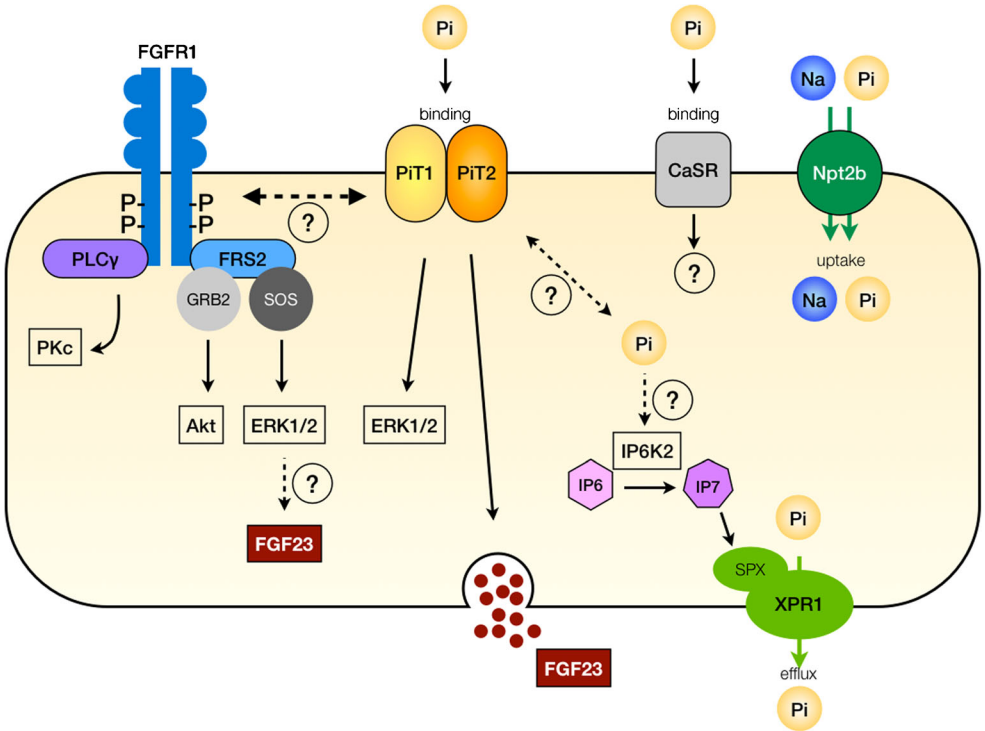

Fig. 1 Schematic view of putative Pi-sensing pathways involved in FGF23 secretion. Upon Pi binding to the PiT1-PiT2 heterodimer, the ERK1/2 MAPK pathway is activated within few minutes, whereas FGF23 secretion is observed within few hours. However, the use of a MEK inhibitor did not block Pi-dependent PiT-mediated secretion of FGF23 indicating that other signaling pathways are involved. Upon stimulation by Pi, FGFR1 has been implicated in FGF23 regulation at the transcription level, a phenomenon that involves the ERK pathway. Since Pi was not demonstrated to bind FGFR1, a functional link between this receptor and the PiT proteins has been suggested. The regulation of intracellular Pi concentration may involve a coordinated control of Pi uptake through the PiTs and Pi efflux through XPR1. The IP6K2 kinase may represent an intracellular Pi sensor by modulating the concentration of IP7 that binds the SPX domain of XPR1 and controls Pi efflux. The role of the binding of Pi to CaSR and the transport of Pi by Npt2b in controlling FGF23 synthesis or secretion requires more investigation but may modulate the above phenomenon

concentration of which changes upon Pi availability, suggesting that, as in yeasts, IP concentration participates to the Pi-sensing mechanism in mammals. The role of IP kinases may therefore be central to the regulation of intracellular Pi, while their role in regulating FGF23 secretion is totally unknown. A recent study illustrated that conditional deletion in mice of Xpr1 in the renal tubule resulted in renal Fanconi-like syndrome with impaired renal Pi reabsorption and impaired bone mineralization [82]. In addition to these Pi-related effects, these mice excrete massive amounts of glucose, amino acids, and albumin, indicating severe kidney damage and suggesting an important role for XPR1 in the kidney that may extend beyond the export or sensing of Pi. Although XPR1 is a promising candidate for controlling intracellular Pi concentration, further studies are needed to establish a link between its expression in the kidney or bone and the regulation of FGF23 synthesis or secretion.

Conclusion and Unresolved Questions

Although the regulation of Pi homeostasis by FGF23 has been extensively studied, the feedback loop by which Pi modulates FGF23 is much less known. Recent efforts have been made to identify the molecular actors involved in the detection of Pi and their role in triggering the expression or secretion of FGF23 (Fig. 1). Important molecules include the PiTs and XPR1 proteins, but also perhaps FGFR1 and CaSR. However, many questions remain. What is the respective role of PiT1 and PiT2 in controlling FGF23 secretion? What are the molecules connecting the PiT proteins to the ERK, Akt, or PKC signaling pathways? Since extracellular and intracellular Pi concentrations are closely related, what is the functional relationship between PiTs and XPR1, and do they function together in a coordinated manner to control FGF23 secretion? Is the sensing of Pi controlling FGF23 secretion limited to bone, or is there a coordination between the intestine, kidneys, bone, and parathyroid glands to integrate the response to variations in serum Pi levels? Finally, if Pi-sensing controlling serum Pi levels proves to be a universal mechanism widely distributed in the body's cells, how can therapeutic strategies be devised to control it? These and many other questions remain challenges for researchers and clinicians and will require in-depth in vivo studies to decipher and understand them.

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Compliance with Ethical Standards

Conflict of Interest Nina Bon, Sarah Beck-Cormier, and Laurent Beck each declare no potential conflicts of interest.

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