REVIEW

Extracellular phosphate sensing in mammals: what do we know?

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Abstract

The critical role of phosphate (Pi) in countless biological processes requires the ability to control its concentration both intracellularly and extracellularly. At the body level, this concentration is finely regulated by numerous hormones, primarily parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). While this control of the body's Pi homeostasis is now well documented, knowledge of the mechanisms that allow the cell and the body to detect extracellular Pi variations is much less known. These systems are well described in bacteria, yeasts and plants, but as will be discussed in this review, knowledge obtained from these organisms is not entirely relevant to the requirements of Pi biology in mammals. In this review, we present the latest findings on extracellular Pi sensing in mammals, and describe the mammalian Pi sensors identified to date, such as SLC20A1 (PIT1)/SLC20A2 (PIT2) heterodimers and the calcium-sensing receptor (CaSR). While there are many questions remaining to be resolved, a clarification of the Pi sensing mechanisms in mammals is critical to understanding the deregulation of Pi balance in certain life-threatening disease states, such as end-stage renal disease and associated vascular calcifications, and to proposing relevant therapeutic approaches.

Key Words

- ▶ metabolism
- ▶ mineralization
- ▶ phosphate homeostasis
- ▶ hormone secretion
- ▶ phosphate signaling

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Introduction

Phosphorus is a vital component of the cell and organism, playing critical roles in many essential processes. It is a mandatory component of the lipid bilayer of cell membranes, nucleoproteins and nucleic acids, and is essential for bone mineralization (Rothfield & Finkelstein 1968, Hansen *et al.* 1976, Kornberg 1979). Moreover, phosphorus is central to the storage and liberation of metabolic energy, cellular signaling, enzyme activity, lipid metabolism, muscle and neurological functions and the delivery of oxygen to the peripheral tissues through the formation of 2,3-diphosphoglycerate (Krebs & Beavo 1979, Bessman & Carpenter 1985, Crook & Swaminathan 1996, Hubbard & Till 2000). Approximately 85% of total

body phosphorus is found in bone, where it is mainly complexed with hydrogen, oxygen and calcium (Ca^{2+}) to form apatite crystals that are deposited on the collagen matrix (Hansen *et al.* 1976). The remainder of phosphorus is found in soft tissue (14%) with only about 1% in extracellular fluids (Crook & Swaminathan 1996).

In the serum, approximately 85% of the phosphorus content is found as unbound inorganic phosphate (Pi) in the form of HPO_4^{2-} and H_2PO_4^- at a 4:1 ratio, at pH 7.4. The remainder (15%) of the phosphorus is bound to proteins or complexed to cations (mainly Ca^{2+} , Mg^{2+} and Na^+). Maintaining a stable concentration of unbound circulating Pi is of major importance for cellular metabolism,

bone mineralization and cardiovascular functions. Accordingly, the regulation of serum Pi levels is a very ancient protective system preserved over the course of evolution that allows the retention of Pi in states of Pi deficiency, or conversely the excretion of Pi in order to avoid Pi toxicity. Serum Pi concentrations are maintained within a narrow range mainly due to the coordinated action of PTH, FGF23 and vitamin D. While significant progress has been made in understanding the regulation of Pi homeostasis over the last 25 years, the mechanism underlying the very first step of this regulation, that is, the way in which Pi informs the cell about its variations in extracellular concentration, is much less known. Information about the Pi sensing mechanism is fundamental to understanding why the humoral and paracrine factors that regulate Pi levels in physiological situations are unable to correct Pi imbalance in disease states.

In this short review, we will present the current knowledge on the possible mechanisms underlying the ability of a mammalian cell to detect variations of extracellular Pi levels leading to its homeostatic regulation throughout the organism.

Sensing extracellular vs intracellular phosphate levels

Because Pi is present almost everywhere in the body and in the cell, in many different chemical forms, in bound and unbound states, the mere definition of 'Pi sensing' is a challenging task. As mentioned previously, systemic Pi sensing at the body level involves the detection of variations in the extracellular Pi concentration by specific cells, which will then respond to these variations by secreting appropriate hormones to maintain the homeostasis of Pi in the whole body. When defined as such, this type of Pi sensing has sometimes been termed 'endocrine sensing' (Chande & Bergwitz 2018), and is the main subject of this review.

However, it is important to keep in mind that in addition to circulating in extracellular fluids, Pi also enters cells where it is used as a basic element to make phospholipids and nucleic acids, or is used as a metabolic fuel to generate ATP and as an indispensable molecule in phosphorylation reactions. Considering this fundamental role, the intracellular concentration of the free available Pi must also be tightly controlled, involving a specific mechanism for the detection of intracellular concentration of Pi. This latter type of detection has been referred as to 'cellular' or 'metabolic' Pi sensing (Chande & Bergwitz 2018, Kritmetapak & Kumar 2019). The Pi

sensing classification as either 'endocrine sensing' or 'cellular/metabolic sensing' has emerged for reasons of simplicity, and refers to the compartments in which Pi concentrations are homeostatically regulated: mainly the circulating fluids or the cellular compartment. However, paradoxically, this distinction can make the identification of the molecules involved in the Pi sensing mechanisms more complex. For instance, 'endocrine' Pi sensing, referring to circulating fluids, may include cell-based mechanisms such as Pi entry in the renal or intestinal cell, which will then trigger 'metabolic' Pi sensing in the respective cells. Therefore, when trying to identify and characterize an 'endocrine' Pi sensor molecule, should it be outside or inside the cell? Similarly, the 'metabolic' sensing of Pi may be influenced by many external hormonal stimuli, including hormones regulated by extracellular Pi. Therefore, in order to better encompass the mechanisms involved in Pi sensing and to better identify putative sensors, it is therefore perhaps more accurate to refer to the terms 'extracellular' or 'intracellular' sensing of Pi. This semantic distinction may seem trivial, but it is, in fact, essential to the understanding of sensing mechanisms and the identification of involved molecules. For instance, an extracellular Pi sensor may be essential to mineralising cells when the local concentration of calcium and Pi in the close vicinity of the cell will trigger cell-independent physical- or chemical-based mechanisms resulting in hydroxyapatite crystal formation (Weiner & Addadi 2011, Kerschnitzki *et al.* 2016). In this example, the sensing of Pi is not a matter of endocrine or metabolic sensing, but rather detecting local extracellular Pi variations. However, while the compartmentalization of Pi sensing as 'extracellular' or 'intracellular' involves different mechanisms and molecules, functional and molecular cross-talks between these two types of sensing are more than likely to exist.

Intracellular Pi sensing is much less known than extracellular Pi sensing, but recent studies have reported that the intracellular concentration of inositol polyphosphates (IP), particularly IP7, changes upon intracellular Pi availability (Wild *et al.* 2016), shedding light on possible intracellular Pi sensing mechanisms. In the latter study, IP7 was shown to bind the SPX (Syg1/Pho81/Xpr1) domain of the retrovirus receptor XPR1 (Battini *et al.* 1999) that was later characterized as a Pi exporter in mammalian cells (Giovannini *et al.* 2013). More recently, Wilson and colleagues identified the inositol hexakisphosphate kinases 1 and 2 (IP6K1 and 2) as being essential to the generation of IP7 upon intracellular Pi level changes, illustrating the central role

of these enzymes in intracellular Pi sensing (Wilson *et al.* 2019). In fact, the role of IP6K1 and -2 is fully compatible with a true intracellular Pi sensor, as they are able to bind Pi and trigger the adaptive response leading to the normalisation of the intracellular Pi concentration by activating its export out of the cell through XPR1 transport activity (Fig. 1A). Finally, it is interesting to observe that in yeasts and plants, SPX domains present in membrane proteins can mediate intracellular signal transmission from variations of extracellular Pi concentration (Hürlimann *et al.* 2009, Ghillebert *et al.* 2011). This illustrates that the boundary between extracellular Pi sensing and intracellular Pi sensing may very well be thinner than we think.

Physiological and pathophysiological adaptations to changes in extracellular Pi

The main source of Pi comes from food which, following digestion, leads to the intestinal absorption of the anion involving two pathways: a passive paracellular pathway and an active transcellular transport pathway (Marks 2019). The paracellular Pi permeability allows a higher Pi absorption from the proximal small intestine lumen, where pH is lower and Pi concentrations are higher (Walling 1977, Knopfel *et al.* 2019). Passive paracellular Pi transport is dependent on the electrochemical gradient of Pi across the intestinal epithelium and is thought to occur through tight junction complexes comprising

Figure 1

Schematic view of putative Pi sensors and Pi sensing pathways in a composite mammalian cell. Knowledge of Pi sensing in mammals comes from a wide variety of cell types in the body, and the mechanisms described may not occur in all cell types. This view is to better identify which molecule can participate in Pi sensing, independently of the physiological endpoint. Sensing of extracellular Pi (Pi_e) must not be confused with sensing of intracellular Pi (Pi_i). In the latter case (A), the intracellular concentration of inositol polyphosphate 7 (IP7), changes upon intracellular Pi availability (Wild *et al.* 2016), upon action of inositol hexakisphosphate kinases 1 and 2 (IP6K1 and 2). Binding of IP7 on the SPX domain of XPR1 triggers Pi efflux from the cell, regulating intracellular Pi concentration (Wilson *et al.* 2019). (B) PiT1 and PiT2 are forming membrane-bound heterodimers allowing for extracellular Pi binding, triggering the ERK1/2 MAPK pathway and FGF23 secretion *in vivo* (Bon *et al.* 2017, 2018). However, the use of a MEK inhibitor did not block Pi-dependent PiT-mediated secretion of FGF23 indicating that other signaling pathways are involved. (C) Upon stimulation by extracellular Pi, FGFR1c has been implicated in FGF23 regulation, a phenomenon that involved the expression of Galnt3 activated through ERK1/2 pathway, leading to the protective O-glycosylation of FGF23. (?) The possibility that FGFR1 and PiT2 functionally interact together to control FGF23 secretion *in vivo* remains to be investigated. (D) The CaSR was recently illustrated as a Pi sensor whereby extracellular Pi binds to arginine residue 62 of CaSR in a non-competitive manner resulting in the activation of CaSR and increased PTH secretion. (E) While PiT1/PiT2 heterodimers and CaSR can be viewed as true extracellular Pi sensors able to detect changes in extracellular Pi levels by binding Pi at their external surface and trigger secondary intracellular events, a molecule that binds Pi does not necessarily represent a Pi sensor. For instance, Pi transporters present at the plasma membrane will bind and transport Pi into the cellular space, and therefore change the intracellular Pi concentration, activating intracellular Pi sensors such as IP6K1/2 (A).

mainly occludins and claudins expressed along the gastrointestinal tract. While these proteins provide a regulated selective paracellular permeability for different ions, it is still unknown whether they truly bear this role for Pi (Amasheh *et al.* 2011, Knopfel *et al.* 2019). The transcellular transport mechanism of intestinal Pi absorption depends on secondary active Pi transport predominantly through the activity of Npt2b/Slc34a2 transporters (Marks 2019). While Npt2b, expressed on the apical membrane of enterocytes, accounts for 90% of Na⁺-dependent Pi transport into the intestinal cell (Sabbagh *et al.* 2009), the mechanisms accounting for the efflux at the basolateral membrane of the enterocytes are still unknown. Npt2b expression is directly upregulated by 1,25(OH)₂D and indirectly by PTH through its action on vitamin D (Segawa *et al.* 2004). Interestingly, an earlier study has demonstrated that Pi increases the expression of Npt2b even in the absence of the vitamin D receptor *in vivo*, suggesting that the regulation by Pi is independent of 1,25(OH)₂D (Segawa *et al.* 2004).

Following intestinal Pi absorption, Pi serum levels will rise, and Pi balance at the organismal level will be obtained mainly through the highly regulated renal excretion of Pi. The primary mechanism responsible for adjusting Pi serum levels is through Npt2a-mediated renal tubular Pi reabsorption (Beck *et al.* 1998), and to a lesser extent through Npt2c transporters (Segawa *et al.* 2009), at least in rodents. While renal Pi homeostasis is tightly regulated by many factors (including acid–base homeostasis, potassium status, and a number of hormones including calcitonin, dopamine, estrogen, glucocorticoids, growth hormone, insulin, insulin-like growth factor 1, EGF, and thyroid hormone (Bergwitz & Jüppner 2010, Levi *et al.* 2019)), the major factors that regulate renal Pi excretion are 1,25(OH)₂D, PTH and FGF23 (Bergwitz & Jüppner 2010). To signal and exert its effects on Pi homeostasis and vitamin D metabolism, FGF23 requires α Klotho as an obligatory co-receptor that forms a ternary complex with FGFRs and the C-terminus of FGF23 (Kuro-O *et al.* 1997, Kurosu *et al.* 2006, Urakawa *et al.* 2006). This allows FGF23 to bind with high affinity to FGFR1c and FGFR3c, and activate ERK signaling (Kurosu *et al.* 2006, Urakawa *et al.* 2006). While studies have shown that in the heart, FGF23 binds to FGFR4 in the absence of α Klotho to induce left ventricular hypertrophy (Faul *et al.* 2011, Grabner *et al.* 2017), no α Klotho-independent action of FGF23 has yet been documented on Pi homeostasis regulation. Accordingly, Erben's group has demonstrated that Klotho's actions on mineral metabolism occur through its role as a co-receptor of FGFRs and that its actions are

FGF23-dependent (Andrukhova *et al.* 2017, Erben 2018). Of primary importance, the secretion of 1,25(OH)₂D, PTH, FGF23 and α Klotho is regulated by Pi levels (Ferrari *et al.* 2005, Fukumoto & Martin 2009, Bergwitz & Jüppner 2010, Jacquillet & Unwin 2019) representing the primary signal that determines the regulating cascade leading to an appropriate organismal Pi balance. Pi has also been shown to regulate directly the expression of Npt2a by modulating TFE3 transcription factor binding to a Pi-response element present in the promoter of the Npt2a gene (Kido *et al.* 1999), although it is still unknown whether this Pi effect on TFE3 has a significant physiological implication.

Homeostatic changes mainly involving vitamin D, PTH and FGF23 to adapt to chronic alterations in serum Pi levels require several hours to several days (Ferrari *et al.* 2005, Ito *et al.* 2007, Bergwitz & Jüppner 2010, Kritmetapak & Kumar 2019). In contrast, an early study suggested the presence of an intestine–kidney axis that would rapidly respond to changes in luminal intestinal Pi concentration by increasing renal Pi excretion within minutes (Berndt *et al.* 2007). This study was based on previous observations in humans showing a rapid increase in renal Pi excretion within 1 h following a high-Pi diet, without changes in PTH, FGF23 or 1,25(OH)₂D serum levels, suggesting the presence of an intestinal Pi sensor (Nishida *et al.* 2006). In their paper, Berndt and colleagues showed that administration of Pi into the duodenum of WT or parathyroidectomized rats was associated with a rapid increase in the fractional excretion of Pi within 10 min, without hormonal changes (Berndt *et al.* 2007). When homogenates of the duodenal mucosa were infused into rats, a corresponding rapid increase in urinary fractional excretion of Pi was illustrated, strongly suggesting the presence of intestinal factors that signal to the kidney to control Pi excretion. This essential mechanism would allow for a rapid crosstalk between intestine and kidneys; however, the exact molecular identity of the intestinal Pi factor is still unknown. Importantly, when similar experiments were carried out in humans, no strong evidence for an intestinal-specific control of renal Pi excretion was shown, probably pointing out that this mechanism is species-dependent (Scanni *et al.* 2014). Moreover, a recent study in rats suggested that the intravenous or intragastric loading of Pi modulated renal excretion of Pi by a PTH-dependent mechanism (Thomas *et al.* 2017), reigniting the controversy over the existence of a short-term pathway and requiring the identification of the putative intestinal phosphatonin to definitively prove the existence of a PTH-independent, intestine-to-kidney short term pathway.

Precise knowledge of the mechanisms underlying the long-term and the putative short-term regulations of Pi homeostasis is crucial to propose an appropriate clinical response in relevant disease states. A persistent hyperphosphatemia has very serious deleterious pathological consequences, among which an increased risk of cardiovascular morbidity and mortality in chronic kidney disease (CKD) patients is a striking example (Clinkenbeard *et al.* 2019). These patients have a notorious propensity to develop vascular calcifications (VC) (Witteaman *et al.* 1986, 1990, Heine *et al.* 2013), which are a strong predictor of elevated cardiovascular mortality (Blacher *et al.* 2001, Schlieper *et al.* 2008). During renal failure, hyperphosphatemia triggers calcification of the medial layer of the arterial wall, but also exacerbates intimal atherosclerosis plaque calcification, which is already present in virtually all CKD patients. Since both types of calcification significantly affect cardiovascular mortality in CKD (London *et al.* 2003), it is crucial to understand the effects of Pi on each. Particularly, the mode of action of Pi that leads to calcification is still a matter of debate. Pi may simply act by precipitating on preexisting crystals, or through its signaling function in promoting the transdifferentiation of vascular smooth muscle cells (VSMC) into osteochondrocyte-like cells (Jono *et al.* 2000, Sage *et al.* 2011, Mokas *et al.* 2016, Hortells *et al.* 2017).

How relevant is the detection of Pi in lower organisms to mammals?

In order to identify Pi sensors in mammalian cells mediating Pi signaling function, an interesting approach has been to study the systems implemented in lower organisms and compare them with the need of more evolved cells and organisms. Excellent review articles on Pi sensing including a description of non-mammalian systems have been written recently, and we encourage readers to refer to them for more details on the detection of Pi in lower organisms (Conrad *et al.* 2014, Qi *et al.* 2016, Chande & Bergwitz 2018, Michigami *et al.* 2018, Kritmetapak & Kumar 2019). What is most striking when studying bacteria, yeast or even plant Pi sensing mechanisms, is that the requirement for Pi is very different than for mammalian cells. Bacteria, yeasts and plants are highly dependent upon their environment when it comes to Pi availability. The quantities of Pi in the soil or in the external environment are very low, in the micromolar range, whereas Pi is in the millimolar range in mammals. Accordingly, plants and unicellular organisms express Pi transporters and Pi sensing molecules that are

adapted to low Pi availability. The 'normal' situation is actually a Pi deprivation for which uptake and sensing of Pi are constantly stimulated by default. It is only when Pi is in higher quantities that the system is turned off. In mammals, there is the reverse situation, since they can move to seek their nutrient needs. The 'normal' situation in mammals is actually to face too much Pi, especially in the modern era, where bioavailable free Pi is extensively used as an additive in industrialized processed food (Ritz *et al.* 2012, Vorland *et al.* 2017). Accordingly, the sensing of extracellular Pi in mammals has evolved to set up endocrine regulatory loops aimed at excreting Pi out of the body in order to avoid Pi toxicity. Another difference between lower organisms and mammals is the actual mechanism of Pi transport through the plasma membrane. In lower organisms, the molecules involved in Pi uptake are coupling the entry of Pi to the efflux of protons, whereas Pi entry in mammals is coupled to sodium uptake. A notable exception is the Pho89 transporter in yeast, which allows co-transport of phosphate and sodium ions within the cell. It is interesting to note that this transporter belongs to the large inorganic phosphate transporter (PiT) family (Transporter Classification Database 2.A.20), which includes the mammalian orthologs (SLC20A1/PiT1 and SLC20A2/PiT2, see subsequent paragraph), and the bacterial PitA and PitB transporters. Unfortunately, yeast studies so far have not been able to attribute a major role to Pho89 in the sensing of Pi (Qi *et al.* 2016), which could be related to its low affinity for Pi and particular transport properties in relation to the extracellular environment of yeast. Accordingly, a large majority of the Pit family of transporters do not transport sodium, but rather H⁺, including the bacterial PitA and PitB transporters, illustrating that their machinery of Pi transport and most probably Pi sensing is based upon different chemistry, stoichiometry, affinity and purpose. These differences in transport and environmental properties may explain why no mammalian Pi sensor has been identified from direct sequence comparison searches using unicellular organisms or plant Pi sensors sequences, or that if sequence homology can be evidenced, the transporters may play different roles in their respective cellular environment.

Identified Pi sensors in mammals

In mammals, several proteins have been recently identified as putative Pi sensors that can play a role in informing the organism of a change in extracellular Pi levels. Our recent work in Pi signaling has led us to identify the PiT1-PiT2 heterodimer complex as an essential membrane

component to mediate Pi-dependent ERK1/2 signaling (Bon *et al.* 2017), which later led to the identification of PiT2 as being a key molecule to mediate FGF23 secretion upon Pi challenge (Bon *et al.* 2018) (Fig. 1B and text subsequently). PiT1 (Slc20a1) and PiT2 (Slc20a2) are high-affinity Na⁺-dependent Pi transporters (Kavanaugh *et al.* 1994, Miller & Miller 1994) that were originally identified as retrovirus receptors (O'Hara *et al.* 1990, Miller *et al.* 1994, van Zeijl *et al.* 1994). Unlike other high-affinity Na⁺-Pi co-transporters described in mammals that belong to the Slc34 family (Wagner *et al.* 2013), PiT proteins are widely expressed, including in key organs involved in Pi homeostasis regulation (Forster *et al.* 2013). However, despite this significant observation, their contribution to Pi homeostasis through their Pi transport capability has not been demonstrated.

Considering their role in Pi signaling (Bon *et al.* 2017), we explored the possibility that the involvement of PiTs in Pi homeostasis may rather derive from their Pi sensing capabilities. To test this hypothesis, we took advantage of the PiT2 KO mouse model and illustrated that the lack of PiT2 *in vivo* fully blunted the secretion of FGF23 in response to variations in dietary Pi loads, without changes in PTH or vitamin D levels (Bon *et al.* 2018). To further confirm these results, we reproduced this effect by stimulating *ex vivo* bone shaft cultures from WT and PiT2 KO mice and confirmed that PiT2 disruption blunted the Pi-dependent regulation of FGF23 secretion independently of PTH and vitamin D (Bon *et al.* 2018). These experiments were the first to report a complete absence of FGF23 secretion in response to Pi both *in vivo* and *ex vivo*. Of important note, we were not able to reproduce these data in osteoblasts and osteocytes in culture, either from established cell lines or primary cultures, despite the fact that this is an approach often used to study the Pi-dependent secretion of FGF23 (Michigami *et al.* 2018, Bon *et al.* 2019, Takashi & Fukumoto 2020). This most likely indicates that the natural 3D environment of osteocytes offered by *ex vivo* cultures of intact bone shafts may be crucial for responding to Pi level variations (Bon *et al.* 2018).

A recent interesting study has shown that high extracellular Pi levels resulted in phosphorylation of the FGF receptor FGFR1c in the absence of canonical ligands (Takashi *et al.* 2019). High Pi appeared to trigger the phosphorylation the FGFR substrate 2 α (FRS2 α), leading to activation of the MEK/ERK pathway and subsequent mechanisms that induces the expression of the polypeptide N-acetylgalactosaminyltransferase 3

(Galnt3) gene. O-glycosylation of FGF23 by the GALNT3 gene product prevented its proteolytic cleavage, leading to an increase in the serum levels of FGF23 in mice fed a high-phosphorus diet (Fig. 1C). Intriguingly, while FGFR1 is central to this mechanism, it is not known how it is activated by extracellular Pi since FGFRs do not bind Pi (Takashi & Fukumoto 2020). It is possible that the 'sensing' of extracellular Pi reported in this publication relates to the ability of Pi to enter the cell and phosphorylate FGFR1. If this is the case, FGFR1 may actually represent an *intracellular Pi* sensor playing a role in the secretion of FGF23. In contrast, the Pi sensing function of PiT2 most likely derives from *extracellular Pi* binding at the plasma membrane and subsequent activation of MAPK, rather than from Pi uptake (Bon *et al.* 2017). The possibility that FGFR1 and PiT2 functionally interact together to control FGF23 secretion *in vivo* remains to be investigated (Fig. 1B and C).

In addition to the control of FGF23 secretion by Pi through PiT2 and FGFR1-mediated sensing, a recent study has identified the calcium-sensing receptor (CaSR) as a Pi sensor controlling PTH secretion. While it has been known for a long time that Pi elicits a concentration-dependent stimulation of PTH (Almaden *et al.* 1996, Slatopolsky *et al.* 1996), the molecular mechanism mediating this effect is still uncertain. Ten years ago, the CaSR was identified in a genome-wide association study as a genetic determinant of serum Pi concentration (Kestenbaum *et al.* 2010). More recently, the crystal structure of the receptor revealed multiple binding sites for PO₄³⁻ ions in the extracellular domain of CaSR, suggesting that the binding of Pi may modify the conformation of CaSR, which therefore could modify the binding properties of Ca²⁺ to the receptor and have indirect consequences on the regulation of Pi homeostasis through Pi sensing (Geng *et al.* 2016). Based on these results, Centeno *et al.* identified arginine residue 62 (R62) as a Pi binding site in CaSR (Centeno *et al.* 2019). Binding of Pi to R62 significantly inhibited CaSR activity in a non-competitive manner resulting in increased PTH secretion, whereas mutation of this residue abolished Pi-induced inhibition of CaSR. Pathophysiologic Pi concentrations elicited a rapid and reversible increase in PTH secretion from human and WT murine parathyroid glands, but not in CaSR knockout glands. These results indicated that CaSR represents a Pi sensor in the parathyroid gland, explaining the stimulatory effect of Pi on PTH secretion (Fig. 1D) (Centeno *et al.* 2019).

Open questions regarding receptors, ligands and involved mechanisms of sensing

The cellular mechanisms that allow the detection of extracellular Pi are only beginning to be understood. A true sensor able to detect changes in extracellular Pi levels is expected to bind Pi at the external surface and trigger secondary intracellular events that will inform the cell of the extracellular Pi changes, leading to relevant cellular responses. A Pi sensor that detects changes in extracellular Pi concentrations would necessarily be expressed at the plasma membrane. Both CaSR and PiT2 meet this definition by triggering the intracellular ERK1/2 MAPK pathway in response to external Pi binding without Pi entry in the cell. As such they are true Pi sensor, respectively triggering PTH and FGF23 secretion upon Pi binding. What is less known is the precise link between the initial fast response of Pi binding (within seconds to minutes), for example, ERK phosphorylation, and the terminal slower cellular response (within minutes to hours), for example, hormonal secretion. Nevertheless, a main difference between CaSR/PiT2- and FGFR1c-mediated sensing is that, in the FGFR1c situation, the initial events of Pi sensing are still to be discovered, particularly how can this receptor inform the cell about extracellular Pi variations without binding extracellular Pi.

Importantly, while a true Pi sensor is expected to bind Pi, a molecule that binds Pi does not necessarily represent a Pi sensor. For instance, a Pi transporter expressed at the plasma membrane that will bind extracellular Pi and transport this anion into the cellular space will participate in changing the intracellular Pi concentration. As the intracellular Pi changes, an adaptive intracellular response will be triggered involving intracellular Pi sensors such as IP6K1/2 (Wild *et al.* 2016, Wilson *et al.* 2019), as discussed in the first paragraph of this review. As such, although the plasma membrane transporter activity is part of the sensing pathway, this is not actually the molecule that acts as a sensor. An interesting example of an important molecule participating in the extracellular Pi sensing is the Na⁺-Pi cotransporter Npt2b (Slc34a2) (Hilfiker *et al.* 1998, Feild *et al.* 1999). The intestinal deletion of Npt2b in mice leads to a decrease in Fgf23 serum levels (Sabbagh *et al.* 2009) and the involvement of Npt2b in an intestine-kidney axis regulating Pi levels (Berndt *et al.* 2007, Kumar 2009) and Pi sensing (Sabbagh & Schiavi 2014) was suggested. However, there is yet no study illustrating a role of Npt2b in specifically triggering downstream intracellular pathways independently of its Pi transport activity, nor regulation of its expression by Pi (Tenenhouse *et al.* 2001). So far, its

possible involvement in Pi sensing pathway is more likely related to its transport activity, which indirectly modifies the intracellular concentrations of Pi that are detected by an intracellular Pi sensor triggering the relevant cellular response (Fig. 1E).

Another question relates to the functional interaction between identified Pi sensors and their roles in different tissues. Considering that both CaSR and PiT2 modulate the ERK pathway, could this mean that their Pi sensing capability are functionally interchangeable? More precisely, can CaSR trigger a FGF23 secretion and PiT2, a PTH secretion, depending on the tissue in which they are expressed? While this issue has yet to be studied in detail, we earlier showed that Pi stimulation of ERK pathway and MGP/OPN expression in MC3T3 osteoblastic cells were unchanged in the presence of either a CaSR agonist (gadolinium) or the G-protein inhibitor pertussis toxin, suggesting that the Pi signaling effect seen in these cells was not dependent upon CaSR (Khoshniat *et al.* 2011), but rather on PiT1/PiT2 expression (Bon *et al.* 2017). This results is somewhat consistent with the fact that Pi binding to PiT2 leads to ERK activation (Bon *et al.* 2017), whereas Pi binding to CaSR leads to ERK inactivation (Centeno *et al.* 2019). It also remains to be determined whether the roles of CaSR and PiT2 on PTH and FGF23 secretion are identical when expressed in different tissues. For instance, what is the contribution of the CaSR to PTH secretion following Pi stimulation in the intestine? Or, what is the physiological role of PiT2 as a Pi sensor in tissues not expressing FGF23?

Notwithstanding these many remaining issues, knowledge about the molecular nature of putative Pi sensors has largely progressed over the last few years. Conversely, knowledge about the precise nature of the involved ligands is still unclear and controversial. In *in vitro* signaling experiments aimed at testing the effect of extracellular Pi on downstream signaling pathways, Pi is traditionally added in the form of Na₂HPO₄⁻ and NaH₂PO₄²⁻ in a 4:1 ratio (pH 7.4), where Na⁺ may be replaced by potassium. In these conditions, the PO₄ anions are supposed to be the ligand triggering the cellular response. However, both in these experiments and in the *in vivo* situation, calcium is present in the fluids at a significant concentration, questioning its role in modulating Pi sensing. We and others have illustrated that both *in vivo* and *in vitro*, the presence of calcium modifies the Pi sensing response (Khoshniat *et al.* 2008, Quinn *et al.* 2013). We earlier showed *in vitro* that calcium was required for Pi to stimulate ERK1/2 phosphorylation and MGP/OPN genes expression and suggested that this effect could originate from

extracellular-related effects of calcium-Pi precipitates (CaP particles) (Khoshniat *et al.* 2008). More recently, Quinn and colleagues illustrated the fundamental role of calcium in vivo in the Pi-dependent secretion of FGF23 (Quinn *et al.* 2013). Based on these findings, a recent study proposes that calciprotein particles, which are nanoparticles of calcium-Pi precipitates bound to the serum protein fetuin-A, are the true signal controlling FGF23 secretion, rather than PO₄ anion alone (Akiyama *et al.* 2020). This study illustrates that deciphering 'Pi sensing', and whether it also depends on calcium or not, is not trivial. While PiT2 has clearly been shown to be involved in Pi-dependent FGF23 secretion both in vivo in mice and *ex vivo* in cultured bone shafts in controlled conditions (Bon *et al.* 2018), it is not known whether PiT2 can bind calciprotein particles. To date, the only structural study that provides strong arguments for a direct binding of PO₄ anions to a Pi sensor was conducted on the crystal structure model of CaSR (Geng *et al.* 2016, Centeno *et al.* 2019). These experiments provided evidence that PO₄ anions directly bind to the CaSR whereby PO₄ acts as a non-competitive antagonist of CaSR efficacy, and were conducted in the presence of various calcimimetic molecules to lower Ca²⁺ concentration and reduce the risk of Ca×P association, most likely excluding a role for Ca²⁺ in the present Pi sensing mechanism (Centeno *et al.* 2019). Similar experiments are clearly required to elucidate the true nature of the ligand responsible for Pi sensing through PiT2 or other putative sensors triggering FGF23 secretion in response to modifications of extracellular Pi (alone or in combination with Ca modifications).

Conclusion

Understanding the Pi sensing mechanisms at the organismal level in mammals is not an easy task, and the very definition of sensing itself can be controversial. In this review, we have highlighted some aspects that are important for understanding the mechanisms that govern the detection of changes in extracellular Pi concentrations. In particular, we have made the reader aware of the complex links that may exist between intracellular and extracellular detection of Pi; the possible existence of short- and long-term detection mechanisms; the importance of calcium in the cellular response to Pi; the endocrine and non-endocrine parameters that influence Pi sensing; the identity of mammalian Pi sensors, both proven and hypothesized; and the fundamental importance of better understanding this field in order to generate appropriate clinical approaches to treat patients with Pi-related diseases.

Declaration of interest

The authors declare to have no financial or other potential conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

- Almaden Y, Canalejo A, Hernandez A, Ballesteros E, Garcia-Navarro S, Torres A & Rodriguez M 1996 Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands in vitro. *Journal of Bone and Mineral Research* **11** 970–976. (<https://doi.org/10.1002/jbmr.5650110714>)
- Akiyama K-I, Miura Y, Hayashi H, Sakata A, Matsumura Y, Kojima M, Tsuchiya K, Nitta K, Shiizaki K, Kurosu H & Kuro-o M 2020 Calciprotein particles regulate fibroblast growth factor-23 expression in osteoblasts. *Kidney International* **97** 702–712. (<https://doi.org/10.1016/j.kint.2019.10.019>)
- Amasheh S, Fromm M & Günzel D 2011 Claudins of intestine and nephron – a correlation of molecular tight junction structure and barrier function. *Acta Physiologica* **201** 133–140. (<https://doi.org/10.1111/j.1748-1716.2010.02148.x>)
- Andruxhova O, Bayer J, Schüler C, Zeitz U, Murali SK, Ada S, Alvarez-Pez JM, Smorodchenko A & Erben RG 2017 Klotho lacks an FGF23-independent role in mineral homeostasis. *Journal of Bone and Mineral Research* **32** 2049–2061. (<https://doi.org/10.1002/jbmr.3195>)
- Battini JL, Rasko JE & Miller AD 1999 A human cell-surface receptor for xenotropic and polytropic murine leukemia viruses: possible role in G protein-coupled signal transduction. *PNAS* **96** 1385–1390. (<https://doi.org/10.1073/pnas.96.4.1385>)
- Beck L, Karaplis AC, Amizuka N, Hewson AS, Ozawa H & Tenenhouse HS 1998 Targeted inactivation of Npt2 in mice leads to severe renal phosphate wasting, hypercalciuria, and skeletal abnormalities. *PNAS* **95** 5372–5377. (<https://doi.org/10.1073/pnas.95.9.5372>)
- Bergwitz C & Jüppner H 2010 Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annual Review of Medicine* **61** 91–104. (<https://doi.org/10.1146/annurev.med.051308.111339>)
- Berndt T, Thomas LF, Craig TA, Sommer S, Li X, Bergstralh EJ & Kumar R 2007 Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. *PNAS* **104** 11085–11090. (<https://doi.org/10.1073/pnas.0704446104>)
- Bessman SP & Carpenter CL 1985 The creatine-creatine phosphate energy shuttle. *Annual Review of Biochemistry* **54** 831–862. (<https://doi.org/10.1146/annurev.bi.54.070185.004151>)
- Blacher J, Guerin AP, Pannier B, Marchais SJ & London GM 2001 Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* **38** 938–942. (<https://doi.org/10.1161/hy1001.096358>)
- Bon N, Couasnay G, Bourguine A, Sourice S, Beck-Cormier S, Guicheux J & Beck L 2017 Phosphate (Pi)-regulated heterodimerization of the high-affinity sodium-dependent Pi transporters PiT1/Slc20a1 and PiT2/Slc20a2 underlies extracellular Pi sensing independently of Pi uptake. *Journal of Biological Chemistry* **293** 2102–2114. (<https://doi.org/10.1074/jbc.M117.807339>)
- Bon N, Frangi G, Sourice S, Guicheux J, Beck-Cormier S & Beck L 2018 Phosphate-dependent FGF23 secretion is modulated by PiT2/Slc20a2.

- Molecular Metabolism* **11** 197–204. (<https://doi.org/10.1016/j.molmet.2018.02.007>)
- Bon N, Beck-Cormier S & Beck L 2019 Interplay between FGF23, phosphate, and molecules involved in phosphate sensing. *Current Molecular Biology Reports* **5** 1–7. (<https://doi.org/10.1007/s40610-019-0109-2>)
- Centeno PP, Herberger A, Mun HC, Tu C, Nemeth EF, Chang W, Conigrave AD & Ward DT 2019 Phosphate acts directly on the calcium-sensing receptor to stimulate parathyroid hormone secretion. *Nature Communications* **10** 4693. (<https://doi.org/10.1038/s41467-019-12399-9>)
- Chande S & Bergwitz C 2018 Role of phosphate sensing in bone and mineral metabolism. *Nature Reviews: Endocrinology* **14** 637–655. (<https://doi.org/10.1038/s41574-018-0076-3>)
- Clinkenbeard EL, Noonan ML, Thomas JC, Ni P, Hum JM, Aref M, Swallow EA, Moe SM, Allen MR & White KE 2019 Increased FGF23 protects against detrimental cardio-renal consequences during elevated blood phosphate in CKD. *JCI Insight* **4** e123817. (<https://doi.org/10.1172/jci.insight.123817>)
- Conrad M, Schothorst J, Kankipati HN, Van Zeebroeck G, Rubio-Teixeira M & Thevelein JM 2014 Nutrient sensing and signaling in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiology Reviews* **38** 254–299. (<https://doi.org/10.1111/1574-6976.12065>)
- Crook M & Swaminathan R 1996 Disorders of plasma phosphate and indications for its measurement. *Annals of Clinical Biochemistry* **33** 376–396. (<https://doi.org/10.1177/000456329603300502>)
- Erben RG 2018 α -Klotho's effects on mineral homeostasis are fibroblast growth factor-23 dependent. *Current Opinion in Nephrology and Hypertension* **27** 229–235. (<https://doi.org/10.1097/MNH.0000000000000415>)
- Faul C, Amaral AP, Oskoue B, Hu MC, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, et al. 2011 FGF23 induces left ventricular hypertrophy. *Journal of Clinical Investigation* **121** 4393–4408. (<https://doi.org/10.1172/JCI46122>)
- Feild JA, Zhang L, Brun KA, Brooks DP & Edwards RM 1999 Cloning and functional characterization of a sodium-dependent phosphate transporter expressed in human lung and small intestine. *Biochemical and Biophysical Research Communications* **258** 578–582. (<https://doi.org/10.1006/bbrc.1999.0666>)
- Ferrari SL, Bonjour JP & Rizzoli R 2005 Fibroblast growth factor-23 relationship to dietary phosphate and renal phosphate handling in healthy young men. *Journal of Clinical Endocrinology and Metabolism* **90** 1519–1524. (<https://doi.org/10.1210/jc.2004-1039>)
- Forster IC, Hernando N, Biber J & Murer H 2013 Phosphate transporters of the SLC20 and SLC34 families. *Molecular Aspects of Medicine* **34** 386–395. (<https://doi.org/10.1016/j.mam.2012.07.007>)
- Fukumoto S & Martin TJ 2009 Bone as an endocrine organ. *Trends in Endocrinology and Metabolism* **20** 230–236. (<https://doi.org/10.1016/j.tem.2009.02.001>)
- Geng Y, Mosyak L, Kurinov I, Zuo H, Sturchler E, Cheng TC, Subramanyam P, Brown AP, Brennan SC, Mun HC, et al. 2016 Structural mechanism of ligand activation in human calcium-sensing receptor. *eLife* **5** e13662. (<https://doi.org/10.7554/eLife.13662>)
- Ghillebert R, Swinnen E, De Snijder P, Smets B & Winderickx J 2011 Differential roles for the low-affinity phosphate transporters Pho87 and Pho90 in *Saccharomyces cerevisiae*. *Biochemical Journal* **434** 243–251. (<https://doi.org/10.1042/BJ20101118>)
- Giovannini D, Touhami J, Charnet P, Sitbon M & Battini JL 2013 Inorganic phosphate export by the retrovirus receptor XPR1 in metazoans. *Cell Reports* **3** 1866–1873. (<https://doi.org/10.1016/j.celrep.2013.05.035>)
- Grabner A, Schramm K, Silswal N, Hendrix M, Yanucil C, Czaya B, Singh S, Wolf M, Hermann S, Stypmann J, et al. 2017 FGF23/FGFR4-mediated left ventricular hypertrophy is reversible. *Scientific Reports* **7** 1993. (<https://doi.org/10.1038/s41598-017-02068-6>)
- Hansen NM, Felix R, Bisaz S & Fleisch H 1976 Aggregation of hydroxyapatite crystals. *Biochimica et Biophysica Acta* **451** 549–559. ([https://doi.org/10.1016/0304-4165\(76\)90150-1](https://doi.org/10.1016/0304-4165(76)90150-1))
- Heine GH, Nangaku M & Fliser D 2013 Calcium and phosphate impact cardiovascular risk. *European Heart Journal* **34** 1112–1121. (<https://doi.org/10.1093/eurheartj/ehs353>)
- Hilfiker H, Hattenhauer O, Traebert M, Forster I, Murer H & Biber J 1998 Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. *PNAS* **95** 14564–14569. (<https://doi.org/10.1073/pnas.95.24.14564>)
- Hortells L, Sosa C, Guillén N, Lucea S, Millán Á & Sorribas V 2017 Identifying early pathogenic events during vascular calcification in uremic rats. *Kidney International* **92** 1384–1394. (<https://doi.org/10.1016/j.kint.2017.06.019>)
- Hubbard SR & Till JH 2000 Protein tyrosine kinase structure and function. *Annual Review of Biochemistry* **69** 373–398. (<https://doi.org/10.1146/annurev.biochem.69.1.373>)
- Hürlimann HC, Pinson B, Stadler-Waibel M, Zeeman SC & Freimoser FM 2009 The SPX domain of the yeast low-affinity phosphate transporter Pho90 regulates transport activity. *EMBO Reports* **10** 1003–1008. (<https://doi.org/10.1038/embor.2009.105>)
- Ito N, Fukumoto S, Takeuchi Y, Takeda S, Suzuki H, Yamashita T & Fujita T 2007 Effect of acute changes of serum phosphate on fibroblast growth factor (FGF)23 levels in humans. *Journal of Bone and Mineral Metabolism* **25** 419–422. (<https://doi.org/10.1007/s00774-007-0779-3>)
- Jacquillet G & Unwin RJ 2019 Physiological regulation of phosphate by vitamin D, parathyroid hormone (PTH) and phosphate (Pi). *Pflügers Archiv* **471** 83–98. (<https://doi.org/10.1007/s00424-018-2231-z>)
- Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H & Giachelli CM 2000 Phosphate regulation of vascular smooth muscle cell calcification. *Circulation Research* **87** E10–E17. (<https://doi.org/10.1161/01.res.87.7.e10>)
- Kavanaugh MP, Miller DG, Zhang W, Law W, Kozak SL, Kabat D & Miller AD 1994 Cell-surface receptors for gibbon ape leukemia virus and amphotropic murine retrovirus are inducible sodium-dependent phosphate symporters. *PNAS* **91** 7071–7075. (<https://doi.org/10.1073/pnas.91.15.7071>)
- Kerschnitzki M, Akiva A, Ben Shoham A, Asscher Y, Wagermaier W, Fratzl P, Addadi L & Weiner S 2016 Bone mineralization pathways during the rapid growth of embryonic chicken long bones. *Journal of Structural Biology* **195** 82–92. (<https://doi.org/10.1016/j.jsb.2016.04.011>)
- Kestenbaum B, Glazer NL, Köttgen A, Felix JF, Hwang SJ, Liu Y, Lohman K, Kritchevsky SB, Hausman DB, Petersen AK, et al. 2010 Common genetic variants associate with serum phosphorus concentration. *Journal of the American Society of Nephrology* **21** 1223–1232. (<https://doi.org/10.1681/ASN.2009111104>)
- Khoshniat S, Julien M, Beck L, Petit M, Rouillon T, Masson M, Gatiús M, Weiss P & Guicheux J 2008 Calcium is required for phosphate-dependent stimulation of MGP and OPN expression in osteoblasts. *Bone* **42** S24. (<https://doi.org/10.1016/j.bone.2007.12.027>)
- Khoshniat S, Bourguin A, Julien M, Petit M, Pilet P, Rouillon T, Masson M, Gatiús M, Weiss P, Guicheux J, et al. 2011 Phosphate-dependent stimulation of MGP and OPN expression in osteoblasts via the ERK1/2 pathway is modulated by calcium. *Bone* **48** 894–902. (<https://doi.org/10.1016/j.bone.2010.12.002>)
- Kido S, Miyamoto K, Mizobuchi H, Taketani Y, Ohkido I, Ogawa N, Kaneko Y, Harashima S & Takeda E 1999 Identification of regulatory sequences and binding proteins in the type II sodium/phosphate cotransporter NPT2 gene responsive to dietary phosphate. *Journal of Biological Chemistry* **274** 28256–28263. (<https://doi.org/10.1074/jbc.274.40.28256>)
- Knopfel T, Himmerkus N, Günzel D, Bleich M, Hernando N & Wagner CA 2019 Paracellular transport of phosphate along the intestine. *American Journal of Physiology: Gastrointestinal and Liver*

- Physiology* **317** G233–G241. (<https://doi.org/10.1152/ajpgi.00032.2019>)
- Kornberg A 1979 The enzymatic replication of DNA. *CRC Critical Reviews in Biochemistry* **7** 23–43. (<https://doi.org/10.3109/10409237909102568>)
- Krebs EG & Beavo JA 1979 Phosphorylation-dephosphorylation of enzymes. *Annual Review of Biochemistry* **48** 923–959. (<https://doi.org/10.1146/annurev.bi.48.070179.004423>)
- Kritmetapak K & Kumar R 2019 Phosphate as a signaling molecule. *Calcified Tissue International* [epub]. (<https://doi.org/10.1007/s00223-019-00636-8>)
- Kumar R 2009 Phosphate sensing. *Current Opinion in Nephrology and Hypertension* **18** 281–284. (<https://doi.org/10.1097/MNH.0b013e32832b5094>)
- Kuro-O M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, *et al.* 1997 Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* **390** 45–51. (<https://doi.org/10.1038/36285>)
- Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, *et al.* 2006 Regulation of fibroblast growth factor-23 signaling by klotho. *Journal of Biological Chemistry* **281** 6120–6123. (<https://doi.org/10.1074/jbc.C500457200>)
- Levi M, Gratton E, Forster IC, Hernando N, Wagner CA, Biber J, Sorribas V & Murer H 2019 Mechanisms of phosphate transport. *Nature Reviews: Nephrology* **15** 482–500. (<https://doi.org/10.1038/s41581-019-0159-y>)
- London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B & Adda H 2003 Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrology, Dialysis, Transplantation* **18** 1731–1740. (<https://doi.org/10.1093/ndt/gfg414>)
- Marks J 2019 The role of SLC34A2 in intestinal phosphate absorption and phosphate homeostasis. *Pflügers Archiv* **471** 165–173. (<https://doi.org/10.1007/s00424-018-2221-1>)
- Michigami T, Kawai M, Yamazaki M & Ozono K 2018 Phosphate as a signaling molecule and its sensing mechanism. *Physiological Reviews* **98** 2317–2348. (<https://doi.org/10.1152/physrev.00022.2017>)
- Miller DG & Miller AD 1994 A family of retroviruses that utilize related phosphate transporters for cell entry. *Journal of Virology* **68** 8270–8276. (<https://doi.org/10.1128/JVI.68.12.8270-8276.1994>)
- Miller DG, Edwards RH & Miller AD 1994 Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. *PNAS* **91** 78–82. (<https://doi.org/10.1073/pnas.91.1.78>)
- Mokas S, Larivière R, Lamallice L, Gobeil S, Cornfield DN, Agharazii M & Richard DE 2016 Hypoxia-inducible factor-1 plays a role in phosphate-induced vascular smooth muscle cell calcification. *Kidney International* **90** 598–609. (<https://doi.org/10.1016/j.kint.2016.05.020>)
- Nishida Y, Taketani Y, Yamanaka-Okumura H, Imamura F, Taniguchi A, Sato T, Shuto E, Nashiki K, Arai H, Yamamoto H, *et al.* 2006 Acute effect of oral phosphate loading on serum fibroblast growth factor 23 levels in healthy men. *Kidney International* **70** 2141–2147. (<https://doi.org/10.1038/sj.ki.5002000>)
- O'Hara B, Johann SV, Klinger HP, Blair DG, Robinson H, Dunn KJ, Sass P, Vitek SM & Robins T 1990 Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. *Cell Growth and Differentiation* **1** 119–127.
- Qi W, Baldwin SA, Muench SP & Baker A 2016 Pi sensing and signalling: from prokaryotic to eukaryotic cells. *Biochemical Society Transactions* **44** 766–773. (<https://doi.org/10.1042/BST20160026>)
- Quinn SJ, Thomsen ARB, Pang JL, Kantham L, Bräuner-Osborne H, Pollak M, Goltzman D & Brown EM 2013 Interactions between calcium and phosphorus in the regulation of the production of fibroblast growth factor 23 in vivo. *American Journal of Physiology: Endocrinology and Metabolism* **304** E310–E320. (<https://doi.org/10.1152/ajpendo.00460.2012>)
- Ritz E, Hahn K, Ketteler M, Kuhlmann MK & Mann J 2012 Phosphate additives in food—a health risk. *Deutsches Arzteblatt International* **109** 49–55. (<https://doi.org/10.3238/arztebl.2012.0049>)
- Rothfield L & Finkelstein A 1968 Membrane biochemistry. *Annual Review of Biochemistry* **37** 463–496. (<https://doi.org/10.1146/annurev.bi.37.070168.002335>)
- Sabbagh Y & Schiavi SC 2014 Role of NPT2b in health and chronic kidney disease. *Current Opinion in Nephrology and Hypertension* **23** 377–384. (<https://doi.org/10.1097/01.mnh.0000447015.44099.5f>)
- Sabbagh Y, O'Brien SP, Song W, Boulanger JH, Stockmann A, Arbeeney C & Schiavi SC 2009 Intestinal npt2b plays a major role in phosphate absorption and homeostasis. *Journal of the American Society of Nephrology* **20** 2348–2358. (<https://doi.org/10.1681/ASN.2009050559>)
- Sage AP, Lu J, Tintut Y & Demer LL 2011 Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells in vitro. *Kidney International* **79** 414–422. (<https://doi.org/10.1038/ki.2010.390>)
- Scanni R, vonRotz M, Jehle S, Hulter HN & Krapf R 2014 The human response to acute enteral and parenteral phosphate loads. *Journal of the American Society of Nephrology* **25** 2730–2739. (<https://doi.org/10.1681/ASN.2013101076>)
- Schlieper G, Krüger T, Djuric Z, Damjanovic T, Markovic N, Schurgers LJ, Brandenburg VM, Westenfeld R, Dimkovic S, Ketteler M, *et al.* 2008 Vascular access calcification predicts mortality in hemodialysis patients. *Kidney International* **74** 1582–1587. (<https://doi.org/10.1038/ki.2008.458>)
- Segawa H, Kaneko I, Yamanaka S, Ito M, Kuwahata M, Inoue Y, Kato S & Miyamoto K-I 2004 Intestinal Na-P(i) cotransporter adaptation to dietary P(i) content in vitamin D receptor null mice. *American Journal of Physiology: Renal Physiology* **287** F39–F47. (<https://doi.org/10.1152/ajprenal.00375.2003>)
- Segawa H, Onitsuka A, Furutani J, Kaneko I, Aranami E, Matsumoto N, Tomoe Y, Kuwahata M, Ito M, Matsumoto M, *et al.* 2009 Npt2a and Npt2c in mice play distinct and synergistic roles in inorganic phosphate metabolism and skeletal development. *American Journal of Physiology: Renal Physiology* **297** F671–F678. (<https://doi.org/10.1152/ajprenal.00156.2009>)
- Slatopolsky E, Finch J, Denda M, Ritter C, Zhong M, Dusso A, MacDonald PN & Brown AJ 1996 Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion in vitro. *Journal of Clinical Investigation* **97** 2534–2540. (<https://doi.org/10.1172/JCI118701>)
- Takashi Y & Fukumoto S 2020 Phosphate-sensing and regulatory mechanism of FGF23 production. *Journal of Endocrinological Investigation* **43** 877–883. (<https://doi.org/10.1007/s40618-020-01205-9>)
- Takashi Y, Kosako H, Sawatsubashi S, Kinoshita Y, Ito N, Tsoumpra MK, Nangaku M, Abe M, Matsuhisa M, Kato S, *et al.* 2019 Activation of unliganded FGF receptor by extracellular phosphate potentiates proteolytic protection of FGF23 by its O-glycosylation. *PNAS* **116** 11418–11427. (<https://doi.org/10.1073/pnas.1815166116>)
- Tenhouse HS, Martel J, Gauthier C, Zhang MY & Portale AA 2001 Renal expression of the sodium/phosphate cotransporter gene, Npt2, is not required for regulation of renal 1 alpha-hydroxylase by phosphate. *Endocrinology* **142** 1124–1129. (<https://doi.org/10.1210/endo.142.3.8029>)
- Thomas L, Bettoni C, Knopfel T, Hernando N, Biber J & Wagner CA 2017 Acute adaptation to oral or intravenous phosphate requires parathyroid hormone. *Journal of the American Society of Nephrology* **28** 903–914. (<https://doi.org/10.1681/ASN.2016010082>)
- Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S & Yamashita T 2006 Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* **444** 770–774. (<https://doi.org/10.1038/nature05315>)

- van Zeijl M, Johann SV, Closs E, Cunningham J, Eddy R, Shows TB & O'Hara B 1994 A human amphotropic retrovirus receptor is a second member of the gibbon ape leukemia virus receptor family. *PNAS* **91** 1168–1172. (<https://doi.org/10.1073/pnas.91.3.1168>)
- Vorland CJ, Stremke ER, Moorthi RN & Hill Gallant KM 2017 Effects of excessive dietary phosphorus intake on bone health. *Current Osteoporosis Reports* **15** 473–482. (<https://doi.org/10.1007/s11914-017-0398-4>)
- Wagner CA, Hernando N, Forster IC & Biber J 2013 The SLC34 family of sodium-dependent phosphate transporters. *Pflügers Archiv: European Journal of Physiology* **466** 139–153. (<https://doi.org/10.2152/jmi.60.27>)
- Walling MW 1977 Intestinal Ca and phosphate transport: differential responses to vitamin D3 metabolites. *American Journal of Physiology* **233** E488–E494. (<https://doi.org/10.1152/ajpendo.1977.233.6.E488>)
- Weiner S & Addadi L 2011 Crystallization pathways in biomineralization. *Annual Review of Materials Research* **41** 21–40. (<https://doi.org/10.1146/annurev-matsci-062910-095803>)
- Wild R, Gerasimaite R, Jung JY, Truffault V, Pavlovic I, Schmidt A, Saiardi A, Jessen HJ, Poirier Y, Hothorn M, *et al.* 2016 Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science* **352** 986–990. (<https://doi.org/10.1126/science.aad9858>)
- Wilson MS, Jessen HJ & Saiardi A 2019 The inositol hexakisphosphate kinases IP6K1 and -2 regulate human cellular phosphate homeostasis, including XPR1-mediated phosphate export. *Journal of Biological Chemistry* **294** 11597–11608. (<https://doi.org/10.1074/jbc.RA119.007848>)
- Witteman JC, Kok FJ, van Saase JL & Valkenburg HA 1986 Aortic calcification as a predictor of cardiovascular mortality. *Lancet* **2** 1120–1122. ([https://doi.org/10.1016/s0140-6736\(86\)90530-1](https://doi.org/10.1016/s0140-6736(86)90530-1))
- Witteman JC, Kannel WB, Wolf PA, Grobbee DE, Hofman A, D'Agostino RB & Cobb JC 1990 Aortic calcified plaques and cardiovascular disease (the Framingham Study). *American Journal of Cardiology* **66** 1060–1064. ([https://doi.org/10.1016/0002-9149\(90\)90505-u](https://doi.org/10.1016/0002-9149(90)90505-u))

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