Cell. Mol. Life Sci. (2011) 68:205–218
DOI 10.1007/s00018-010-0527-z

Cellular and Molecular Life Sciences

REVIEW

The emergence of phosphate as a specific signaling molecule in bone and other cell types in mammals

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Received: 23 March 2010 / Revised: 2 August 2010 / Accepted: 31 August 2010 / Published online: 17 September 2010

Abstract Although considerable advances in our understanding of the mechanisms of phosphate homeostasis and skeleton mineralization have recently been made, little is known about the initial events involving the detection of changes in the phosphate serum concentrations and the subsequent downstream regulation cascade. Recent data has strengthened a long-established hypothesis that a phosphate-sensing mechanism may be present in various organs. Such a phosphate sensor would detect changes in serum or local phosphate concentration and would inform the body, the local environment, or the individual cell. This suggests that phosphate in itself could represent a signal regulating multiple factors necessary for diverse biological processes such as bone or vascular calcification. This review summarizes findings supporting the possibility that phosphate represents a signaling molecule, particularly in bone and cartilage, but also in other tissues. The involvement of various signaling pathways (ERK1/2), transcription factors (Fra-1, Runx2) and phosphate transporters (PiT1, PiT2) is discussed.

Keywords Phosphate sensing · Phosphate homeostasis · Bone mineralization · Signaling · SLC20a1 · SLC20a2

Abbreviations

$1\alpha,25(OH)_2D_3$	1α,25-dihydroxyvitamin D
ALP	Alkaline phosphatase
Pi	Inorganic phosphate
IGF-1	Insulin growth factor-1
ESRD	End-stage renal disease
FGF-23	Fibroblast growth factor 23
HSMC	Human aortic smooth muscle cell
MGP	Matrix gla protein
PTH	Parathyroid hormone
PFA	Phosphonoformic acid
RANKL	Receptor activator of nuclear factor kappa B (NF-κB) (RANK) ligand
VSMC	Vascular smooth muscle cell

Introduction

Phosphorus is the second most abundant mineral in the human body and plays a critical role in vital processes. In body fluids, phosphorus is present mainly as inorganic phosphate (Pi) in the form of $H_2PO_4^-/HPO_4^{2-}$ with a 1:4 ratio at physiological pH values. In cells and tissues, it is found in a variety of organic substances, including sugar phosphates, phosphoproteins, phospholipids and nucleic acids. Approximately 85% of phosphorus is in bone, and is primarily complexed with calcium (Ca^{2+}) in hydroxyapatite crystals deposited onto the collagen matrix [1].

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The remainder is in soft tissue with only about 1% in extracellular fluids [2]. Hence, phosphorus has structural roles in phospholipids of cell membranes, nucleoproteins and nucleic acids [3, 4], and is essential for bone mineralization [1]. Moreover, Pi 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 [2, 5–10]. Prolonged deficiency of Pi results in hypophosphatemia along with serious biological consequences, including impaired mineralization of bone resulting in osteomalacia or rickets, central nervous system dysfunction, increased erythrocyte membrane rigidity, abnormal leukocyte and platelet function, rhabdomyolysis and muscle weakness, and cardiac dysfunction and respiratory failure [11–15]. On the other hand, hyperphosphatemia is now known to decrease life expectancy and lead to seizures, cardiac dysrhythmias, muscle weakness and tetany, decreased visual acuity, soft tissue calcification, renal failure and eventually death [16–21]. Therefore, controlling the serum Pi concentration is critical for the well being of the organism.

The normal range of serum Pi changes from the neonatal period through adolescence to reach adult values towards the end of puberty (Table 1). Pi concentrations are highest in the neonatal period (1.88–2.4 mM), but fall rapidly in the first months of life and then more slowly to reach childhood levels (1.45–1.80 mM). The concentrations then remain fairly constant until adolescence, when they fall to adult values (0.85–1.44 mM) [22] (Table 1). A normal Pi serum value is the result of a properly controlled absorption, reabsorption and bodily Pi consumption. Absorption of Pi occurs primarily in the ileum, whereas Pi reabsorption occurs in the kidney, principally along the proximal convoluted tubules [23]. In states of neutral Pi balance, the amount of Pi absorbed in the intestine (approximately 0.8–1.5 g per 24 h) is equivalent to the amount excreted in the urine, reflecting that serum Pi homeostasis is maintained primarily through the control of renal Pi reabsorption.

The regulation of Pi homeostasis is classically described as a complex process involving the interplay between parathyroid hormone (PTH) and the vitamin D endocrine system. Whereas 1α,25-dihydroxyvitamin D ($1\alpha,25(OH)_2D_3$) increases the efficiency of Pi absorption in the intestine [24–26], PTH decreases the efficiency of renal Pi reabsorption [27–29]. Over the past 15 years, considerable advances has been made in the understanding of the mechanisms that specifically control Pi balance [22, 30–32]. Several factors, such as fibroblast growth factor 23 (FGF-23), klotho, secreted frizzled-related protein 4 (sFRP-4), stanniocalcin-1 and -2, dentin matrix protein 1 and matrix extracellular glycoprotein have emerged as major regulators of Pi homeostasis and suggest the existence of an elaborate network of humoral interactions and feedback loops involving intestine, kidney, parathyroid gland, and bone (Fig. 1). As this topic is beyond the scope of this short review, the reader can refer to numerous recent reviews concerning these various factors and their role in controlling Pi homeostasis [14, 30, 33–36].

Despite the considerable progress made in our understanding of Pi homeostasis, there are still many gaps in our knowledge. Particularly, a necessary first step in the regulation of Pi homeostasis is the ability of a cell or an organism to detect changes in Pi concentrations in its environment. This would imply the existence of a Pi-sensing mechanism able to detect changes in the concentration of Pi. While the existence of such a mechanism still lacks evidence in mammals, a number of recent observations suggest that Pi may act as a signaling molecule able to modulate diverse cellular functions. This review focuses on the role of Pi as a signaling molecule at both the cellular and whole-body level. Its role in bone and cartilage will be highlighted and questions regarding its action mechanisms will be discussed.

Mammalian phosphate transporters

Because a cell's interior is electronegative relative to the exterior, the movement of Pi into the cell does not occur by simple diffusion. In mammals, Na^+-coupled Pi cotransporters mediate the transport of Pi across cell membranes [23, 37]. Currently, identified Na^+-Pi cotransporters in mammals have been grouped into three dissimilar families, based on their amino acid sequence and structural similarity [38, 39]. These families differ not only by their affinities for Pi, but also by their tissue distribution,

Table 1 Normal serum values of Pi, Ca, magnesium and potassium in children and adults

	Pi (mM)	Total Ca (mM)	Ionized Ca (mM)	Mg (mM)	K (mM)
Neonatal period	1.88–2.4	1.8–1.9	1.0–1.3	0.5–0.75	3.9–5.9
Childhood	1.45–1.8	2.2–2.6	1.1–1.4	0.6–0.9	3.5–5
Adulthood	0.85–1.44	2.2–2.6	1.1–1.4	0.7–0.9	3.5–5

Pi inorganic phosphate, *Ca* calcium, *Mg* magnesium, *K* potassium

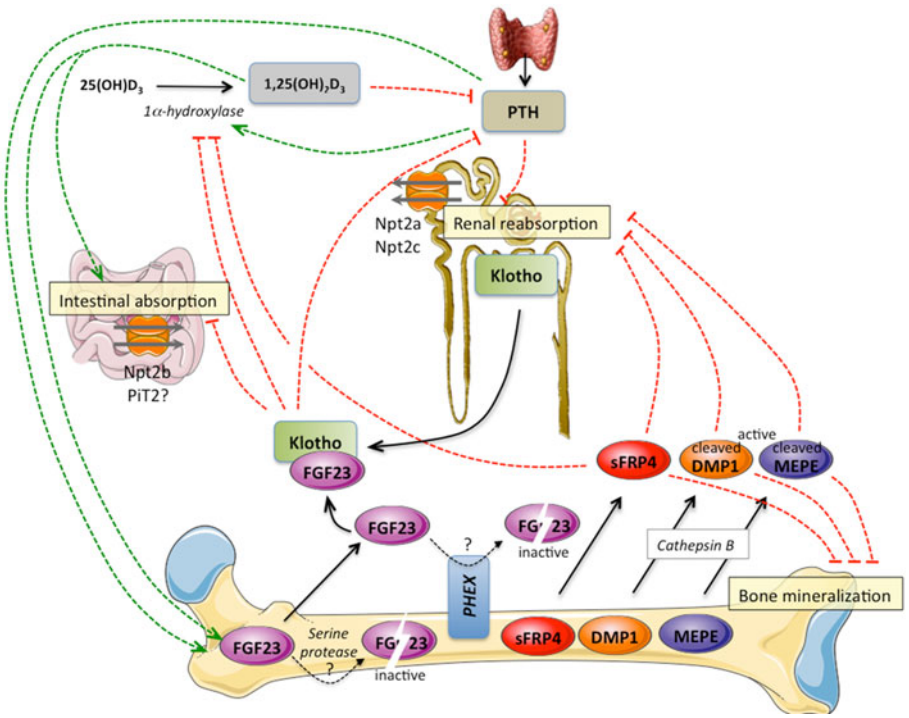

Fig. 1 Simplified overview of the interrelationships between organs, hormones and transporters involved in the maintenance of phosphate homeostasis. Major regulators that inhibit (*red lines*) or stimulate (*green lines*) phosphate absorption or reabsorption, and bone mineralization are shown. The reader can refer to the text for more details, and to references [14, 30, 33–35]

physiological regulations and roles. Whilst they are often referred to in the literature as "type I, II and III" Na^+-Pi cotransporters, a numbering system based on the chronological identification of their Pi transport activity, it must be noted that all these transporters are multi-spanning membrane proteins and are therefore classified as type III integral membrane proteins according to the classification of membrane proteins proposed by Singer in 1990 [40–42]. The human gene nomenclature database (http://www.genenames.org/) has assigned these transporters to the solute carrier series SLC17, SLC34, and SLC20. Hence, the first identified (i.e. "type I") membrane protein bearing a Na^+-Pi cotransport activity belongs to the SLC17 series and is known as NPT1; the second identified (i.e. "type II") Na^+-Pi cotransporter belongs to the SLC34 series and is known as NPT2; and the last identified (i.e. "type III") mammalian Na^+-Pi cotransporter belongs to the SLC20 series and is known as PiT (Fig. 2).

Although the SLC17 transporters display Pi uptake capacity [43], they also transport other anions and molecules [44–48] and their physiological roles remain to be fully established [49, 50]. The SLC34 transporters include NPT2a, NPT2b and NPT2c. Npt2a (SLC34A1) is primarily located at the brush border of proximal tubular epithelial cells, is recognized as the main transporter responsible for the renal reabsorption of Pi, and is a target for most humoral factors regulating Pi homeostasis [39, 51, 52]. Npt2b (SLC34A2) is expressed broadly and has recently been shown to be the main transporter for intestinal Pi absorption [53, 54]. Npt2c (SLC34A3) is localized in the same cells as Npt2a, and is not expressed in bone [55]. Its role is still unclear, and it has been suggested it plays a role during body growth [56]. Recently, the Miyamoto group showed that Npt2c has important physiological roles in Pi and Ca homeostasis by modulating the vitamin D/fibroblast growth factor 23 axis, but also in bone mineralization [55, 57]. The SLC20 family of transporters includes PiT1 (SLC20A1) and PiT2 (SLC20A2) [58]. These widely expressed proteins were originally described as retroviral receptors, hence were named Glvr-1 and Ram-1 [59, 60], and were later shown to be Na^+-Pi cotransporters [61]. According to their physiological function, they were renamed after their prokaryotic paralogs: PiT1 for Glvr-1 and PiT2 for Ram-1. The possible physiological roles of PiT1 and PiT2 have been recently reviewed [58, 62]. Of importance for the present article, it should be noted that PiT1 may have important roles in vascular and bone physiology [63–65], whereas PiT2 may play a role in Pi reabsorption [66].

Phosphate and skeletal tissues

During skeletal growth and bone remodeling, calcium and Pi are required for the formation of biological apatites. The rate at which mineralization occurs is dependent, in part, on the local availability of Pi and calcium [1, 67]. In the absence of these ions, or in case of abnormal absorption or reabsorption, mineralization is impaired, resulting in the formation of poorly mineralized bone that is characteristic

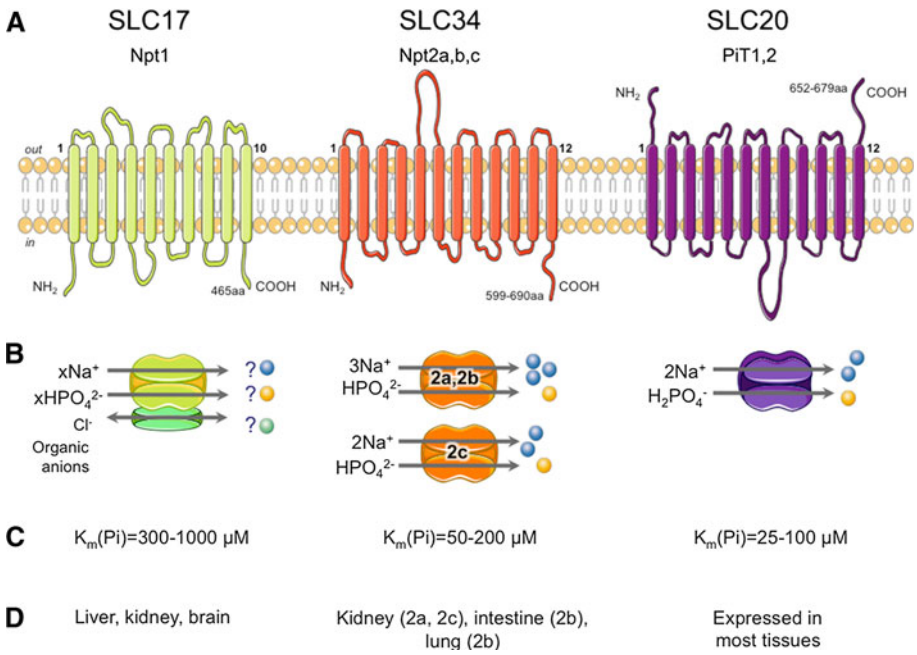

Fig. 2 The three families of mammalian Na^+-phosphate cotransporters. **a** Assignment to solute carrier series and schematic representation of membrane topology according to current accepted models, as indicated. **b** Substrate specificity and stoechiometry of Pi transport. **c** Affinity for phosphate (Michaelis–Menten K_m value). **d** Main site of expression in adults

of osteomalacia or rickets [68]. However, increasing evidence indicates that Pi is also required in events other than mineralization, proposing Pi itself as a signaling molecule capable of modulating multiple cellular functions in bone [69].

Pi modulates osteoblastic proliferation

Several studies have recently implicated Pi as an important molecule capable of modulating osteoblastic proliferation. Naviglio et al. [70] reported that Pi treatment of human osteosarcoma U2OS cells resulted in a decrease in both intracellular cAMP levels and adenylate cyclase activity, and in cell growth inhibition. The use of pharmacological agents that maintain a high level of cAMP, such as forskolin, prevented the cell growth inhibition in response to Pi. A large-scale quantitative proteomic investigation conducted in MC3T3-E1 cells, a model of preosteoblastic cells often used for studying osteoblast differentiation and activity [71], showed that a 24-h Pi treatment modulates the expression levels of a large set of proteins [69]. A pathway/function analysis of these proteins revealed wide-ranging effects from transcriptional regulators to signal transduction molecules. Several upregulated proteins in response to Pi were found to be involved in the regulation of cell cycle, proliferation and DNA synthesis [69]. Flow cytometric analysis of MC3T3-E1 cells showed that the number of cells in the S-phase increased in response to Pi treatment, suggesting an increase in the proliferation in response to Pi [69, 72, 73]. Moreover, Kanatani et al. [74] have recently shown that an increase in extracellular Pi from 2 to 4 mM, dose-dependently stimulated DNA synthesis in MC3T3-E1 cells. By using an Insulin growth factor-1 (IGF-1) antiserum or a neutralizing IGF-1 antibody, these investigators inhibited partially but significantly the Pi-induced proliferation. This suggested that Pi can modulate osteoblastic proliferation at least in part via IGF-1, which is known as one of the main growth factors involved in osteoblastic cell proliferation [75]. However, the interaction with the IGF-1 signaling pathway may not be restricted to Pi since calcium was also shown to stimulate osteoblastic proliferation in relation to IGF-1 status [76], although IGF-1-independent effects of calcium on DNA synthesis were also described [77].

Pi modulates osteoblastic differentiation and activity

Inorganic phosphate has also been shown to play a role in osteoblastic cell differentiation and activity. These are complex processes that involve the temporally-regulated expression of multiple markers. During the early osteoblastic differentiation, the expression level of alkaline phosphatase (ALP) increases. In the presence of organic phosphates, such as β-glycerophosphate, ALP contributes to the local production or liberation of phosphate, thus leading to an increased Pi concentration in the extracellular environment [67]. The potential role of Pi as a signaling molecule during the course of osteoblast differentiation was first suggested by Beck et al. [78]. They showed that a decrease in extracellular Pi formation due to the inhibition of ALP activity correlated with a decrease in osteopontin expression, a molecule involved in the regulation of mineralization [78]. Later on, they showed that osteopontin expression was strongly induced in direct response to

increased Pi levels in the culture medium [79]. The authors speculated that the physiological significance of the regulation of osteopontin expression by Pi generated via ALP was to prevent the induction of an excessive mineralization before osteoblasts cease proliferation [79]. An elegant work from Yoshiko and colleagues illustrated the role of Pi in bone mineralization in vitro and in vivo through the identification of an osteoblast autonomous Pi regulatory system that regulates bone mineralization. They showed that Pi stimulated the expression of stanniocalcin 1, which itself increased PiT1 accumulation in both osteoblast cultures and in vivo, resulting in increased Pi uptake and increased mineralization [65]. Once mineralized, osteoblasts may become osteocytes or bording cells or die by apoptosis. The work of Adams et al. [80] has linked extracellular Pi to a decrease in the mitochondrial membrane permeability leading to the apoptosis of osteoblasts. It should be noted, however, that osteoblasts are much less sensitive to Pi-induced apoptosis than chondrocytes, and that calcium is required during this process [80, 81]. In a recent study conducted in the murine odontoblast-like cell line MO6-G3, we showed an up-regulation of PiT1 and PiT2 expressions by high extracellular Pi concentrations, which correlated with ERK1/2 phosphorylation and calcium/phosphate crystal formation [82]. Although more detailed studies are necessary, our observations support the idea that crystal formation more than Pi itself may be required for the increase in ERK phosphorylation and PiT1 and PiT2 expression [82]. In line with this work, we recently obtained similar data in mouse primary osteoblasts and MC3T3-E1 cells where the Pi stimulation of MGP expression via the ERK1/2 pathway [83] correlated with the presence of calcium in the medium and could be abolished by the use of crystal formation inhibitors (Khoshniat et al., unpublished observation).

Pi modulates bone resorption

Bone resorption is due to the resorbing activity of specialized cells called osteoclasts [84]. Osteoclast precursors come from hematopoietic cells and need to couple with osteoblasts in order to differentiate into mature resorbing osteoclasts. Thus, the receptor activator of nuclear factor kappa B (NF-κB) (RANK) ligand (RANKL) at the surface of osteoblasts interacts with RANK at the surface of osteoclasts and allows the differentiation into mature osteoclasts [85–88], while osteoprotegerin is a molecule that inhibits osteoclast differentiation by binding to RANKL and preventing the RANK–RANKL interaction [86, 89].

Inorganic phosphate depletion is well known to stimulate osteoclastic bone resorption [90–92] while increased Pi inhibits both osteoclast differentiation and the bone resorption process [93]. The effect of an increase in Pi concentration has recently been studied in a culture of bone marrow cells. These data show that high extracellular Pi concentrations inhibit both the osteoclast differentiation and the osteoclast bone resorbing activity [94]. Furthermore, it has been shown that Pi inhibits the differentiation of osteoclastic precursors by stimulating osteoprotegerin expression in osteoblastic cells, and that extracellular Pi inhibits osteoclastic activity at least in part by the direct induction of osteoclasts apoptosis [94]. These findings were confirmed and expanded by Mozar et al. in a recent study where they showed that increased extracellular Pi concentration inhibited both osteoclastic differentiation and bone resorption activity induced by RANKL. Pi was found to inhibit the RANKL-induced JNK and Akt activation, while RANKL-induced p38 and ERK 1/2 phosphorylation were not affected [95].

Pi modulates chondrocyte differentiation

Early studies suggested a possible role of Pi in modulating chondrocyte differentiation. Using an organ culture technique as a model, Bingham and Raisz [96] showed in 1974 that bone shaft growth and mineralization from rat fetuses increase with a Pi treatment from 1.5 to 4.5 mM. Such concentrations of Pi lead to crystal formation by precipitation. However, using a low-calcium medium, they showed that growth and mineralization were still impaired [96]. Along the same line of evidence, a study from Kakuta et al. supports the view that metabolic regulation of the Pi pool size may be a rate-limiting factor in the mineralization of cartilage. These investigators analyzed sections of epiphyseal growth cartilage which revealed that very low levels of Pi are present in pre-mineralized cartilage. At the mineralization front, however, a large increase in Pi is correlated with mineral formation together with a decrease in the concentration of low molecular weight compounds that could provide the initial source of Pi for the development of mineral [97]. In a more recent study, Wang et al. [98] examined the potential role of Pi and calcium in the regulatory pathways that mediate chondrogenesis and cartilage maturation, using the CFK2 cells as a chondrocytic model. They showed that the response to external Pi induced a rapid up-regulation of PiT1 protein expression at the plasma membrane, associated with an increase in Pi transport. However, this effect was temporally restricted to an early stage of CFK2 differentiation, suggesting that extracellular Pi plays a role in the commitment of chondrogenic cells to differentiation [98]. The effect of Pi on the maturation of chondrocytes was also shown by the suppression of type II collagen and PTH receptor expression, and acceleration of type X collagen expression in the ATDC5 cell line, a model of growth plate chondrocytes [99, 100]. Additional data

obtained in osteoarthritic cartilage showed that IL-8 promotes hypertrophic terminal chondrocyte differentiation by modulating chondrocyte PiT1 expression and sodium-dependent Pi uptake [101]. These results demonstrate that Pi can play a role in the early and late differentiation of chondrocytes, by stimulating the expression of various chondrocytic markers. The exact molecular mechanisms involved in these effects have not yet been deciphered and need further investigation.

The terminal differentiation of growth plate chondrocytes involves a hypertrophic conversion associated with matrix mineralization and ultimately on their apoptosis [102]. During the calcification process, chondrocytes experience high levels of extracellular Pi [103]. Several studies have shown that exposing chondrocytes to a high level of Pi leads to their terminal maturation and subsequent matrix mineralization [100, 101, 104–107]. The role of Pi in these processes is highlighted by the mineralization defects that could be observed in the case of inherited hypophosphatemias [108]. Finally, the work of Mansfield et al. [106] has linked the elevation in environmental Pi concentration and the concomitant rise in intracellular Pi levels to rapid chondrocytic death through the apoptotic pathway. This effect of Pi is increased in the presence of calcium [109].

Phosphate and extraskeletal tissues

The ability of Pi to alter gene expression or cell function is not restricted to bone-forming cells. Regulation of osteopontin expression by Pi has also been demonstrated in NIH3T3 fibroblasts [79], cells that do not retain an ability to differentiate into osteoblast-like cells, showing that the regulation of osteopontin by phosphate is not restricted to cells in the osteoblast lineage.

Inorganic phosphate has been linked to the regulation of Runx2 and osteocalcin in human vascular smooth muscle cells [110, 111]. When cultured in media containing normal physiological levels of Pi (1.4 mM), human aortic smooth muscle cell (HSMC) grew in monolayers and did not mineralize. In contrast, HSMC cultured in media containing Pi levels comparable to those seen in hyperphosphatemic individuals (>1.4 mM) showed dose-dependent increases in cell culture calcium deposition. High Pi also enhanced the expression of osteocalcin and Runx2. This data suggest that high Pi may directly stimulate HSMC to undergo phenotypic changes that predispose to calcification and may help explain the phenomenon of human metastatic calcification under hyperphosphatemic conditions. The effects of high Pi were suggested to be mediated by PiT1. When HSMC were stably transduced with shRNA directed against PiT1, Pi uptake as well as Pi-induced mineralization and osteogenic differentiation markers Runx2 and osteopontin were reduced [112].

Low-external phosphate concentration has been shown to stimulate the SLC34 Na^+-Pi cotransporter in the murine kidney [113, 114]. Kido et al. showed that feeding mice with a low-Pi diet for 4 days led to a twofold increase in *Npt2a* mRNA abundance. They identified and characterized a DNA sequence responsible for the Pi response, designated as a phosphate response element in the *Npt2a* gene. This response element shares a region with 9 of 10 bp identity to yeast phosphate-responsive transcription factor Pho4 binding element. At the center of this region is a CACGTG motif sufficient to confer the transactivation by dietary Pi deprivation. The authors have also identified the mouse transcription factor mE3 (TFE3), with structural features very similar to those of Pho4, that is up-regulated by low Pi diet. These results emphasize the role of Pi as a direct regulator of gene transcription.

The group of Cho has published a series of papers during the past 5 years describing the effect of high or low dietary Pi on the growth of diverse organs, including brain [115, 116], liver [117] and lungs [118–121]. Their studies clearly show that Pi alters the growth of these organs, most probably through the Akt/mTOR pathway, as well as the Raf/MEK/ERK pathway. However, the precise mechanisms are still to be clarified since both high and low Pi diets act similarly on both of these pathways. Moreover, the Pi transporters that may be involved in this process are not clearly identified since, for example, either Npt2a [118] or Npt2b [119–121] have been implicated in the same organ, and SLC20 transporters were not investigated.

Finally, changes in extracellular Pi (and intracellular Pi) were thought to be responsible for the increased survival of cultured fetal rat cortical neurons after an excitotoxic or oxidative insult [122]. Pi was also implicated as a triggering signal for PTH induction and secretion in parathyroid tissue in vivo and in vitro [123–125].

Phosphate as a signaling molecule: mechanistic issues

As the biological role of Pi as a signaling molecule is emerging in mammals, questions regarding its mechanism of action have arisen. One of the first issues to address is whether any known signaling pathways are activated and if there is the need for a nuclear response. Another critical aspect is whether or not Pi needs to be transported into the cell to fulfill its signaling role. A close examination of the available data reveals that answering this latter point is not straightforward. Moreover, in the event that Pi acts as an extracellular signal, the requirement of membrane proteins capable of mediating the effects of Pi within the cell has to be conceptualized.

What are the signaling pathways involved?

The specific intracellular signaling pathways activated by Pi are poorly elucidated. Beck et al. have found that Pi selectively activates the extracellular signal regulated kinase (ERK1/2) signaling pathway to mediate its effect on osteopontin expression in MC3T3 cells [83, 126]. Increased extracellular Pi concentration caused a rapid and biphasic phosphorylation of ERK1/2 but did not activate the other mitogen-activated protein kinase (MAPK) signaling proteins, p38 or the c-jun N-terminal kinase (JNK). Other pathways including PI3-kinase, protein kinase A, and protein kinase G were not involved. These observations are in agreement with a study demonstrating an increase in ERK1/2 phosphorylation, but not p38 or JNK, in response to bisphosphonates that is further induced by the addition of 3 mM Pi [127]. In a recent study, we obtained similar results and confirmed that Pi effect induces the specific activation of the ERK1/2 signaling pathway in osteoblasts [83]. We have shown that this pathway is required for the effect of Pi on the expression of matrix gla protein (MGP), a known inhibitor of calcification. We have also obtained similar results in ATDC5 and primary chondrocytes. Most importantly, we have shown that, as in osteoblasts, Pi stimulates the ERK1/2 pathway in chondrocytes to mediate its effects on MGP expression, suggesting a negative feedback loop for the control of mineralization induced by Pi in cartilage [128]. Although further investigation is needed to fully understand the mechanisms by which an increased intracellular Pi might regulate gene expression and ultimately cell function, this data collectively demonstrates that several specific signaling pathways are involved in the effects of Pi in the regulation of gene expression.

Are transcription factors involved?

Very few data are available on the transcription factors that might be involved in the gene regulation by extracellular Pi, and most of the studies have been conducted in bone-forming cells. In osteoblastic MC3T3-E1, chondrocytic ATDC5 and osteocytic MLO-Y4 cell lines, Runx2 was shown to be exported from the nucleus to the cytoplasm in response to extracellular Pi, but its expression was not regulated at the mRNA level by Pi treatment [129]. Accordingly, osteocalcin gene expression was repressed by the lack of Runx2 in the nucleus after Pi treatment [129], which appears contradictory to the physiological expression of osteocalcin occurring during MC3T3-E1 differentiation [73]. Runx2 was also suggested to mediate the effects of Pi in an in vitro model of vascular calcification [110]. In this study, treatment with high level Pi enhanced the expression of the osteoblastic differentiation markers osteocalcin and Runx2 by vascular smooth muscle cells (VSMCs). The ability of Pi to regulate gene transcription and cellular function represents a potentially novel extracellular signaling mechanism. Accordingly, Beck et al. [73] have identified a discrete set of genes increased by Pi in MC3T3-E1 cells including the transcription factor Nrf2 which was shown to be regulated at the transcriptional level. These authors further compared transcriptome and proteome datasets with the goal of identifying a number of candidate proteins that are post-transcriptionally regulated by elevated Pi [69]. From their data, Fra-1, a member of the AP-1 family of transcription factors, seems to be such a candidate as it is not regulated at the mRNA level but its protein level increases in response to Pi. Moreover, the use of inhibitors of transcription and translation did not affect the effects of Pi on Fra-1 expression, thus suggesting a post translational regulation of Fra-1 in response to Pi [69]. Accordingly, previous studies have shown that phosphorylation of Fra-1 by the MAPK ERK1/2 resulted in Fra-1 protection from proteosomal degradation [130] and subsequent post-translational stabilization [131]. Interestingly, in a recent report, we have observed that the regulation of MGP expression by Pi through the ERK1/2 signaling pathway involves the stimulation of the DNA binding activity of Fra-1 to specific sequences in the MGP promoter [83].

Does Pi have to enter the cell?

It is commonly believed that Pi must enter the cell to affect cell functions, hence involving Pi transporters at the plasma membrane. To investigate this issue, most studies have used phosphonoformic acid (PFA), a well-documented competitive inhibitor of Na^+-dependent Pi transport mediated by SLC34 (Npt) Pi transporters [132, 133]. Limited data are available on the effect of PFA on SLC20 (PiT) proteins. Bai et al. [134] expressed mouse PiT2 in *Xenopus* oocytes and documented a 40% decrease in Pi uptake by using a 50-fold excess of PFA (5 mM). However, when used at concentrations which inhibit renal SLC34 transporters, Ravera et al. [135] found no effect of PFA on Pi uptake in any of the PiT isoforms studied, confirming previous observations of Villa-Bellosta et al. [136]. Using Pi uptake and electrophysiology on PiT mRNA-injected *Xenopus* oocytes, Ravera et al. [135] unequivocally showed that PFA, in contrast to its action as a competitive inhibitor of SLC34 Pi transporters, does not inhibit Pi transport mediated by PiT. Confirming these data, a recent study from Villa-Bellosta et al. [137] showed, by using Pi uptake assays, that PFA is a very poor inhibitor of Pi uptake mediated by rat PiT1 and PiT2 both in oocytes and in native rat VSMCs. The authors conclude that, in contrast to its action as a competitive inhibitor of SLC34 Na^+-Pi cotransporters, PFA does not inhibit Pi transport mediated

by PiT. PiT transporters, which prefers $H_2PO_4^-$ as a substrate, and SLC34, which prefers HPO_4^{2-}, transport most efficiently in the acidic and alkaline ranges, respectively [62]. This difference in preferred Pi species may explain why PFA inhibits Pi transport by SLC34, but not by SLC20.

It has been reported that PFA blocks Pi-induced calcification in smooth muscle cells [110, 112] as well as matrix calcification in osteoblast-like cells [138] and osteoblast subcultures [65]. As PFA does not block PiT1-mediated Pi transport, the effect of PFA in inhibiting calcification is probably more related to the ability of phosphonates and bisphosphonates (with which PFA shares a similar structure [139]) to inhibit the formation of calcium phosphate crystals, than to inhibition of Pi transport through PiT1 [137]. The studies aimed at illustrating the role of extracellular Pi in signaling processes were conducted in fibroblasts, osteoblasts, chondrocytes and VSMCs in which only SLC20 Pi transporters (and not SLC34) were expressed. Most of these studies used PFA as an inhibitor of Pi transport and drew conclusions based on this property that now need to be re-considered in view of the lack of PFA effect on PiT-mediated Pi transport. As a consequence, and based on the actual available literature, it is not possible to get a clear answer as to whether Pi needs to enter the cells to mediate its signaling effect, and whether PiT proteins are implicated in this process. We and others have recently generated mice carrying conditional and null alleles of the Pi transporter PiT1 [140, 141]. Since there is no specific PiT1 inhibitor, such animal models will be invaluable tools to investigate the role of SLC20 Pi transporters in mediating the effects of Pi on cell functions.

Phosphate sensing in mammals

Although long-term Pi homeostasis is controlled by a variety of hormones, peptides and small molecules that regulate the efficiency of Pi absorption in the intestine and Pi excretion in the renal tubule, recent data suggest the existence of a Pi-sensing mechanism on the surface of cells that is able to detect changes in Pi concentration [142].

In vitro, renal and intestinal cells are able to respond to changes in Pi concentration in the extracellular medium. A low-Pi medium increased Pi uptake, whereas a high-Pi medium decreased Pi uptake in renal [143] and intestinal cells [144, 145]. Osteoblast and bone-marrow stromal cells respond to changes in external Pi concentrations by altering the expression, localization or secretion of growth factors, transcription factors or enzymes [129, 144, 146, 147].

In vivo, a chronic elevation of Pi concentration in the serum, which can result from genetic syndromes or renal insufficiency, is associated with severe defects in humans, such as vascular calcification and secondary hyperparathyroidism [63, 148]. Genetic disorders due to mutations in GALNT3 [149], FGF23 [150] or Klotho [151] genes cause familial tumoral calcinosis, a rare autosomal recessive disorder manifesting with severe hyperphosphatemia and widespread ectopic calcifications [152]. Likewise, targeted deletion of either FGF23 or Klotho in mice leads to hyperphosphatemia and vascular calcification [153–155]. Hyperphosphatemia and vascular calcification are also prevalent in patients with end-stage renal disease (ESRD) [148]. Elevated serum Pi concentration (greater than 2.1 mM) was shown to be positively correlated with mortality in ESRD patients [156], and even small elevations in Pi concentration in the high-normal range (1.1–1.45 mM) have been correlated with increased risk of cardiovascular mortality in ESRD patients and in the general population [157, 158]. A recent review of the literature shows that there is a graded independent association between serum Pi levels and mortality, mainly cardiovascular events, and the progression of renal disease [20]. Lowering serum Pi levels with a non-calcium-containing Pi binder slows the progression of vascular calcification in ESRD patients [159, 160].

The hyperphosphatemia seen in ESRD patients will also eventually lead to secondary hyperparathyroidism with attendant increased PTH synthesis, secretion, and parathyroid cell proliferation. Secondary hyperparathyroidism can be controlled by a Pi-restricted diet, as was shown in dogs [161, 162]. Importantly, these studies showed that dietary Pi restriction corrected the secondary hyperparathyroidism independently from changes in serum calcium and $1\alpha,25(OH)_2D_3$ levels. In addition, in vitro studies have shown that the effect of serum Pi on the parathyroid was direct. Although high Pi concentration stimulated PTH secretion, this direct effect required the maintenance of tissue architecture [123, 124, 163]. The work of Silver and colleagues suggests that the effect of Pi 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 [164]. Two of the PTH mRNA-binding proteins, acting as stabilizing or degrading factors, were identified as AU-rich binding factor (AUF1) and upstream of N-ras (Unr). Their regulated binding to the PTH cis-acting element located on its 3′UTR determine the PTH mRNA half-life [164].

The existence of a Pi-sensing mechanism in the parathyroid gland was suggested in the experiments of Martin et al. [165]. These investigators showed that, when uremic rats were administered a low-Pi diet by force-feeding, this induced a decrease in PTH and serum Pi concentration within 15 min. The infusion of Pi into the duodenum or the circulation led to a rapid increase in PTH, without change in Pi or calcium concentration, suggesting that the parathyroid gland is capable of sensing Pi in the absence of

changes of serum calcium. In addition, Berndt et al. [166] recently demonstrated that Pi administration into the duodenum of intact or parathyroidectomized rats was associated with very rapid change in the fractional excretion of phosphorus in the kidney, with no change in PTH, FGF-23 or s-FRP-4 concentration. Thus, the intestine is able to sense an increase in luminal Pi concentration and signal to the kidney [166]. Enteric-renal signaling mechanisms also exist in the case of other ions, including sodium [167, 168], potassium [169] and calcium [170].

The molecular nature of the Pi sensor in mammals still remains to be identified. Bacteria and yeasts both have well-developed Pi sensing mechanisms [171–173]. In *Escherichia coli*, the phosphate transporter PstS and other periplasmic proteins (PstC, Pst and PstB/PhoU) detect low external Pi concentration and increase the efficiency of Pi retention in the bacteria [172]. In *Saccharomyces cerevisiae*, a low external Pi concentration inactivates the Pho80–Pho85 complex by the cyclin-dependent kinase inhibitor Pho81, resulting in the activation of the transcription factor Pho4 and subsequent induction of the Pi transporter Pho84, along with other *PHO* genes [173]. Recent data now identify Pho84 as the essential component of the Pi-sensing system, mediating the rapid activation of PKA following Pi restriction [174]. Pho84 was also identified as a Pi transceptor, by mediating PKA activation following Pi triggering, without transporting Pi [175]. Although the response to Pi variations in the environment has been well characterized in unicellular organisms, no equivalent Pi-sensing system has been found to date in mammals. In mammals, extracellular Pi regulates the activity of specific Na^+-dependent Pi transporters and modulates the cell cycle, as in unicellular organisms [176–178]. In parathyroid glands, PTH secretion is regulated by extracellular Pi [123, 124]. This effect of Pi can be mediated by specific molecules in the parathyroid cell membrane or by metabolic signals associated with increased cellular Pi concentrations. One such mediator may be a Na^+-Pi cotransporter. The work of Miyamoto and colleagues identified PiT1 as being the only Na^+-Pi cotransporter present in the parathyroids and being regulated by changes in extracellular Pi concentrations. As such, they proposed that PiT1 could play a role in sensing Pi in parathyroid cells, although formal demonstration is still lacking [179–181]. On the other hand, Salaün et al. proposed that PiT2 could serve as a Pi sensor. They showed that PiT2 transport-deficient mutants were still able to "sense" Pi by retaining their oligomerization properties in response to high extracellular Pi concentrations [182]. It is not known whether a Pi-sensing mechanism in mammals would involve a local cell-to-cell regulation or a central or hormonal control. Since PiT1 and PiT2 are expressed ubiquitously, their putative role in sensing extracellular Pi may relate to a cell-to-cell-based regulation, enabling a fine tuning depending on individual Pi cellular needs. Of course, the involvement of PiT transporters and their mode of action remains entirely a hypothesis, and physiological evidence is lacking.

Conclusion

Considerable progress has been made over the past decade in our understanding of the mechanisms of Pi homeostasis and skeleton mineralization. Still, very little is known about the initial events involving the detection of a change in the Pi serum concentration and the subsequent appropriate regulation cascade. Future studies should be directed at uncovering the nature and identity of the Pi-sensing mechanism. This knowledge is essential to the proper understanding of the global regulation of Pi homeostasis and would most certainly change the current concepts of this field. It is now clear that Pi represents a signal molecule capable of modulating the expression or regulation of multiple factors necessary for diverse biological processes. Increased knowledge of Pi biology and mode of action is essential for the development of therapeutic strategies that may prevent the consequences of hyperphosphataemia in patients with chronic renal failure.

Acknowledgments The authors gratefully acknowledge Joanna Ashton-Chess for critical reading of the manuscript. This work was partially supported by grants from INSERM, ANR (PHYSIOPATH ANR-07-PHYSIO-017-01), La Fondation pour la Recherche Médicale (AAP "Vieillissement ostéoarticulaire"), BIOREGOS and Région des Pays de la Loire. Marion Julien received a fellowship from INSERM/Région des Pays de la Loire and La Fondation pour la Recherche Médicale, Solmaz Khoshniat from the French Ministry of Research and la Fondation pour la Recherche Médicale and Annabelle Bourgine from INSERM/Région des Pays de la Loire.

References

1. Hansen NM, Felix R, Bisaz S, Fleisch H (1976) Aggregation of hydroxyapatite crystals. Biochim Biophys Acta 451:549–559
2. Crook M, Swaminathan R (1996) Disorders of plasma phosphate and indications for its measurement. Ann Clin Biochem 33(Pt 5):376–396
3. Kornberg A (1979) The enzymatic replication of DNA. CRC Crit Rev Biochem 7:23–43
4. Rothfield L, Finkelstein A (1968) Membrane biochemistry. Annu Rev Biochem 37:463–496
5. Lehninger AL, Wadkins CL (1962) Oxidative phosphorylation. Annu Rev Biochem 31:47–78
6. Hubbard SR, Till JH (2000) Protein tyrosine kinase structure and function. Annu Rev Biochem 69:373–398
7. Krebs EG, Beavo JA (1979) Phosphorylation-dephosphorylation of enzymes. Annu Rev Biochem 48:923–959
8. Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. Annu Rev Biochem 69:183–215

9. Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. Annu Rev Biochem 67:481–507
10. Bessman SP, Carpenter CL (1985) The creatine–creatine phosphate energy shuttle. Annu Rev Biochem 54:831–862
11. Prié D, Beck L, Friedlander G, Silve C (2004) Sodium-phosphate cotransporters, nephrolithiasis and bone demineralization. Curr Opin Nephrol Hypertens 13:675–681
12. Knochel JP, Barcenas C, Cotton JR, Fuller TJ, Haller R, Carter NW (1978) Hypophosphatemia and rhabdomyolysis. J Clin Invest 62:1240–1246
13. Knochel JP (1977) The pathophysiology and clinical characteristics of severe hypophosphatemia. Arch Intern Med 137:203–220
14. Berndt T, Kumar R (2009) Novel mechanisms in the regulation of phosphorus homeostasis. Physiology (Bethesda) 24:17–25
15. Hruska K, Slatopolsky E (1997) Disorders of phosphorus, calcium, and magnesium metabolism. In: Schrier R, Gottschalk C (eds) Diseases of the kidney. Little and Brown, Boston
16. Rutecki GW, Cugino A, Jarjoura D, Kilner JF, Whittier FC (1997) Nephrologists' subjective attitudes towards end-of-life issues and the conduct of terminal care. Clin Nephrol 48:173–180
17. Weisinger JR, Bellorin-Font E (1998) Magnesium and phosphorus. Lancet 352:391–396
18. Shiber JR, Mattu A (2002) Serum phosphate abnormalities in the emergency department. J Emerg Med 23:395–400
19. Hruska KA, Mathew S, Lund RJ, Memon I, Saab G (2009) The pathogenesis of vascular calcification in the chronic kidney disease mineral bone disorder: the links between bone and the vasculature. Semin Nephrol 29:156–165
20. Kanbay M, Goldsmith D, Akcay A, Covic A (2009) Phosphate - the silent stealthy cardiorenal culprit in all stages of chronic kidney disease: a systematic review. Blood Purif 27:220–230
21. Hruska KA, Mathew S, Lund R, Qiu P, Pratt R (2008) Hyperphosphatemia of chronic kidney disease. Kidney Int 74:148–157
22. Pettifor JM (2008) What's new in hypophosphataemic rickets? Eur J Pediatr 167:493–499
23. Murer H, Hernando N, Forster I, Biber J (2000) Proximal tubular phosphate reabsorption: molecular mechanisms. Physiol Rev 80:1373–1409
24. Tanaka Y, Deluca HF (1973) The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. Arch Biochem Biophys 154:566–574
25. DeLuca HF (2004) Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 80:1689S–1696S
26. Berndt TJ, Schiavi S, Kumar R (2005) "Phosphatonins" and the regulation of phosphorus homeostasis. Am J Physiol Renal Physiol 289:F1170–F1182
27. Berndt T, Knox F (1992) Renal regulation of phosphate excretion. In: Seldin D, Giebish G (eds) The kidney: physiology and pathophysiology. Raven Press, New York, pp 2511–2532
28. Chase LR, Aurbach GD (1967) Parathyroid function and the renal excretion of 3'5'-adenylic acid. Proc Natl Acad Sci USA 58:518–525
29. Muff R, Fischer JA, Biber J, Murer H (1992) Parathyroid hormone receptors in control of proximal tubule function. Annu Rev Physiol 54:67–79
30. Kiela PR, Ghishan FK (2009) Recent advances in the renal-skeletal-gut axis that controls phosphate homeostasis. Lab Invest 89:7–14
31. Strom TM, Jüppner H (2008) PHEX, FGF23, DMP1 and beyond. Curr Opin Nephrol Hypertens 17:357–362
32. Johnston J, Ramos-Valdes Y, Stanton LA, Ladhani S, Beier F, Dimattia GE (2010) Human stanniocalcin-1 or -2 expressed in mice reduces bone size and severely inhibits cranial intramembranous bone growth. Transgenic Res. doi:10.1007/s11248-010-9376-7
33. Jüppner H (2007) Novel regulators of phosphate homeostasis and bone metabolism. Therapeutic apheresis and dialysis: official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis. Jpn Soc Dial Ther 11(Suppl 1):S3–S22
34. Shaikh A, Berndt T, Kumar R (2008) Regulation of phosphate homeostasis by the phosphatonins and other novel mediators. Pediatr Nephrol 23:1203–1210
35. Renkema K, Alexander RT, Bindels R, Hoenderop J (2008) Calcium and phosphate homeostasis: concerted interplay of new regulators. Ann Med 40:82–91
36. Prie D, Friedlander G (2010) Genetic disorders of renal phosphate transport. N Engl J Med 362:2399–2409
37. Werner A, Kinne RK (2001) Evolution of the Na-P(i) cotransport systems. Am J Physiol Regul Integr Comp Physiol 280:R301–R312
38. Beck L, Silve C (2001) Molecular aspects of phosphate homeostasis in mammals. Nephrologie 22:149–159
39. Prie D, Urena Torres P, Friedlander G (2009) Latest findings in phosphate homeostasis. Kidney Int 75:882–889
40. Singer SJ (1990) The structure and insertion of integral proteins in membranes. Annu Rev Cell Biol 6:247–296
41. Chou KC, Elrod DW (1999) Prediction of membrane protein types and subcellular locations. Proteins 34:137–153
42. Chou KC, Cai YD (2005) Using GO-PseAA predictor to identify membrane proteins and their types. Biochem Biophys Res Commun 327:845–847
43. Werner A, Moore ML, Mantei N, Biber J, Semenza G, Murer H (1991) Cloning and expression of cDNA for a Na/Pi cotransport system of kidney cortex. Proc Natl Acad Sci USA 88:9608–9612
44. Busch AE, Schuster A, Waldegger S, Wagner CA, Zempel G, Broer S, Biber J, Murer H, Lang F (1996) Expression of a renal type I sodium/phosphate transporter (NaPi-1) induces a conductance in *Xenopus oocytes* permeable for organic and inorganic anions. Proc Natl Acad Sci USA 93:5347–5351
45. Yabuuchi H, Tamai I, Morita K, Kouda T, Miyamoto K, Takeda E, Tsuji A (1998) Hepatic sinusoidal membrane transport of anionic drugs mediated by anion transporter Npt1. J Pharmacol Exp Ther 286:1391–1396
46. Uchino H, Tamai I, Yamashita K, Minemoto Y, Sai Y, Yabuuchi H, Miyamoto K, Takeda E, Tsuji A (2000) p-aminohippuric acid transport at renal apical membrane mediated by human inorganic phosphate transporter NPT1. Biochem Biophys Res Commun 270:254–259
47. Uchino H, Tamai I, Yabuuchi H, China K, Miyamoto K, Takeda E, Tsuji A (2000) Faropenem transport across the renal epithelial luminal membrane via inorganic phosphate transporter Npt1. Antimicrob Agents Chemother 44:574–577
48. Cheret C, Doyen A, Yaniv M, Pontoglio M (2002) Hepatocyte nuclear factor 1 alpha controls renal expression of the Npt1–Npt4 anionic transporter locus. J Mol Biol 322:929–941
49. Reimer RJ, Edwards RH (2004) Organic anion transport is the primary function of the SLC17/type I phosphate transporter family. Pflugers Arch 447:629–635
50. Urano W, Taniguchi A, Anzai N, Inoue E, Kanai Y, Yamanaka M, Endou H, Kamatani N, Yamanaka H (2010) Sodium-dependent phosphate cotransporter type 1 sequence polymorphisms in male patients with gout. Ann Rheum Dis 69:1232–1234
51. Murer H, Hernando N, Forster I, Biber J (2003) Regulation of Na/Pi transporter in the proximal tubule. Annu Rev Physiol 65:531–542
52. 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. Proc Natl Acad Sci USA 95:5372–5377

53. 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. Proc Natl Acad Sci USA 95:14564–14569
54. Sabbagh Y, O'Brien SP, Song W, Boulanger JH, Stockmann A, Arbeeny C, Schiavi SC (2009) Intestinal npt2b plays a major role in phosphate absorption and homeostasis. J Am Soc Nephrol 20:2348–2358
55. Segawa H, Onitsuka A, Furutani J, Kaneko I, Aranami F, Matsumoto N, Tomoe Y, Kuwahata M, Ito M, Matsumoto M, Li M, Amizuka N, Miyamoto K (2009) Npt2a and Npt2c in mice play distinct and synergistic roles in inorganic phosphate metabolism and skeletal development. Am J Physiol Renal Physiol 297:F671–F678
56. Segawa H, Kaneko I, Takahashi A, Kuwahata M, Ito M, Ohkido I, Tatsumi S, Miyamoto K (2002) Growth-related renal type II Na/Pi cotransporter. J Biol Chem 277:19665–19672
57. Segawa H, Onitsuka A, Kuwahata M, Hanabusa E, Furutani J, Kaneko I, Tomoe Y, Aranami F, Matsumoto N, Ito M, Matsumoto M, Li M, Amizuka N, Miyamoto K (2009) Type IIc sodium-dependent phosphate transporter regulates calcium metabolism. J Am Soc Nephrol 20:104–113
58. Collins JF, Bai L, Ghishan FK (2004) The SLC20 family of proteins: dual functions as sodium-phosphate cotransporters and viral receptors. Pflugers Arch 447:647–652
59. 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. Proc Natl Acad Sci USA 91:78–82
60. Miller DG, Miller AD (1994) A family of retroviruses that utilize related phosphate transporters for cell entry. J Virol 68:8270–8276
61. 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. Proc Natl Acad Sci USA 91:7071–7075
62. Virkki LV, Biber J, Murer H, Forster IC (2007) Phosphate transporters: a tale of two solute carrier families. Am J Physiol Renal Physiol 293:F643–F654
63. Giachelli CM (2009) The emerging role of phosphate in vascular calcification. Kidney Int 75:890–897
64. Li X, Giachelli CM (2007) Sodium-dependent phosphate cotransporters and vascular calcification. Curr Opin Nephrol Hypertens 16:325–328
65. Yoshiko Y, Candeliere GA, Maeda N, Aubin JE (2007) Osteoblast autonomous Pi regulation via Pit1 plays a role in bone mineralization. Mol Cell Biol 27:4465–4474
66. Villa-Bellosta R, Ravera S, Sorribas V, Stange G, Levi M, Murer H, Biber J, Forster IC (2009) The Na^+-Pi cotransporter PiT-2 (SLC20A2) is expressed in the apical membrane of rat renal proximal tubules and regulated by dietary Pi. Am J Physiol Renal Physiol 296:F691–F699
67. Bellows CG, Heersche JN, Aubin JE (1992) Inorganic phosphate added exogenously or released from beta-glycerophosphate initiates mineralization of osteoid nodules in vitro. Bone Miner 17:15–29
68. Kumar R, Riggs B (1980) Pathologic bone physiology. In: Urist M (ed) Fundamental and clinical bone physiology. Lippincott, Philadelphia, pp 394–406
69. Conrads K, Yi M, Simpson K, Lucas D, Camalier C, Yu L, Veenstra T, Stephens R, Conrads T, Beck G (2005) A combined proteome and microarray investigation of inorganic phosphate-induced pre-osteoblast cells. Mol Cell Proteomics 4:1284–1296
70. Naviglio S, Spina A, Chiosi E, Fusco A, Illiano F, Pagano M, Romano M, Senatore G, Sorrentino A, Sorvillo L, Illiano G (2006) Inorganic phosphate inhibits growth of human osteosarcoma U2OS cells via adenylate cyclase/cAMP pathway. J Cell Biochem 98:1584–1596
71. Quarles LD, Yohay DA, Lever LW, Caton R, Wenstrup RJ (1992) Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: an in vitro model of osteoblast development. J Bone Miner Res 7:683–692
72. Conrads K, Yu L, Lucas D, Zhou M, Chan K, Simpson K, Schaefer C, Issaq H, Veenstra T, Beck G, Conrads T (2004) Quantitative proteomic analysis of inorganic phosphate-induced murine MC3T3-E1 osteoblast cells. Electrophoresis 25:1342–1352
73. Beck G, Moran E, Knecht N (2003) Inorganic phosphate regulates multiple genes during osteoblast differentiation, including Nrf2. Exp Cell Res 288:288–300
74. Kanatani M, Sugimoto T, Kano J, Chihara K (2002) IGF-I mediates the stimulatory effect of high phosphate concentration on osteoblastic cell proliferation. J Cell Physiol 190:306–312
75. Baylink DJ, Finkelman RD, Mohan S (1993) Growth factors to stimulate bone formation. J Bone Miner Res 8(Suppl 2):S565–S572
76. Sugimoto T, Kanatani M, Kano J, Kobayashi T, Yamaguchi T, Fukase M, Chihara K (1994) IGF-I mediates the stimulatory effect of high calcium concentration on osteoblastic cell proliferation. Am J Physiol 266:E709–E716
77. Schmid C, Keller C, Schlapfer I, Veldman C, Zapf J (1998) Calcium and insulin-like growth factor I stimulation of sodium-dependent phosphate transport and proliferation of cultured rat osteoblasts. Biochem Biophys Res Commun 245:220–225
78. Beck GR Jr, Sullivan EC, Moran E, Zerler B (1998) Relationship between alkaline phosphatase levels, osteopontin expression, and mineralization in differentiating MC3T3-E1 osteoblasts. J Cell Biochem 68:269–280
79. Beck G, Zerler B, Moran E (2000) Phosphate is a specific signal for induction of osteopontin gene expression. Proc Natl Acad Sci USA 97:8352–8357
80. Adams CS, Mansfield K, Perlot RL, Shapiro IM (2001) Matrix regulation of skeletal cell apoptosis. Role of calcium and phosphate ions. J Biol Chem 276:20316–20322
81. Meleti Z, Shapiro IM, Adams CS (2000) Inorganic phosphate induces apoptosis of osteoblast-like cells in culture. Bone 27:359–366
82. Wittrant Y, Bourgine A, Khoshniat S, Alliot-Licht B, Masson M, Gatius M, Rouillon T, Weiss P, Beck L, Guicheux J (2009) Inorganic phosphate regulates Glvr-1 and -2 expression: role of calcium and ERK1/2. Biochem Biophys Res Commun 381:259–263
83. Julien M, Khoshniat S, Lacreusette A, Gatius M, Bozec A, Wagner EF, Wittrant Y, Masson M, Weiss P, Beck L, Magne D, Guicheux J (2009) Phosphate-dependent regulation of MGP in osteoblasts: role of ERK1/2 and Fra-1. J Bone Miner Res 24:1856–1868
84. Teitelbaum SL (2000) Bone resorption by osteoclasts. Science 289:1504–1508
85. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L (1997) A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390:175–179
86. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176

87. Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett FS 3rd, Frankel WN, Lee SY, Choi Y (1997) TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 272:25190–25194
88. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602
89. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
90. Baylink D, Wergedal J, Stauffer M (1971) Formation, mineralization, and resorption of bone in hypophosphatemic rats. J Clin Invest 50:2519–2530
91. Bruin WJ, Baylink DJ, Wergedal JE (1975) Acute inhibition of mineralization and stimulation of bone resorption mediated by hypophosphatemia. Endocrinology 96:394–399
92. Thompson ER, Baylink DJ, Wergedal JE (1975) Increases in number and size of osteoclasts in response to calcium or phosphorus deficiency in the rat. Endocrinology 97:283–289
93. Yates AJ, Oreffo RO, Mayor K, Mundy GR (1991) Inhibition of bone resorption by inorganic phosphate is mediated by both reduced osteoclast formation and decreased activity of mature osteoclasts. J Bone Miner Res 6:473–478
94. Kanatani M, Sugimoto T, Kano J, Kanzawa M, Chihara K (2003) Effect of high phosphate concentration on osteoclast differentiation as well as bone-resorbing activity. J Cell Physiol 196:180–189
95. Mozar A, Haren N, Chasseraud M, Louvet L, Maziere C, Wattel A, Mentaverri R, Morliere P, Kamel S, Brazier M, Maziere JC, Massy ZA (2008) High extracellular inorganic phosphate concentration inhibits RANK–RANKL signaling in osteoclast-like cells. J Cell Physiol 215:47–54
96. Bingham PJ, Raisz LG (1974) Bone growth in organ culture: effects of phosphate and other nutrients on bone and cartilage. Calcif Tissue Res 14:31–48
97. Kakuta S, Golub EE, Shapiro IM (1985) Morphochemical analysis of phosphorus pools in calcifying cartilage. Calcif Tissue Int 37:293–299
98. Wang D, Canaff L, Davidson D, Corluka A, Liu H, Hendy GN, Henderson JE (2001) Alterations in the sensing and transport of phosphate and calcium by differentiating chondrocytes. J Biol Chem 276:33995–34005
99. Fujita T, Meguro T, Izumo N, Yasutomi C, Fukuyama R, Nakamuta H, Koida M (2001) Phosphate stimulates differentiation and mineralization of the chondroprogenitor clone ATDC5. Jpn J Pharmacol 85:278–281
100. Magne D, Bluteau G, Faucheux C, Palmer G, Vignes-Colombeix C, Pilet P, Rouillon T, Caverzasio J, Weiss P, Daculsi G, Guicheux J (2003) Phosphate is a specific signal for ATDC5 chondrocyte maturation and apoptosis-associated mineralization: possible implication of apoptosis in the regulation of endochondral ossification. J Bone Miner Res 18:1430–1442
101. Cecil DL, Rose DM, Terkeltaub R, Liu-Bryan R (2005) Role of interleukin-8 in PiT-1 expression and CXCR1-mediated inorganic phosphate uptake in chondrocytes. Arthritis Rheum 52:144–154
102. Magne D, Julien M, Vinatier C, Merhi-Soussi F, Weiss P, Guicheux J (2005) Cartilage formation in growth plate and arteries: from physiology to pathology. Bioessays 27:708–716
103. Anderson HC (2003) Matrix vesicles and calcification. Curr Rheumatol Rep 5:222–226
104. Alini M, Carey D, Hirata S, Grynpas MD, Pidoux I, Poole AR (1994) Cellular and matrix changes before and at the time of calcification in the growth plate studied in vitro: arrest of type X collagen synthesis and net loss of collagen when calcification is initiated. J Bone Miner Res 9:1077–1087
105. Mansfield K, Rajpurohit R, Shapiro IM (1999) Extracellular phosphate ions cause apoptosis of terminally differentiated epiphyseal chondrocytes. J Cell Physiol 179:276–286
106. Mansfield K, Teixeira CC, Adams CS, Shapiro IM (2001) Phosphate ions mediate chondrocyte apoptosis through a plasma membrane transporter mechanism. Bone 28:1–8
107. Teixeira CC, Mansfield K, Hertkorn C, Ischiropoulos H, Shapiro IM (2001) Phosphate-induced chondrocyte apoptosis is linked to nitric oxide generation. Am J Physiol Cell Physiol 281:C833–C839
108. Sabbagh Y, Carpenter TO, Demay MB (2005) Hypophosphatemia leads to rickets by impairing caspase-mediated apoptosis of hypertrophic chondrocytes. Proc Natl Acad Sci USA 102:9637–9642
109. Mansfield K, Pucci B, Adams CS, Shapiro IM (2003) Induction of apoptosis in skeletal tissues: phosphate-mediated chick chondrocyte apoptosis is calcium dependent. Calcif Tissue Int 73:161–172
110. 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. Circ Res 87:E10–E17
111. Giachelli CM, Jono S, Shioi A, Nishizawa Y, Mori K, Morii H (2001) Vascular calcification and inorganic phosphate. Am J Kidney Dis 38:S34–S37
112. Li X, Yang HY, Giachelli CM (2006) Role of the sodium-dependent phosphate cotransporter, Pit-1, in vascular smooth muscle cell calcification. Circ Res 98:905–912
113. 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. J Biol Chem 274:28256–28263
114. Biber J, Forgo J, Murer H (1988) Modulation of Na + -Pi cotransport in opossum kidney cells by extracellular phosphate. Am J Physiol 255:C155–C161
115. Jin H, Hwang SK, Yu K, Anderson HK, Lee YS, Lee KH, Prats AC, Morello D, Beck GR Jr, Cho MH (2006) A high inorganic phosphate diet perturbs brain growth, alters Akt-ERK signaling, and results in changes in cap-dependent translation. Toxicol Sci 90:221–229
116. Jin H, Hwang SK, Kwon JT, Lee YS, An GH, Lee KH, Prats AC, Morello D, Beck GR Jr, Cho MH (2008) Low dietary inorganic phosphate affects the brain by controlling apoptosis, cell cycle and protein translation. J Nutr Biochem 19:16–25
117. Xu CX, Jin H, Lim HT, Kim JE, Shin JY, Lee ES, Chung YS, Lee YS, Beck G Jr, Lee KH, Cho MH (2008) High dietary inorganic phosphate enhances cap-dependent protein translation, cell-cycle progression, and angiogenesis in the livers of young mice. Am J Physiol Gastrointest Liver Physiol 295:G654–G663
118. Chang SH, Yu KN, Lee YS, An GH, Beck GR Jr, Colburn NH, Lee KH, Cho MH (2006) Elevated inorganic phosphate stimulates Akt-ERK1/2-Mnk1 signaling in human lung cells. Am J Respir Cell Mol Biol 35:528–539
119. Jin H, Chang SH, Xu CX, Shin JY, Chung YS, Park SJ, Lee YS, An GH, Lee KH, Cho MH (2007) High dietary inorganic phosphate affects lung through altering protein translation, cell

cycle, and angiogenesis in developing mice. Toxicol Sci 100:215–223
120. Jin H, Xu CX, Lim HT, Park SJ, Shin JY, Chung YS, Park SC, Chang SH, Youn HJ, Lee KH, Lee YS, Ha YC, Chae CH, Beck GR Jr, Cho MH (2009) High dietary inorganic phosphate increases lung tumorigenesis and alters Akt signaling. Am J Respir Crit Care Med 179:59–68
121. Xu CX, Jin H, Chung YS, Shin JY, Hwang SK, Kwon JT, Park SJ, Lee ES, Minai-Tehrani A, Chang SH, Woo MA, Noh MS, An GH, Lee KH, Cho MH (2009) Low dietary inorganic phosphate affects the lung growth of developing mice. J Vet Sci 10:105–113
122. Glinn M, Ni B, Irwin RP, Kelley SW, Lin SZ, Paul SM (1998) Inorganic Pi increases neuronal survival in the acute early phase following excitotoxic/oxidative insults. J Neurochem 70:1850–1858
123. 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. J Clin Invest 97:2534–2540
124. 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. J Bone Miner Res 11:970–976
125. Silver J, Kilav R, Naveh-Many T (2002) Mechanisms of secondary hyperparathyroidism. Am J Physiol Renal Physiol 283:F367–F376
126. Beck G, Knecht N (2003) Osteopontin regulation by inorganic phosphate is ERK1/2-, protein kinase C-, and proteasome-dependent. J Biol Chem 278:41921–41929
127. Fujita T, Izumo N, Fukuyama R, Meguro T, Yasutomi C, Nakamuta H, Koida M (2001) Incadronate and etidronate accelerate phosphate-primed mineralization of MC4 cells via ERK1/2-Cbfa1 signaling pathway in a Ras-independent manner: further involvement of mevalonate-pathway blockade for incadronate. Jpn J Pharmacol 86:86–96
128. Julien M, Magne D, Masson M, Rolli-Derkinderen M, Chassande O, Cario-Toumaniantz C, Cherel Y, Weiss P, Guicheux J (2007) Phosphate stimulates matrix Gla protein expression in chondrocytes through the extracellular signal regulated kinase signaling pathway. Endocrinology 148:530–537
129. Fujita T, Izumo N, Fukuyama R, Meguro T, Nakamuta H, Kohno T, Koida M (2001) Phosphate provides an extracellular signal that drives nuclear export of Runx2/Cbfa1 in bone cells. Biochem Biophys Res Commun 280:348–352
130. Vial E, Marshall CJ (2003) Elevated ERK-MAP kinase activity protects the FOS family member FRA-1 against proteasomal degradation in colon carcinoma cells. J Cell Sci 116:4957–4963
131. Casalino L, De Cesare D, Verde P (2003) Accumulation of Fra-1 in ras-transformed cells depends on both transcriptional autoregulation and MEK-dependent posttranslational stabilization. Mol Cell Biol 23:4401–4415
132. Busch AE, Wagner CA, Schuster A, Waldegger S, Biber J, Murer H, Lang F (1995) Properties of electrogenic Pi transport by a human renal brush border Na^+/Pi transporter. J Am Soc Nephrol 6:1547–1551
133. Szczepanska-Konkel M, Yusufi AN, VanScoy M, Webster SK, Dousa TP (1986) Phosphonocarboxylic acids as specific inhibitors of Na^+-dependent transport of phosphate across renal brush border membrane. J Biol Chem 261:6375–6383
134. Bai L, Collins JF, Ghishan FK (2000) Cloning and characterization of a type III Na-dependent phosphate cotransporter from mouse intestine. Am J Physiol Cell Physiol 279:C1135–C1143
135. Ravera S, Virkki LV, Murer H, Forster IC (2007) Deciphering PiT transport kinetics and substrate specificity using electrophysiology and flux measurements. Am J Physiol Cell Physiol 293:C606–C620
136. Villa-Bellosta R, Bogaert YE, Levi M, Sorribas V (2007) Characterization of phosphate transport in rat vascular smooth muscle cells: implications for vascular calcification. Arterioscler Thromb Vasc Biol 27:1030–1036
137. Villa-Bellosta R, Sorribas V (2009) Phosphonoformic acid prevents vascular smooth muscle cell calcification by inhibiting calcium-phosphate deposition. Arterioscler Thromb Vasc Biol 29:761–766
138. Suzuki A, Ghayor C, Guicheux J, Magne D, Quillard S, Kakita A, Ono Y, Miura Y, Oiso Y, Itoh M, Caverzasio J (2006) Enhanced expression of the inorganic phosphate transporter Pit-1 is involved in BMP-2-induced matrix mineralization in osteoblast-like cells. J Bone Miner Res 21:674–683
139. Fleisch HA, Russell RG, Bisaz S, Muhlbauer RC, Williams DA (1970) The inhibitory effect of phosphonates on the formation of calcium phosphate crystals in vitro and on aortic and kidney calcification in vivo. Eur J Clin Invest 1:12–18
140. Beck L, Leroy C, Beck-cormier S, Forand A, Salaun C, Paris N, Bernier A, Urena-Torres P, Prié D, Ollero M, Coulombel L, Friedlander G (2010) The phosphate transporter PiT1 (Slc20a1) revealed as a new essential gene for mouse liver development. PLoS One 5:e9148
141. Festing MH, Speer MY, Yang HY, Giachelli CM (2009) Generation of mouse conditional and null alleles of the type III sodium-dependent phosphate cotransporter PiT-1. Genesis 47:858–863
142. Kumar R (2009) Phosphate sensing. Curr Opin Nephrol Hypertens 18:281–284
143. Markovich D, Verri T, Sorribas V, Forgo J, Biber J, Murer H (1995) Regulation of opossum kidney (OK) cell Na/Pi cotransport by Pi deprivation involves mRNA stability. Pflugers Arch 430:459–463
144. Segawa H, Kaneko I, Yamanaka S, Ito M, Kuwahata M, Inoue Y, Kato S, Miyamoto K (2004) Intestinal Na-P(i) cotransporter adaptation to dietary P(i) content in vitamin D receptor null mice. Am J Physiol Renal Physiol 287:F39–F47
145. Rizzoli R, Fleisch H, Bonjour JP (1977) Role of 1, 25-dihydroxyvitamin D3 on intestinal phosphate absorption in rats with a normal vitamin D supply. J Clin Invest 60:639–647
146. Goseki-Sone M, Yamada A, Hamatani R, Mizoi L, Iimura T, Ezawa I (2002) Phosphate depletion enhances bone morphogenetic protein-4 gene expression in a cultured mouse marrow stromal cell line ST2. Biochem Biophys Res Commun 299:395–399
147. Goseki-Sone M, Yamada A, Asahi K, Hirota A, Ezawa I, Iimura T (1999) Phosphate depletion enhances tissue-nonspecific alkaline phosphatase gene expression in a cultured mouse marrow stromal cell line ST2. Biochem Biophys Res Commun 265:24–28
148. Delmez JA, Slatopolsky E (1992) Hyperphosphatemia: its consequences and treatment in patients with chronic renal disease. Am J Kidney Dis 19:303–317
149. Topaz O, Shurman DL, Bergman R, Indelman M, Ratajczak P, Mizrachi M, Khamaysi Z, Behar D, Petronius D, Friedman V, Zelikovic I, Raimer S, Metzker A, Richard G, Sprecher E (2004) Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoral calcinosis. Nat Genet 36:579–581
150. Benet-Pages A, Orlik P, Strom TM, Lorenz-Depiereux B (2005) An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia. Hum Mol Genet 14:385–390
151. Ichikawa S, Imel EA, Kreiter ML, Yu X, Mackenzie DS, Sorenson AH, Goetz R, Mohammadi M, White KE, Econs MJ

(2007) A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. J Clin Invest 117:2684–2691
152. Chefetz I, Kohno K, Izumi H, Uitto J, Richard G, Sprecher E (2009) GALNT3, a gene associated with hyperphosphatemic familial tumoral calcinosis, is transcriptionally regulated by extracellular phosphate and modulates matrix metalloproteinase activity. Biochim Biophys Acta 1792:61–67
153. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T (2004) Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest 113:561–568
154. Stubbs JR, Liu S, Tang W, Zhou J, Wang Y, Yao X, Quarles LD (2007) Role of hyperphosphatemia and 1, 25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. J Am Soc Nephrol 18:2116–2124
155. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390:45–51
156. Block GA (2000) Prevalence and clinical consequences of elevated Ca x P product in hemodialysis patients. Clin Nephrol 54:318–324
157. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G (2005) Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. Circulation 112:2627–2633
158. Foley RN (2009) Phosphate levels and cardiovascular disease in the general population. Clin J Am Soc Nephrol 4:1136–1139
159. Loghman-Adham M (1999) Phosphate binders for control of phosphate retention in chronic renal failure. Pediatr Nephrol 13:701–708
160. Russo D, Miranda I, Ruocco C, Battaglia Y, Buonanno E, Manzi S, Russo L, Scafarto A, Andreucci VE (2007) The progression of coronary artery calcification in predialysis patients on calcium carbonate or sevelamer. Kidney Int 72:1255–1261
161. Slatopolsky E, Bricker NS (1973) The role of phosphorus restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. Kidney Int 4:141–145
162. Lopez-Hilker S, Dusso AS, Rapp NS, Martin KJ, Slatopolsky E (1990) Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. Am J Physiol 259:F432–F437
163. Nielsen PK, Feldt-Rasmussen U, Olgaard K (1996) A direct effect in vitro of phosphate on PTH release from bovine parathyroid tissue slices but not from dispersed parathyroid cells. Nephrol Dial Transplant 11:1762–1768
164. Silver J, Naveh-Many T (2009) Phosphate and the parathyroid. Kidney Int 75:898–905
165. Martin DR, Ritter CS, Slatopolsky E, Brown AJ (2005) Acute regulation of parathyroid hormone by dietary phosphate. Am J Physiol Endocrinol Metab 289:E729–E734
166. 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. Proc Natl Acad Sci USA 104:11085–11090
167. Lennane RJ, Carey RM, Goodwin TJ, Peart WS (1975) A comparison of natriuresis after oral and intravenous sodium loading in sodium-depleted man: evidence for a gastrointestinal or portal monitor of sodium intake. Clin Sci Mol Med 49:437–440
168. Lennane RJ, Peart WS, Carey RM, Shaw J (1975) A comparison on natriuresis after oral and intravenous sodium loading in sodium-depleted rabbits: evidence for a gastrointestinal or portal monitor of sodium intake. Clin Sci Mol Med 49:433–436
169. Lee FN, Oh G, McDonough AA, Youn JH (2007) Evidence for gut factor in K^+ homeostasis. Am J Physiol Renal Physiol 293:F541–F547
170. Conigrave AD, Brown EM (2006) Taste receptors in the gastrointestinal tract. II. L-amino acid sensing by calcium-sensing receptors: implications for GI physiology. Am J Physiol Gastrointest Liver Physiol 291:G753–G761
171. Hsieh YJ, Wanner BL (2010) Global regulation by the seven-component Pi signaling system. Curr Opin Microbiol 13:198–203
172. Lamarche MG, Wanner BL, Crepin S, Harel J (2008) The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis. FEMS Microbiol Rev 32:461–473
173. Mouillon JM, Persson BL (2006) New aspects on phosphate sensing and signalling in *Saccharomyces cerevisiae*. FEMS Yeast Res 6:171–176
174. Giots F, Donaton MC, Thevelein JM (2003) Inorganic phosphate is sensed by specific phosphate carriers and acts in concert with glucose as a nutrient signal for activation of the protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. Mol Microbiol 47:1163–1181
175. Popova Y, Thayumanavan P, Lonati E, Agrochao M, Thevelein JM (2010) Transport and signaling through the phosphate-binding site of the yeast Pho84 phosphate transceptor. Proc Natl Acad Sci USA 107:2890–2895
176. Carroll AS, O'Shea EK (2002) Pho85 and signaling environmental conditions. Trends Biochem Sci 27:87–93
177. Wilson WA, Roach PJ (2002) Nutrient-regulated protein kinases in budding yeast. Cell 111:155–158
178. Silver J (2001) Cycling with the parathyroid. J Clin Invest 107:1079–1080
179. Miyamoto K, Tatsumi S, Morita K, Takeda E (1998) Does the parathyroid 'see' phosphate? Nephrol Dial Transplant 13:2727–2729
180. Miyamoto K, Tatsumi S, Segawa H, Morita K, Nii T, Fujioka A, Kitano M, Inoue Y, Takeda E (1999) Regulation of PiT-1, a sodium-dependent phosphate co-transporter in rat parathyroid glands. Nephrol Dial Transplant 14(Suppl 1):73–75
181. Tatsumi S, Segawa H, Morita K, Haga H, Kouda T, Yamamoto H, Inoue Y, Nii T, Katai K, Taketani Y, Miyamoto KI, Takeda E (1998) Molecular cloning and hormonal regulation of PiT-1, a sodium-dependent phosphate cotransporter from rat parathyroid glands. Endocrinology 139:1692–1699
182. Salaun C, Marechal V, Heard JM (2004) Transport-deficient Pit2 phosphate transporters still modify cell surface oligomers structure in response to inorganic phosphate. J Mol Biol 340:39–47