

PERSPECTIVES

Bone Mineralization and Regulation of Phosphate Homeostasis

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Abstract

The physiological process of biomineralization (calcification) that occurs in bone tissue takes place throughout an individual's life. Complex biological systems carefully orchestrating crosstalk between skeletal tissue and mineralization modulators include environmental and physiological factors acting as promoters or inhibitors. They consist of local mineralization promoters synthesized by osteoblasts, the phosphatases, namely, tissue-nonspecific alkaline phosphatase (TNAP) and phosphatase orphan 1 (PHOSPHO1). The local inhibitors are produced by osteoblasts and osteocytes and include inorganic pyrophosphate (PPi) and organic non-collagenous proteins or peptides of the extracellular matrix, such as osteopontin. While these modulators act in a paracrine/autocrine manner, locally synthesized fibroblast growth factor 23 (FGF23) regulates systemic phosphate levels by creating bone–kidney–parathyroid feedback loops. Locally, the active role in regulation of mineralization and phosphate homeostasis is played by inorganic forms of phosphate itself, and in particular the Pi/PPi ratio. The upper control of this ratio is mediated by circulating effects of FGF23 on phosphate homeostasis. Bone cell products allow bone to play an active role in coordinating its mineralization with total systemic phosphate regulation. *IBMS BoneKEy*. 2011 June;8(6):286-300.

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Bone Tissue

The physiological process of biomineralization (calcification) that occurs in bone tissue takes place throughout the entire human life cycle, both during growth and development and in adult life, and is characterized by age- and gender-dependent differences. Complex biological systems carefully orchestrating crosstalk between skeletal tissue and mineralization modulators include environmental and physiological factors acting as promoters or inhibitors. This *Perspective* reviews major local and systemic bone mineralization regulators, emphasizing feedback loop relations with phosphate homeostasis.

Bone is a specialized connective tissue. Physiologically, the mineralization process is restricted to bone tissue and teeth. Bone tissue consists of several types of functioning cells and a complex extracellular matrix (ECM). Osteoblasts, present at the

bone surface, are specialized for formation and secretion of both inorganic and organic constituents of the ECM. Secreted hydroxyapatite (HA), calcium hydroxy phosphate crystals, is deposited in the spaced “hole regions” of triple helical fibrils of collagen 1 under the influence of non-collagenous proteins (1;2). Osteocytes, representing 90-95% of all bone cells, are old osteoblasts embedded within the matrix that they have produced. Osteocytogenesis, the process of osteoblast differentiation into osteocytes, is accompanied by down-regulation of many previously expressed bone markers, including alkaline phosphatase and collagen type I (3), and increased expression of genes coding for non-collagenous bone matrix polyanionic proteins, such as matrix extracellular phosphoglycoprotein (MEPE) and dentin matrix protein 1 (DMP1) (4). Osteoclasts are the third type of bone cell. They are bone-resorbing cells that appear at the bone

surface and play a crucial role in bone remodeling processes.

Bone Mineralization Is Locally Regulated by Phosphate Molecules

Local inorganic and organic molecules synthesized by bone cells are involved in the

strictly regulated process of bone calcification, acting in an autocrine/paracrine manner. Additional circulating factors produced by other tissues or even by bone itself function as endocrine hormones in feedback loops to ensure normal bone calcification (Fig. 1).

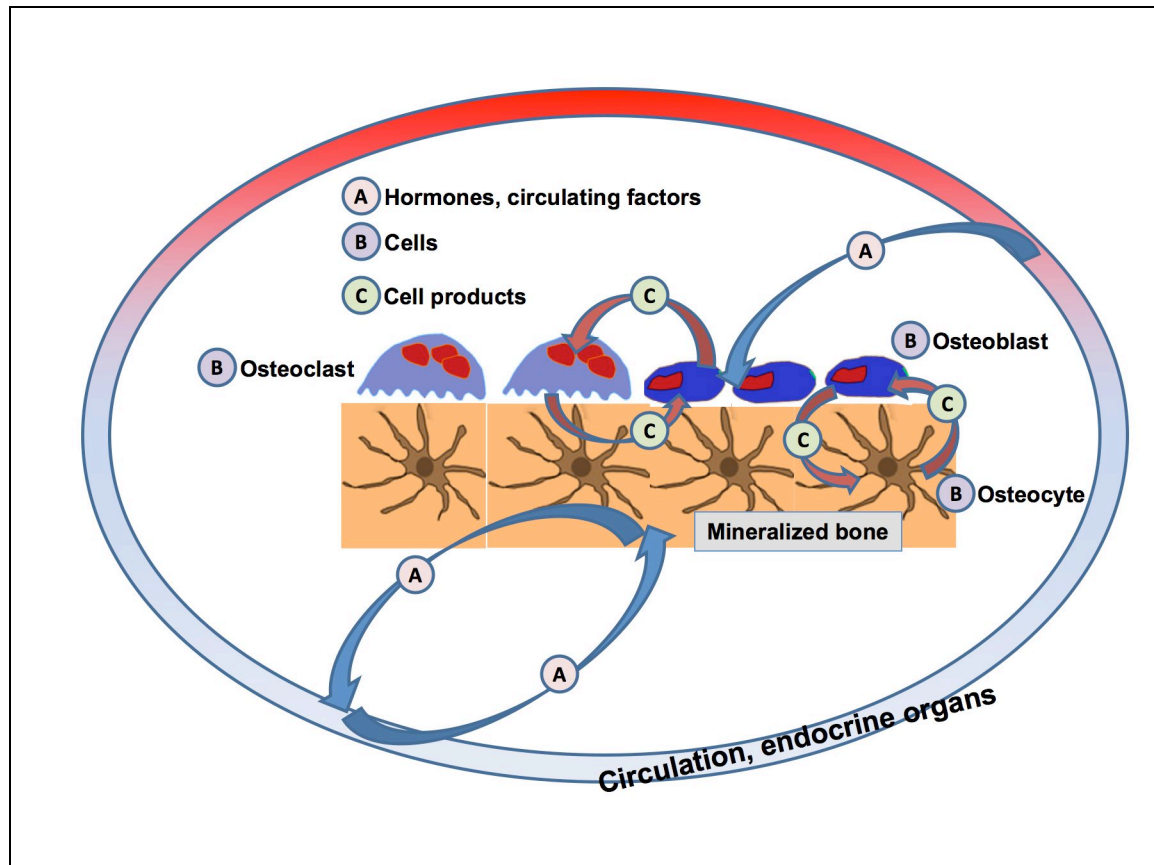


Fig. 1. Three levels of bone mineralization control: (A) systemic hormones (B) bone cells (osteoblasts, osteoclasts, and osteocytes) and (C) cell products, acting in a paracrine/autocrine manner. Delicate feedback loops between all three control levels regulate normal mineralization.

The mineral ions Ca^{2+} and Pi (in the form of $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$) play a central role in controlling essential biological activities. Phosphate is an obligate component of cellular structures and functions. Maintaining phosphate balance is essential for physiological activities in all tissues, including cellular signal transduction, biochemical pathways, energy metabolism, and bone mineralization (5). Both extremes of serum phosphorous-imbalanced levels, hyper- and hypophosphatemia, reflect systemic disorders (6;7). The body receives the nutrient phosphorous as organic and

inorganic forms absorbed in the intestine. Phosphate is taken into cells from the circulation, stored in the skeleton as a calcium-phosphate complex, and excreted by the kidney through urine. More than 80-85% of the total phosphorous distributed in the body is present in bone (8;9). Therefore, impaired bone mineralization can result either from dietary phosphate deficiency or from excessive retention of phosphate in the body (9). Until recently, the regulation of phosphate homeostasis was thought to be a passive process mediated largely by the well-known calcitropic hormones

parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃). Here we focus on the central role played by inorganic forms of phosphate itself, namely the Pi/PPi ratio, in regulation of linked bone mineralization and phosphate homeostasis. Pyrophosphate (PPi) consists of two molecules of inorganic phosphate (Pi) joined by a hydrolyzable high-energy ester bond. PPi is a by-product of many intracellular metabolic reactions of most tissues. It is present in both intra- and extracellular compartments and body fluids, including plasma (10;11). The normal reported PPi plasma range for adults is 1.19-5.65 μM (11), while the Pi plasma range is 0.85-1.44 mM (8). Extracellular PPi deficiency leads to excess HA formation in the skeleton (12), whereas PPi elevation results in decreased skeletal mineralization (11;12). Bone mineralization is dependent on a tight local balance between extracellular levels of Pi

and PPi (13). Pi is locally generated by osteoblasts expressing an ecto-enzyme tissue-nonspecific alkaline phosphatase (TNAP, an isoenzyme of alkaline phosphatase), which hydrolyzes extracellular PPi to Pi (14). The controlled co-expression of TNAP with fibrillar collagen 1 directly promotes the deposition of HA in bone matrix (14). Controlling the Pi/PPi balance through a complex regulation of PPi formation and degradation, as part of HA formation, links bone mineralization with phosphate homeostasis.

Pi and PPi balance regulation

The intracellular phosphate pool is obtained by Pi uptake from the blood, and hydrolysis of ATP and AMP (15). The first step of forming crystalline HA occurs within osteoblast organelles, named membrane-bound matrix vesicles (MVs) [Fig. 2(A)].

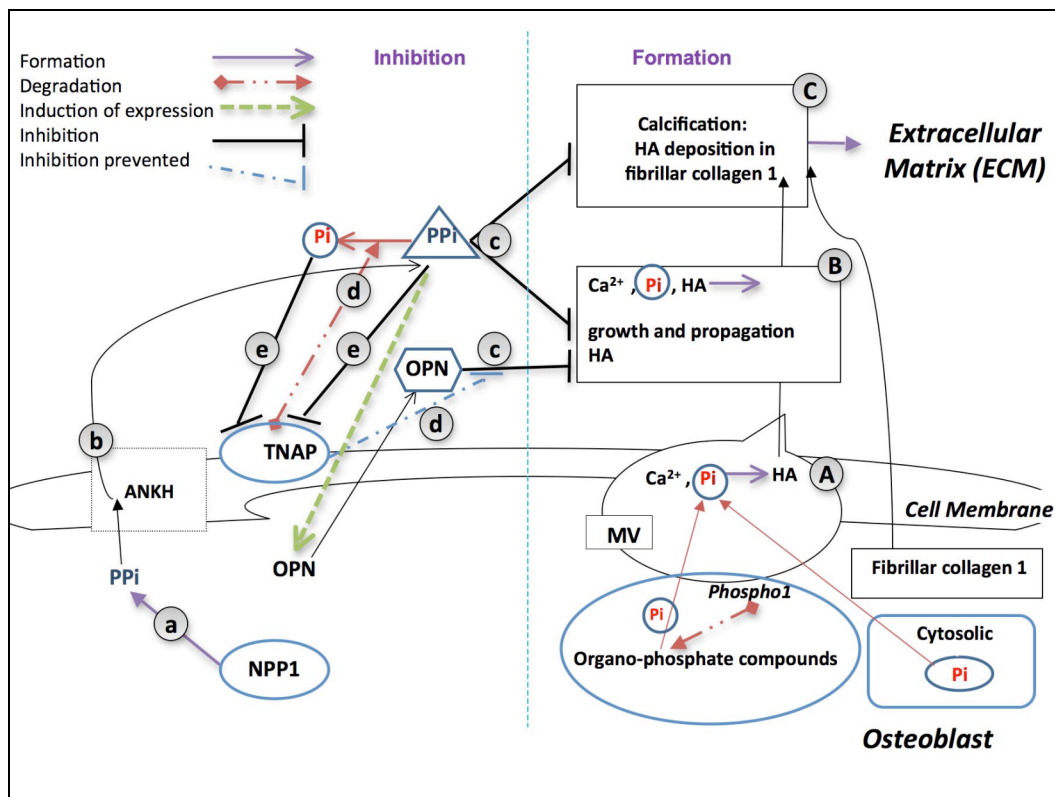


Fig. 2. Regulatory intercellular and intracellular signaling pathways operated by the osteoblast, to control the local Pi/PPi balance required for physiological bone mineralization. Right side: formation of bone matrix; (A) accumulation of Ca²⁺ and Pi within MVs initiates HA formation. (B) and (C) extracellular HA growth, propagation and deposition into bone matrix. Left side: inhibition of mineralization by a low Pi/PPi ratio; (a) and (b) PPi formed within the cell is transported to the ECM. (c) PPi and also OPN inhibit mineralization. (d) TNAP upregulates mineralization by degrading PPi and by dephosphorylation of OPN. (e) In turn, Pi and PPi downregulate TNAP activity.

In these vesicles the inorganic ions Ca^{2+} and Pi are accumulated (16), while PPI is excluded (17). Subsequently, the MV membrane is ruptured and/or broken down, and HA crystals spill out into the extracellular fluid, where their further growth and propagation occurs, to become part of the ECM (18) [Fig. 2(B), (C)]. Extracellular PPI is the key regulator inhibiting the ability of Pi to crystallize with calcium in the ECM, thereby inhibiting HA formation (19) [Fig. 2(C)]. PPI intracellular formation, transport and degradation at the ECM are regulated by specialized gene products expressed by osteoblasts. PPI formation is carried out by nucleotide pyrophosphatase phosphodiesterase 1 (NPP1), which generates intracellular PPI from nucleoside triphosphates (20) [Fig. 2(A)]. PPI transport to the cell exterior is mediated by the multiple-pass transmembrane protein ANKH (a homolog of the mouse progressive ankylosis (ank) gene product), which transfers the generated PPI from the cytosol to the ECM [Fig. 2(B)], where its inhibitory effects on HA crystal growth and propagation are exerted (20-23). The ratio of extracellular Pi/PPI concentrations is crucial for the inhibitory process (20). Hypermineralization is caused in mice by mutations in either *NPP1* or *ANK* (23;24). PPI hydrolysis takes place in the ECM by the TNAP enzyme, located on the osteoblast cell surface (14;25) [Fig. 2(D)]. As PPI is a potent mineral-binding small molecule antagonizing the ability of Pi to crystallize with calcium to form HA (13), its enzymatic removal is essential for bone mineralization. Thus, TNAP plays a crucial role in restricting bone levels of PPI. TNAP promotes bone mineralization by providing Pi available for HA formation (26); at the same time TNAP maintains proper low extracellular PPI concentrations preventing its inhibitory effect, and allowing normal bone mineralization (25;27). Accumulation of PPI in skeletal tissues and elevated plasma PPI levels have been observed in patients with TNAP deficiency and impaired pyrophosphatase function caused by hereditary genetic defects; such high PPI levels lead to deficiency in free extracellular Pi and are characterized by hypophosphatemia and hypomineralization (rickets and osteomalacia) in both humans

(11;28) and mice (29). These shared phenotypes in the same individual, hypophosphatemia and mineralization disorders, distinguish the potential linked regulation of Pi homeostasis with bone calcification. Maintaining linked Pi homeostasis with normal bone mineralization might involve crosstalk between bone extracellular PPI, the local key regulator, and circulating hormones and their signaling cascades.

While TNAP controls PPI levels directly as its substrate, another phosphatase, namely, PHOSPHO1 (phosphatase, orphan 1) seems to be indirectly involved in PPI regulation. PHOSPHO1 is a cytosolic enzyme with specificity for several organophosphate compounds (phosphoethanolamine and phosphocholine). It is expressed at 100-fold higher levels in mineralizing than in non-mineralizing tissues and is active within osteoblast MVs (30;31). PHOSPHO1 deficiency in genetically modified mice causes elevated extracellular PPI levels, as a result of altered expression of all three molecules involved directly in PPI metabolism. While PPI production and transport were enhanced (high NPP1 activity and high ANK expression), its degradation was decreased (reduced TNAP activity). Moreover, these modified mice displayed multiple skeletal deformations including spontaneous fractures, bowed long bones, osteomalacia and scoliosis in early life (32). The latter study also generated mice with double ablation of both PHOSPHO1 and TNAP function, in which an essentially complete absence of mineralization and perinatal lethality were observed despite the availability of systemic Pi. These findings suggest independent, non-redundant mineralization roles played by the two phosphatases. Accordingly, Yadav *et al.* (32) suggested different pathways for the roles of these phosphatases in bone mineralization, to eventually achieve combined mineralization and Pi/PPI balance control. The mineralization initiation with Pi generation and accumulation within MVs is thought to depend on PHOSPHO1 hydrolyzing organophosphate compounds and Pi influx via Pi transporters [Fig. 2(A)]. This step is followed by extravesicular Pi

generation, carried out through TNAP hydrolyzing PPi (or ATP) (33) [Fig. 2(D)]. The last step of ECM mineralization depends on the presence of collagen fibrils and TNAP's pyrophosphatase activity. TNAP causes further mineralization by maintaining the extracellular Pi/PPi balance: it degrades the mineralization inhibitor PPi and thereby generates Pi.

An additional mineralization inhibitor is osteopontin (OPN), a 34-kDa, acidic, highly phosphorylated extracellular glycoprotein. It is normally present as a major component of the non-collagenous bone matrix, with strong mineral-binding properties (34). Dephosphorylation of OPN by TNAP prevents much of its mineral-binding and crystal growth-inhibiting activity (35). Both PPi and OPN are found at high levels in bone, both inhibit mineralization by binding to growing HA crystals via negatively charged phosphate residues (36;37), and both are inactivated by TNAP [Fig. 2(C) and (D)]. OPN acts independently of PPi to inhibit bone calcification, and also as a mediator of PPi effects (38). OPN synthesis and secretion by osteoblasts are increased by accumulated levels of extracellular PPi (37;39). On the other hand, inhibition of TNAP by PPi (in the presence of OPN or other matrix phosphoproteins) [Fig. 2(E)] maintains the phosphorylated form of OPN to further regulate bone mineral growth. Thus, the local Pi/PPi ratio directly represses OPN expression, independently of phosphate uptake. Another distinct mechanism that regulates bone mineralization seems to be plasma Pi-dependent: high levels of Pi have been demonstrated to inhibit TNAP activity through regulation dependent on intracellular uptake via Na⁺/Pi co-transporters (40;41).

This led Addison and colleagues (35) to suggest three mechanisms by which PPi prevents mineralization. The first involves direct binding to growing HA crystals and further prevention of the apposition of mineral ions. The additional two mechanisms include PPi direct effects on osteoblast activity, specifically: (a) induction of OPN expression by osteoblasts, via intracellular signaling pathways (MAPK),

thus providing coordinated actions of both PPi and OPN for negative control of HA crystal growth and deposition; and (b) use of a feedback mechanism in which osteoblasts respond to the proper Pi/PPi ratio by inhibiting TNAP activity. As a result, hydrolysis of PPi into its constituent Pi ions is slowed, and the phosphorylated active form of OPN is maintained. These two mechanisms operated by osteoblasts lead to increased levels of both mineralization inhibitors PPi and OPN, whose concentrations are controlled by linked counter-regulatory mechanisms, leading to a combined mineralization inhibitory effect.

Bone Mineralization Is Systematically Regulated by Circulating Hormones

Bone mineralization is a dynamic process that involves adjustments and adaptations to changing physiological conditions. Regulatory bone mineralization mediators, especially the Pi/PPi balance, are targets for local systemic hormonal control, which in turn is subject to upstream signals in a feedback loop. A coordinated regulation of gene expression at transcriptional and post-transcriptional levels in a well-defined sequence maintains the physiological integrity of bone tissue as well as phosphate homeostasis. Deviation from physiological conditions is expressed as pathological conditions, hypo- or hyperphosphatemia. Systemic calcium and phosphate homeostasis has traditionally been thought to be regulated by the PTH-1,25(OH)₂D₃ axis. Recently, however, an additional player has been identified, namely fibroblast growth factor 23 (FGF23), which together with the above form the intestine–bone–kidney–parathyroid gland feedback loop.

The role of FGF23 in phosphate homeostasis and bone

Circulating levels of FGF23, a 30-kDa member of the large FGF family of proteins, originate primarily from osteocytes, and to a lesser extent from osteoblasts in mineralized bone (42). Normal human serum levels of FGF23 are very low [~1 pM, (43)]. Its target organs are assumed to be dependent on tissue expression of Klotho, a single-pass transmembrane protein essential as a

cofactor for the binding of FGF23 to its receptor (FGFR) (44). Identified target tissues expressing Klotho include the distal convoluted tubules in the kidney, the choroid plexus in the brain, and the parathyroid gland (44-46). It is not detectably expressed in skeletal tissues (47). FGF23 hormone is regarded as a phosphaturic factor, inhibiting renal reabsorption of phosphate, indispensable for maintaining phosphate homeostasis. Studies of human genetic and acquired diseases, as well as genetically-modified animal models and tissue culture, have demonstrated that both extremely high and low serum FGF23 levels are associated with bone impairments (48-50). Some tumors overexpress FGF23 and are

associated with tumor-induced osteomalacia (TIO) (51).

FGF23 excess leads to hypophosphatemia (52;53), and its deficiency to hyperphosphatemia (49;50;54;55). Controlling phosphate serum levels is achieved through the hormonal cascade of the bone–kidney–parathyroid gland axis. The participating hormones, namely FGF23, $1,25(\text{OH})_2\text{D}_3$, and PTH, are modulated by dietary and serum phosphorous levels and by counter-regulatory loops, created by their reciprocal actions on plasma Pi levels (56-59). In this manner, high phosphorous intake and $1,25(\text{OH})_2\text{D}_3$ induce expression of FGF23, which increases renal Pi excretion (60;61) [Fig. 3(A), (B), (H)].

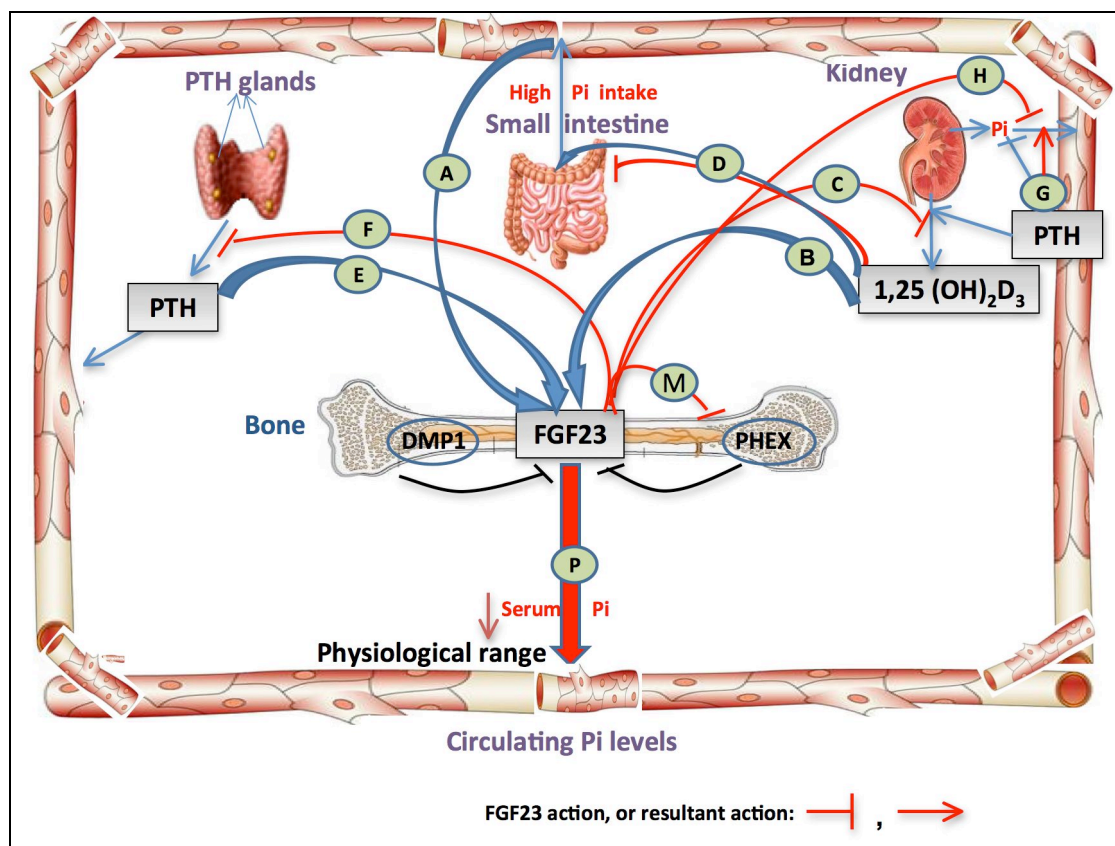


Fig. 3. Negative regulation of phosphate homeostasis by FGF23. FGF23 is negatively regulated by bone signaling mechanisms (DMP1 and PHEX), and is positively regulated by systemic factors (serum Pi, $1,25(\text{OH})_2\text{D}_3$, PTH). A-H: intestine–bone–kidney–parathyroid gland feedback loops. (A) High absorbed dietary Pi stimulates FGF23 secretion. (B) $1,25(\text{OH})_2\text{D}_3$ also stimulates FGF23 secretion. (C) In turn, FGF23 suppresses kidney production of $1,25(\text{OH})_2\text{D}_3$, resulting in (D) decreased potential $1,25(\text{OH})_2\text{D}_3$ -dependent Pi absorption by the intestine. (E) PTH also up-regulates FGF23 synthesis and secretion, and in turn (F) PTH synthesis is decreased by FGF23. (G) As a result, potential PTH-dependent inhibition of renal Pi reabsorption is decreased. (H) Overall elevated levels of FGF23 result in inhibited renal Pi reabsorption (phosphaturic action). (M) Local bone FGF23 excess results in inhibited mineralization. (P) The sum consequence of FGF23 action is to decrease excess serum Pi to a physiological range.

In turn, elevated plasma FGF23 levels decrease $1,25(\text{OH})_2\text{D}_3$ production, PTH synthesis and secretion, resulting in reduced intestinal phosphorous absorption (55;57;58) [Fig. 3(C), (F), (D)]. The parathyroid gland–bone axis is involved in systemic phosphate regulation and PTH acts directly on bone osteocytes and osteoblasts, through its receptor (PTH1R) to increase FGF23 expression (59;62;63), and FGF23 in turn (as mentioned above), suppresses PTH expression (46;59;64) [Fig. 3(E), (F), and (G)]. The overall action of FGF23 to prevent potential hyperphosphatemia by decreasing serum phosphate is primarily an outcome of its phosphaturic activity and of the induced reduction in $1,25(\text{OH})_2\text{D}_3$ production (52;55;65) [Fig. 3(H), (C), (D), and (P)].

FGF23-null mice, or humans with homozygous missense mutations in *FGF23*, develop severe hyperphosphatemia, elevated $1,25(\text{OH})_2\text{D}_3$ levels, soft tissue calcifications, and display abnormalities in bone mineralization (49;50;55;66).

However, an important question that remains unclear is whether FGF23 prevents mineralization as a result of the combined effects of its hormonal indirect negative regulation of phosphate homeostasis and direct local actions in bone. In 2008, Wang *et al.* (67) showed that FGF23 overexpression in fetal rat calvaria cell cultures causes enhanced phosphorylation of its receptor (FGFR1), which results in matrix mineralization suppression. The potential induced mineralization inhibition by FGF23 excess was rescued by an inhibitor (SU5402) of FGFR1 activity. These results indicate that FGF23 overexpression suppresses matrix mineralization independently of FGF23 systemic effects on Pi homeostasis.

Of interest is the fact that the obligate co-receptor for FGF23-receptor interactions Klotho is not detectably expressed in skeletal tissues. It is therefore possible that FGF23-FGFR signaling pathways may use the soluble form of Klotho functioning as a humoral factor (47;68), which participates in FGF23-specific signaling in bone.

Currently, a few local bone-derived factors are implicated in both the regulation of FGF23 expression and the extent of mineralization. These include phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*), and dentin matrix acidic phosphoprotein 1 (*DMP1*). *PHEX* was identified as the mutated gene responsible for

X-chromosome-linked hypophosphatemia (XLH) in humans. The *PHEX*-derived enzyme is located on the cell surface of osteoblasts and osteocytes (65). *DMP1* is the mutated gene responsible for autosomal recessive hypophosphatemic rickets (ARHR), expressed by late stage osteoblasts and osteocytes (69). XLH and ARHR present similar phenotypes, characterized by elevated FGF23 levels and rickets/osteomalacia. Similarly, either *PHEX* or *DMP1* deficiency in mice results in an excess of FGF23, hypophosphatemia, aberrant vitamin D metabolism and rickets/osteomalacia (65;70). Although it seems obvious that *PHEX* and *DMP1* are acting upstream in regulating FGF23 production (55), the molecular mechanisms by which inactivating mutations of *PHEX* or *DMP1* result in elevated FGF23 levels are still elusive. It has been suggested that the normal low expression levels of FGF23 in osteocytes are under negative regulation due to combined, direct or indirect, suppressive signal mechanisms donated by osteocyte *PHEX* and *DMP1* proteins (69;71), which simultaneously facilitate matrix mineralization (72). Therefore, it may be assumed that *PHEX* and *DMP1* activity, through autocrine/paracrine pathways in bone, coordinates phosphate homeostasis both by decreasing plasma phosphate levels via incorporation of Pi into the ECM, and by suppressing FGF23 levels to allow renal phosphate reabsorption and increased mineralization.

In addition, *PHEX* and *DMP1* proteins seem to be involved in mineralization modulation through their relations with a family of non-collagenous bone and dentin matrix polyanionic proteins, named SIBLING proteins (Small Integrin-Binding Ligand, N-linked Glycoproteins). The SIBLING proteins map to a tightly clustered region on chromosome 4q in humans and

chromosome 5 in mice (73). All SIBLING proteins are likely to play key biological roles in mineralization of bone and dentin (74). Osteoblastic proteases degrade SIBLING proteins to released small protease-resistant phosphorylated ASARM (Acidic Serine- and Aspartate-Rich Motif) peptides (75;76). *DMP1* and *OPN* are members of this family of genes. MEPE is another SIBLING protein, expressed mainly by osteocytes. It was discovered because of its expression in tumors that cause TIO and is associated with elevated levels of FGF23 (73). The ASARM peptides MEPE, DMP1 and OPN share extensive conserved homology (~ 60%) (73). The PHEX enzyme is able to bind to the ASARM motif of the SIBLING protein. This interaction could release the ASARM peptide *in vivo*; also, PHEX binds to free ASARM peptide, neutralizing its activity by sequestration and hydrolysis (76-78). Martin *et al.* (76) showed, *in vitro* and *in vivo*, that ASARM peptide is responsible for inhibition of mineralization in HYP (X-linked hypophosphatemic rickets) mouse osteoblasts. MEPE- and DMP1-derived ASARM peptides inhibit mineralization by direct binding to HA, as a function of the peptide state of phosphorylation, and the extent of its cleavage by PHEX (76;78-80). Administration of these peptides *in vitro* induces increased expression of FGF23, likely by competing with other PHEX substrates and/or disrupting PHEX activity (76;78;81). In the hypomineralized bones of XLH patients, loss of PHEX activity results in an accumulation of SIBLING-derived ASARM peptides and increased expression of FGF23, resulting in osteomalacia. In osteoblast cultures, mineralization inhibition can be rescued by normal PHEX proteolytic digestion activity (79). Thus, PHEX activity *in vivo* may be regarded as a regulatory mechanism to limit and prevent inhibition of mineralization by ASARM peptides and to suppress the expression of FGF23. Such regulated and coordinated mineralization inhibition may indicate the existence of physiologic loop mechanisms involving PHEX, SIBLING proteins and FGF23 activities. However, while phosphorylated SIBLING-derived ASARM peptides may inhibit mineralization in culture or *in vitro*, it must be demonstrated whether such

physiologic function regulates the mineralization process *in vivo*. A recent work (82) has demonstrated that the ASARM peptides may induce hypophosphatemia *in vivo*, in mice with X-linked hypophosphatemic rickets (HYP) or autosomal recessive hypophosphatemic rickets (ARHR or DMP1 null mice), and proposed a physiological role for them. In normal mice, the free ASARM peptides are assumed to have direct mineralization inhibition effects, and to be involved in an indirect pathway regulating FGF23 expression and processing. The indirect pathway is operated by a feedback regulatory loop involving FGF23, 1,25(OH)₂D₃, ASARM peptides and PHEX-DMP1 interaction. In contrast to normal mice, HYP and ARHR mice have defective PHEX and DMP1, respectively. In these mice, overexpression of FGF23 is the result of an absence of PHEX-DMP1 interaction.

An indirect involvement of FGF23 in the bone mineralization process might be due to its regulated expression of the calciotropic hormones (PTH and 1,25(OH)₂D₃), which in turn regulate local bone mineralization factors. The latter might include PTH's influence on the expression of *RUNX2* (P_i-regulated osteoblast master transcription factor) (8), and OPN and 1,25(OH)₂D₃ promotion of TNAP expression (83). OPN might be involved further in bone mineralization via its action as a suppressor of PTH transcriptional events in osteoblasts (84;85).

Further studies are needed to determine the detailed molecular interactions of FGF23 with genes responsible for skeletal mineralization. FGF23 overexpression results in mineralization inhibition. Physiologic mineralization inhibitors include P_{Pi} (or P_i/P_{Pi} balance), OPN, and the ASARM peptides (DMP1, OPN and MEPE). Since mineralization is a tightly regulated process, one may assume that the activity of all inhibitors must be interrelated and coordinated. Moreover, all inhibitor molecules (P_{Pi}, OPN, DMP1-, OPN- and MEPE-derived ASARM peptides, and FGF23), are locally produced by osteoblasts and osteocytes. PHEX, whose activity is central for phosphate homeostasis and bone

mineralization, is also expressed by these cells. Thus mineralization coordination likely involves gene-gene interactions of the genes involved in regulation of mineralization inhibitors, by involving crosstalk between the associated signaling pathways, in an autocrine/paracrine manner. For example, as has been summarized above, PPI and OPN interact to achieve proper mineralization inhibition; this is achieved by PPI-induced signaling pathways in osteoblasts to result in OPN expression and in modulated TNAP action on both PPI and OPN. It seems that OPN is involved in mineralization inhibition through additional pathways. Both the intact OPN molecule and OPN-derived ASARM peptides bind directly to HA, resulting in inhibited mineralization. But, the ASARM peptides are

released through specific pathways involving PHEX activity, indicating the need for coordinated actions. As another example, PPI, Pi, and osteocalcin (the most abundant of the non-collagenous proteins) were also shown to inhibit PHEX activity *in vitro*, probably through their shared, negatively charged groups interacting with the PHEX enzyme (86).

Conclusion

In summary, osteoblast and osteocyte products are involved in a hypothetical multiple factor interaction to affect Pi/PPI balance, PHEX activity and FGF23 levels essential for a coordinated bone mineralization process with systemic regulation of phosphorous levels (Fig. 4).

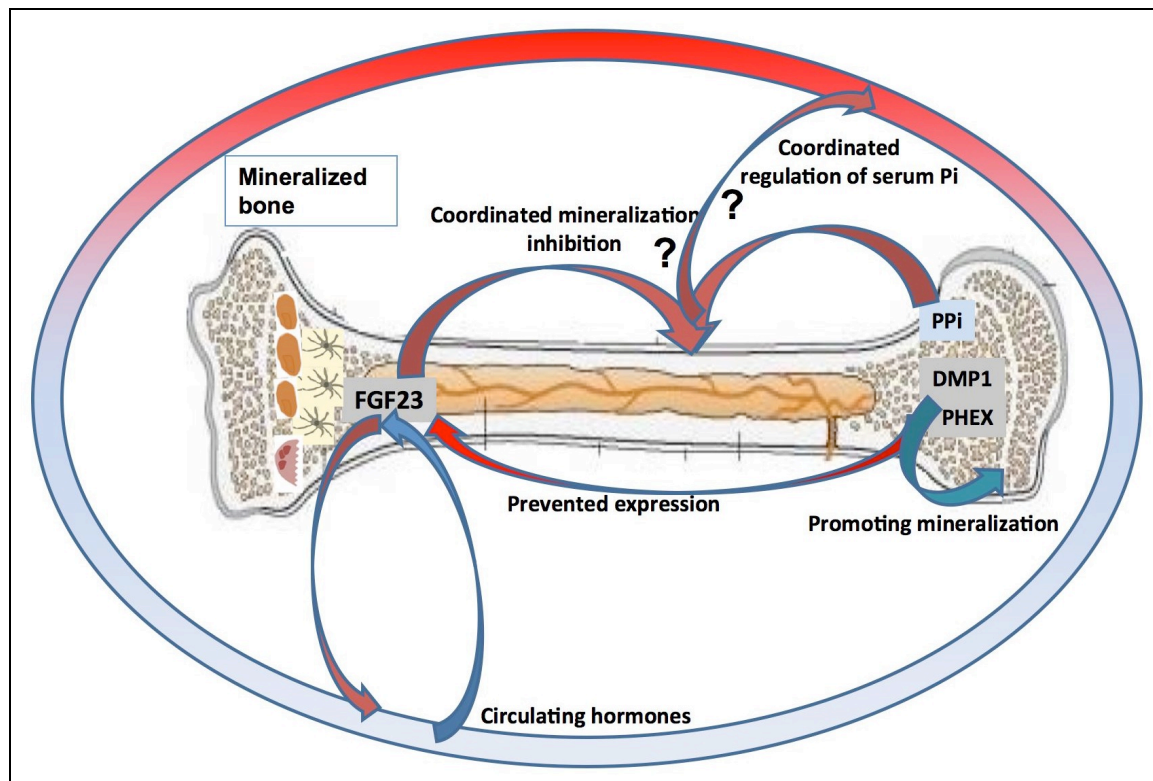


Fig. 4. Physiological bone mineralization and phosphate homeostasis are hypothesized to be coordinated by linked actions of PPI, PHEX enzyme and FGF23.

Consequently, bone is distinguished as an endocrine organ actively participating in maintaining body phosphate homeostasis. Bone tissue employs inorganic phosphate molecules both as a mineral deposit in the calcified bone matrix, and as a central regulator of the mineralization process.

Thereby, the skeleton is able to fulfill its functional uniqueness – providing skeletal strength and flexibility, body support, inner organ protection and sites for voluntary muscle attachment – while at the same time playing a key role as the body reservoir and regulator for maintaining calcium and

phosphate homeostasis. Further understanding of the coordinated activities involved in bone mineralization and phosphate homeostasis could be used beneficially for the therapeutic management of mineralization disorders and phosphate dysregulation.

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