FGF23, PHEX, and MEPE regulation of phosphate homeostasis and skeletal mineralization

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Quarles, L. Darryl. FGF23, PHEX, and MEPE regulation of phosphate homeostasis and skeletal mineralization. Am J Physiol Endocrinol Metab 285: E1–E9, 2003;10.1152/ajpendo.00016.2003.—There is evidence for a hormone/enzyme/extracellular matrix protein cascade involving fibroblastic growth factor 23 (FGF23), a phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), and a matrix extracellular phosphoglycoprotein (MEPE) that regulates systemic phosphate homeostasis and mineralization. Genetic studies of autosomal dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemia (XLH) identified the phosphaturic hormone FGF23 and the membrane metalloprotease PHEX, and investigations of tumor-induced osteomalacia (TIO) discovered the extracellular matrix protein MEPE. Similarities between ADHR, XLH, and TIO suggest a model to explain the common pathogenesis of renal phosphate wasting and defective mineralization in these disorders. In this model, increments in FGF23 and MEPE, respectively, cause renal phosphate wasting and intrinsic mineralization abnormalities. FGF23 elevations in ADHR are due to mutations of FGF23 that block its degradation, in XLH from indirect actions of inactivating mutations of PHEX to modify the expression and/or degradation of FGF23 and MEPE, and in TIO because of increased production of FGF23 and MEPE. Although this model is attractive, several aspects need to be validated. First, the enzymes responsible for metabolizing FGF23 and MEPE need to be established. Second, the physiologically relevant PHEX substrates and the mechanisms whereby PHEX controls FGF23 and MEPE metabolism need to be elucidated. Finally, additional studies are required to establish the molecular mechanisms of FGF23 and MEPE actions on kidney and bone, as well as to confirm the role of these and other potential “phosphatonin,” such as frizzled related protein-4, in the pathogenesis of the renal and skeletal phenotypes in XLH and TIO. Unraveling the components of this hormone/enzyme/extracellular matrix pathway will not only lead to a better understanding of phosphate homeostasis and mineralization but may also improve the diagnosis and treatment of hypophosphatemic disorders.

phosphatonin; Hyp; X-linked hypophosphatemia; rickets; osteomalacia; hypophosphatemia

OUR UNDERSTANDING OF HORMONAL REGULATION of phosphate homeostasis is rapidly expanding. The classical parathyroid hormone (PTH)/vitamin D axis is not sufficient to account for the physiological complexity of renal phosphate handling and bone mineralization (58). Rather, there is emerging evidence for other phosphaturic hormones, collectively called phosphatonins (35), as well as other local factors regulating the mineralization process in hypophosphatemic disorders, referred to as minihormones (57, 78). Studies of two inherited hypophosphatemic disorders, autosomal dominant hypophosphatemic rickets (ADHR) (2), and X-linked hypophosphatemia (XLH) (70), and the sporadic tumor-induced osteomalacia (TIO, also called oncogenic osteomalacia or OOM) (17, 60) have, respectively, identified a circulating phosphaturic factor, fibroblastic growth factor 23 (FGF23), a cell surface metalloprotease, PHEX, and the matrix extracellular phosphoglycoprotein (MEPE) that appear to be etiologic factors in these hypophosphatemic disorders. There is substantial evidence that FGF23, PHEX, and MEPE constitute a novel hormone/enzyme/extracellular matrix protein axis that regulates phosphate homeostasis and skeletal mineralization.

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XLH RICKETS, A DISEASE CAUSED BY INACTIVATING PHEX MUTATIONS

XLH, the most common inherited form of rickets, is a dominant disorder characterized by defective calcification of cartilage and bone, growth retardation, impaired renal tubular reabsorption of inorganic phosphate (P_i), aberrant regulation of 1,25-dihydroxyvitamin D_3 [1,25(OH)_2D_3] production, and resistance to phosphorus and vitamin D therapy (12, 26, 32, 58, 70, 77). XLH is caused by inactivating mutations of the cell surface metalloprotease PHEX, or phosphate-regulating gene with homologies to endopeptidases on the X chromosome (32, 70). The mouse gene and locus are referred to as Phex and Hyp, respectively (5, 26, 69). The PHEX gene is conserved across species. The mouse PheX cDNA sequence is highly homologous to that of humans (5, 26, 29). Spontaneous and induced mutations of Phex have been identified in the Hyp, Gy, and Ska1 mouse homologs of XLH (5, 14, 41, 69). The Hyp mouse has a 3′ deletion of the Phex gene that leads to the production of a truncated endopeptidase lacking the catalytic domain (5). The Gy mouse is a more complex model characterized by a 5′ deletion of Phex as well as a portion of the spermine synthetase gene that lies upstream (41). The Ska1 mouse results from ethyl-nitrosourea-induced point mutation leading to exon skipping, thereby creating a selective Phex deficiency (14). Bony fish species Danio rerio is the simplest organism having PHEX (8), indicating that the presence of this gene is associated with the emergence of hydroxypatite-containing bone structures.

PHEX is a member of the M13 family of type II cell-surface membrane zinc-dependent proteases that include nepriysin (NEP), two endothelin-converting enzymes (ECE-1 and -2), the KELL antigen, and damage-induced neuronal endopeptidase (DINE)/X-converting enzyme (XCE) (24, 52, 72, 73). Several different mechanisms have been considered to explain how inactivation of an enzyme results in phosphaturia and defective mineralization. Theoretically, on the basis of the known function of members of the M13 enzyme family (72), Phex could function to convert a prohormone to a phosphate-conserving factor, inactivate a phosphaturic hormone, or possibly interact with other membrane proteins to modulate their activity. In addition, the physiological function of Phex may be dependent on the available substrates that, in turn, may be organ or cell type specific. Because the predominant expression of Phex is in cartilage, bone, and teeth (5, 19, 29, 38, 61, 62, 71), where it has been shown to be present in osteoblasts, osteocytes, and odontoblasts, locally produced substrates in the skeleton may be involved in the pathogenesis of XLH. Phex is also found at extraskeletal sites in lower abundance, including brain, lung, ovary, testis, heart, and parathyroid glands. This wider distribution of Phex raises the possibility of additional tissue-specific functions and multiple substrates, similar to related endopeptidases. For example, NEP inactivates atrial natriuretic factor in kidney, substance P in the lung, and encephalins in the brain (72).

The physiologically relevant Phex substrates have not been identified. Recent in vitro studies indicate that recombinant Phex cleaves certain NEP substrates, such as ZAAL-pNA and leu(enkephalin) (28, 67), as well as PTH-related protein (9) and amyloid peptide–(1–40) (67). These substrates, however, are not likely to have a role in phosphate homeostasis. Further studies are needed to identify physiologically relevant substrates for Phex. Recent information regarding the crystal structure of the related enzyme NEP (52) and the high conservation between NEP and PHEX suggests that PHEX has a similar three-dimensional structure. If so, the putative substrate-binding pocket of PHEX may be able to accommodate only oligopeptide substrates.

The examination of the murine homologs of XLH and osteoblasts derived from Hyp mice suggests that Phex may have multiple substrates, including factors that regulate the production/degradation of a phosphaturic hormone, referred to as phosphatonin (68), as well as an inhibitor of mineralization, referred to as minihinbin (57, 78).

The initial direct data supporting the presence of a phosphaturic hormone, phosphatonin, in XLH/Hyp were parabiosis experiments showing that Hyp mice transferred the phosphaturic phenotype to the normal mouse (42). Studies in parathyroidectomized Hyp mice demonstrate that this systemic phosphaturic activity was not due to PTH (43). Subsequently, cross-kidney transplant studies confirmed that the phosphaturia resulted from a circulating factor, rather than a primary renal defect (48). Osteoblasts derived from Hyp mice produce a factor capable of inhibiting sodium-dependent phosphate transport in renal tubular cells (49), implicating bone as a possible source of phosphatonin. The potential for cells within the osteoblast lineage to produce phosphaturic factors is also evident from certain forms of the McCune-Albright syndrome, in which activating mutations of Gsα are associated with bone fibrous dysplastic lesions, rickets/osteomalacia, and humoral-induced phosphaturia (15, 79). Investigations of ADHR and TIO have identified several candidates for phosphatonins (see TIO, AN ACQUIRED DISORDER RESEMBLING ADHR AND XLH, IDENTIFIES FGF23, MEPE, AND OTHER PHOSPHATONINS).

There is also strong support for the presence of separate factors responsible for the impaired mineralization. The ability to dissociate defective mineralization from hypophosphatemia in mouse models of XLH is consistent with additional, possibly intrinsic, factors in the skeleton that regulate osteoblastic function and are responsible for impaired mineralization independent of hypophosphatemia. In this regard, study of homozygous female Ska1 mice indicated that inactivation of Phex exerts a dominant effect with regard to hypophosphatemia, whereas it exerts a semidominant effect with regard to impaired mineralization (14). In human heterozygous females and hemizygous males with XLH, however, a gene-dosage effect is not consis-
ently observed with regard to the skeleton (12, 76), possibly due to the greater variability in human subjects compared with inbred mouse strains. Regardless, the bone and cartilage defect in Hyp mice is more severe than can be explained by hypophosphatemia alone. Whereas Hyp mice display severe rickets and osteomalacia, hypophosphatemia caused by deletion of the renal sodium-dependent phosphate transporter in Npt-2 null mice displays an age-dependent “normalization” of bone abnormalities (6), suggesting that adaptive responses occur in osteoblasts, when Phex is active, to minimize the effects of hypophosphatemia on bone.

An intrinsic defect in bone is also supported by the finding that Phex deficiency in osteoblasts results in abnormal mineralization of extracellular matrix independent of hypophosphatemia. A primary defect in Phex-deficient osteoblasts is supported by the findings that transplantation of osteoblasts from Hyp mice into normal animals produces abnormal bone with impaired mineralization (21, 22). In addition, clonal osteoblasts and stromal cells isolated from Hyp mice both produce diffusible autocrine/paracrine factors that may either block mineralization of extracellular matrix in normal osteoblasts (78) or cause an osteogenic cell differentiation defect, leading to diminished collagen deposition and calcium accumulation (44). Finally, the restoration of Phex function to osteoblasts, by use of either a mouse osteocalcin (OG2) promoter (39) or a pro-α1(1) collagen gene promoter (4) to drive osteoblastic expression of Phex, fails to rescue the hypophosphatemia in Hyp mice but results in partial correction of the skeletal phenotype in vivo, as evidenced by a small but significant increase in dry ashed weight and bone mineral density of femurs derived from OG2 Phex-Hyp mice (39). Osteoblasts derived from Hyp mice overexpressing Phex under the control of the pro-α1(1) collagen promoter also were shown to mineralize and downregulate MEPE expression ex vivo. These findings are consistent with hypophosphatemia masking the direct effect of Phex to regulate osteoblastic function (4).

The identities of phosphatonin and minihbin are unknown, but the discovery of FGF23 as a circulating phosphaturic factor causing ADHR (2) and the identification of MEPE in TIO (60) have provided strong candidates for these factors.

**ADHR IS CAUSED BY MUTATIONS INHIBITING THE PROTEOLYSIS OF FGF23**

Genetic studies of ADHR have identified missense mutations in FGF23 as the cause of this hypophosphatemic disorder (2). The FGF23 gene located on human chromosome 12p13 is one of 22 known members of the FGF family (56, 65). The full-length FGF23 is an ~26-kDa (251 amino acids) protein with an NH2-terminal region containing the FGF homology domain and a novel 71-amino acid COOH terminus (65). Cleavage of FGF23 at the 176-RXXR-179 motif generates biologically inactive NH2- and COOH-terminal fragments (66). In ADHR, mutations of the 176-RXXR-179 site prevent cleavage and inactivation of FGF23 (2). It is presumed that the resulting increase in circulating levels of cleavage-resistant FGF23 is responsible for phosphaturia due to direct actions of this hormone on the renal proximal tubule. However, the alternative possibility, that mutations in FGF23 result in gain of functions not normally possessed by wild-type FGF23, has not been totally excluded.

The enzyme responsible for metabolizing FGF23 has not been identified. The RXXR motif is similar to the consensus cleavage site for mammalian precursor convertases (PCs) belonging to the family of serine proteases of the subtilisin/kexin-type; therefore, it is likely that PCs are responsible for FGF23 degradation (47). Moreover, all cell lines and expression systems for generating recombinant proteins tested to date contain enzymes capable of metabolizing FGF23 into its NH2- and COOH-terminal fragments (64, 65, 75), suggesting that the FGF23-processing enzyme is widely expressed. One unsubstantiated report, however, suggests that recombinant Phex may cleave FGF23 at the RXXR motif or a nearby site (10), but other studies have failed to confirm Phex-dependent cleavage of the FGF23 peptide containing the RXXR site (28). Additional studies are needed to determine how Phex may regulate FGF23 metabolism.

Nevertheless, several clinical studies using newly developed assays indicate that FGF23 is present in the circulation of normal individuals, albeit at low levels, consistent with a physiological role of FGF23 in regulating serum phosphorus (74, 81). In support of this, phosphate loading resulted in significant increases in serum FGF23 levels (1), and a positive correlation between FGF23 levels and serum phosphate has been observed in hyperphosphatemic subjects with end-stage renal disease (74). In addition, FGF23 is elevated in subjects with TIO as well as in some subjects with XLH (74, 81). In XLH patients, serum phosphate is negatively correlated with FGF23 levels, suggesting that, in this setting, FGF23 is causing the hypophosphatemia (74).

Animal studies establish that FGF23 is a phosphaturic hormone (63, 65, 66, 80). In this regard, recombinant mutant FGF23, which is resistant to proteolytic cleavage, consistently induces hypophosphatemia, suppresses 1,25(OH)2D3 levels, and leads to rickets/osteoalacia when administered in vivo (63, 65, 66). The in vivo administration to and/or overexpression in mice of full-length wild-type FGF23 (65), however, has more variable effects, possibly related to its rapid metabolism. In addition, in vivo studies indicate that activity is limited to full-length FGF23, whereas the NH2-terminal fragment is inactive (66). The necessity for full-length FGF23 is curious, because the FGF domain, which presumably contains FGR receptor-binding domains, is in the purportedly inactive NH2-terminal fragment. Nevertheless, preliminary reports of FGF23-null mice confirm the essential and nonredundant role of FGF23 in regulating phosphate homeostasis (64). In this regard, FGF23 deficiency results in hyperphos-
phatemic disorder that has phenotypic features similar to ADHR and XLH (17, 57, 60). Differential gene expression profiling of these tumors by a variety of techniques has identified the presence of FGF23 as well as PHEX in TIO tumors (17, 65). Additional studies confirmed that FGF23 is secreted from TIO tumors (73). In addition, removal of the TIO tumor is associated with reductions in circulating FGF23 concentrations and correction of the hypophosphatemia and abnormal 1,25(OH)2D3 metabolism (74, 81) consistent with a role of FGF23 in the pathogenesis of TIO. Additional data are needed to establish a cause-effect relationship between overproduction of FGF23 and the renal and skeletal abnormalities of TIO, particularly since many other factors are produced by these tumors that could contribute to defective mineralization and hypophosphatemia (35, 60), including MEPE and frizzled related protein 4 (FRP-4).

The exact cell type that produces FGF23 under normal physiological conditions has also not been determined. Current reports, largely based on RT-PCR analysis, indicated that FGF23 is produced in liver, lymph nodes, thymus, and heart (in low abundance), but not in kidney (65), and initial unconfirmed reports indicated that FGF23 is not expressed in bone marrow (65) or osteoblasts (39) but that FGF23 transcripts can be amplified from RNA derived from total bone (unpublished observations). Further studies are needed to clarify the cell type and tissue expression of FGF23.

The molecular mechanisms of FGF23 actions are currently being elucidated. Recombinant FGF23, under some experimental conditions, acts directly on the proximal tubule in vitro, purportedly through the FGF receptor 3C to decrease the expression of both the sodium-phosphate cotransport type 2a and the renal 1α,25-hydroxyvitamin D hydroxylase (63). These studies also show that FGF23 action requires heparin, which facilitates FGF interactions with FGF receptors. In addition, recombinant FGF23 binds to FGF receptor 3C and FGF receptor 2C but not to FGF receptor 1C (80). The distribution of FGF receptor 3C, which is expressed in the proximal tubule and cartilage, and FGF receptor 2C, which is expressed in osteoblasts, is consistent with the likely renal and skeletal effects of FGF23 (13, 53). The function of FGF receptor 3 (FGFR3) to mediate the phosphaturic actions of FGF23, however, needs to be confirmed, because neither FGFR3-deficient states nor activating mutations of the FGFR3 have been reported to alter renal handling of phosphorus (16, 18, 45). Moreover, the failure of some studies to demonstrate any actions of FGF23 on phosphate transport in OK cells (65) implies that the in vivo actions of FGF23 to regulate phosphate transport may be indirect. Additional investigations are required to define the molecular targets of FGF23 actions and to establish the precise mechanisms whereby FGF23 causes the renal and skeletal phenotypes.

Finally, FGF23 mutations are necessary but not sufficient to fully account for the variability in the ADHR phenotype. In this regard, the severity of the skeletal manifestations in ADHR is heterogeneous, ranging from bone pain without overt bone abnormalities to severe lower-extremity deformities (2). Moreover, some patients with ADHR and FGF23 mutations have a remission in renal phosphate wasting after puberty (23, 34). These observations suggest the presence of other factors that modulate FGF23 metabolism and/or function of FGF23.

**TIO, AN ACQUIRED DISORDER RESEMBLING ADHR AND XLH, IDENTIFIES FGF23, MEPE, AND OTHER PHOSPHATONINS**

TIO, also called OOM, is an acquired hypophosphatemic disorder that has phenotypic features similar to ADHR and XLH (17, 57, 60). Differential gene expression profiling of these tumors by a variety of techniques has identified the presence of FGF23 as well as PHEX in TIO tumors (17, 65). Additional studies confirmed that FGF23 is secreted from TIO tumors (73). In addition, removal of the TIO tumor is associated with reductions in circulating FGF23 concentrations and correction of the hypophosphatemia and abnormal 1,25(OH)2D3 metabolism (74, 81) consistent with a role of FGF23 in the pathogenesis of TIO. Additional data are needed to establish a cause-effect relationship between overproduction of FGF23 and the renal and skeletal abnormalities of TIO, particularly since many other factors are produced by these tumors that could contribute to defective mineralization and hypophosphatemia (35, 60), including MEPE and frizzled related protein 4 (FRP-4).

The matrix extracellular phosphoglycoprotein MEPE, also named OF45 (25), was isolated and cloned from a TIO tumor cDNA library (60). Independently, others isolated and cloned the rat and mouse homolog on the basis of its ability to regulate mineralization (55). The human MEPE gene, located on chromosome 4q21, encodes a 525-amino acid protein (40). The mouse and rat MEPE proteins are 433 amino acids and 435 amino acids, respectively, that have only a 50% overall homology to the human MEPE but share conserved motifs, including a signal peptide, N-glycosylation sites, an SDGD glycosaminoglycan-attachment site, RGD cell-attachment regions, phosphorylation sites for PKC, tyrosine kinase, and CAM-dependent kinases, and a COOH-terminal acid serine aspartate-rich (ASARM) region.

Although initially thought to be a candidate for phosphatonin, the effect of MEPE on renal phosphate transport is controversial. MEPE-deficient mice have no abnormalities of serum phosphate concentrations, as might be expected if MEPE played an important role in serum phosphate homeostasis (25). Rather, MEPE may be the postulated mineralization inhibitor minhibin (57). In this regard, MEPE appears to be a multifunctional protein with distinct domains capable of influencing the process of extracellular matrix mineralization and cell attachment. MEPE is expressed in bone and teeth (3, 40) and, similar to PHEX, displays a temporal maturation-dependent expression in osteoblast cultures, and is highly expressed within the osteocytes that are imbedded within bone (40). Current evidence indicates that MEPE inhibits bone formation and mineralization (25). MEPE-deficient mice develop increased bone mass resulting from an increase in osteoblast number and osteoblast activity, with unaltered osteoclast number and osteoclast surface in knockout animals (25). MEPE knockout osteoblasts also produce significantly more mineralized nodules in ex vivo cell cultures than wild-type osteoblasts (25). At present, it is not clear whether MEPE’s actions are through the full-length protein or degradation fragments. Low-molecular-weight fragments have been identified in conditioned media from cells secreting recombinant MEPE (25, 30). In addition, NEP and
cathepsin B cleavage sites have been identified in MEPE, and cathepsin B has been shown to cleave MEPE in vitro (30). Nevertheless, the possibility that MEPE is minhibin is supported by several observations. First, MEPE transcripts are increased in poorly mineralizing bone derived from Hyp mice (3), suggesting that MEPE may be involved in the mineralization defect in XLH. Second, the targeted overexpression of PHEX to osteoblasts is associated with reductions in MEPE expression and normalization of osteoblast-mediated mineralization ex vivo (4). Finally, although MEPE does not appear to be directly cleaved by Phex, recent studies show that Phex can inhibit cathepsin B-dependent cleavage of MEPE in vitro (30), consistent with MEPE involvement in the mineralization abnormalities in XLH/Hyp through indirect actions of Phex.

Serial analysis of gene expression (SAGE) analysis identified another secreted protein, FRP-4, for which there is growing evidence of a potential role in the pathogenesis of both the renal and skeletal abnormalities (35). First, preliminary studies indicate that recombinant FRP-4 is phosphaturic when administered to rats in vivo (7, 35). Second, FRPs act as secreted decoy receptors, blocking Wnt-dependent signaling by competing for binding of Wnt to the low-density lipoprotein receptor (LRP) and frizzled receptor complex. The potential importance of this is derived from the observations that Wnt-signaling pathways are an important regulation of osteoblast-mediated bone formation. For example, LRP5V171 mutation causes high bone density through its actions to increase Wnt signaling in bone (11). Finally, there is evidence for involvement of the Wnt/β-catenin pathway in regulating the expression of FGFs (33). Further studies will be needed to understand the interrelationships between FGF23 and Wnt signaling pathways in TIO and XLH.

Finally, SAGE analysis also identified increments in the type III sodium-dependent Pi transporter Pit-1/Glvr-1 and the ANK pyrophosphate transporter (17). The function of these transporters in the tumors is not certain, but they are known to be involved in regulating mineralization of the extracellular matrix. ANK, through the transport of inorganic pyrophosphate, acts as an inhibitor of hydroxyapatite formation. Inactivating mutations of ANK in humans cause craniometaphyseal dysplasia (CMD) (51), and ANK-deficient mice have excessive mineralization articular cartilage and periarticular mineralization (31), features completely different from those of TIO. Pit-1 is involved in regulating Pi handling in osteoblast and chondrocytes (27, 50, 54). Although these factors are unlikely to be the primary defect in TIO, they point to downstream factors that might be regulated by FGF23 and/or PHEX. They are a precedent for interactions between putative anion transporters and members of the M13 family of endopeptidases to which PHEX belongs. For example, XK associates with the Kell endopeptidase in red blood cell membranes (36), and mutations of XK are responsible for a hereditary chorea-acanthocytosis disorder called the McLeod neuroacanthocytosis disorder (36, 37). It is possible that a similar interaction might exist between PHEX and factors regulating the mineralization process in osteoblasts.

A SINGLE-ENZYME/SINGLE-SUBSTRATE MODEL TO EXPLAIN THE COMMON PATHOGENESIS OF ADHR, XLH, AND TIO?

The phenotypic similarities between ADHR, XLH, and TIO and the finding that FGF23 is elevated in these disorders (74, 81) formed the basis for an initial enzyme/substrate model (Fig. 1) to explain the common pathogenesis of these hypophosphatemic disorders (68). According to this model, FGF23 is phosphatonin, the phosphaturic hormone that inhibits sodium-dependent phosphate uptake in the renal proximal tubule. This model presumes that only full-length FGF23 is phosphaturic and postulates that the cell surface enzyme PHEX degrades FGF23 into inactive fragments. On the basis of these assumptions, this model predicts that FGF23 is increased in ADHR because of mutations in FGF23 that render it resistant to PHEX-dependent cleavage, in XLH because inactivating mutations of PHEX prevent the normal degradation of FGF23, and in TIO because overproduction of FGF23 by the tumor overwhelms the inactivation capacity of degradative mechanisms. Currently, three fundamental aspects of this model have been established, namely, that mutations preventing the metabolism of FGF23 cause ADHR (2), that FGF23 possesses phosphaturic actions (62, 64), and that serum concentrations of FGF23 are elevated in some subjects with TIO and XLH (74, 81).

Although this model has support, especially for ADHR and possibly TIO, there are a number of inconsistencies and unexplained observations, particularly with regard to XLH, that raise concerns about whether this simple PHEX-FGF23 hypothesis is correct. First and foremost, the underlying premise that FGF23 is the phosphatonin in XLH has not been fully substan-

![Fig. 1. Single-enzyme/single-substrate model to explain fibroblastic growth factor 23 (FGF23) and phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX) regulation of phosphate homeostasis. In this previously proposed model (68), full-length FGF23 is the biologically active phosphaturic factor that is degraded by PHEX cleavage at the RXXR site.](http://ajpendo.physiology.org/)
tiated. FGF23 is not elevated in all patients with XLH, and the observed association between elevated FGF23 levels and hypophosphatemia has not been proven to be a cause-effect relationship. In addition, there is “phosphaturic activity” in Hyp-derived osteoblasts that may not be FGF23 (49), and there are other potential phosphatonin, such as FRP-4 (35), which could be the phosphaturic factor in XLH/Hyp.

Second, it has not been established that FGF23 is a substrate for PHEX. Using a rabbit reticulolysate expression system, others have reported that wild-type FGF23 is cleaved by recombinant PHEX in vitro, whereas the mutation of the RXRXR putative cleavage site in FGF23 was shown to prevent recombinant PHEX cleavage of FGF23 (10). These studies, however, did not define the actual PHEX cleavage site, identify the FGF23 fragments, or test an inactive PHEX control to exclude nonspecific effects of contaminating enzymes. In contrast, enzymes other than PHEX present in the expression systems are known to cleave FGF23 at the RXRXR site (75). In addition, the theoretical notion that PHEX preferentially metabolizes small-molecular-weight proteins on the basis of its predicted three-dimensional structure (52) would also exclude full-length FGF23 as a substrate because of its size. If FGF23 accumulation is not due to the direct inhibition of its metabolism by inactivating PHEX mutations, then other mechanisms whereby PHEX mutations impact on FGF23 production and metabolism need to be more fully explored. Other possibilities include indirect actions of PHEX to degrade FGF23 (e.g., PHEX regulates another enzyme that metabolizes FGF23) or direct actions of PHEX on other substrates to stimulate FGF23 biosynthesis and secretion (i.e., a multistep cascade).

The third reason to modify the simple single-enzyme/single-substrate hypothesis is that it does not readily account for the role of MEPE in the apparent intrinsic defect in osteoblast-mediated mineralization found in XLH. Because PHEX can inhibit cathepsin B-dependent cleavage of MEPE in vitro (30), it is possible that PHEX indirectly regulates MEPE metabolism in vitro through actions to modify the activity of other enzymes. Other data show that inactivation of PHEX is associated with increased activity of the related M13 family members NEP and ECEL1/DIN, and that phosphoramidon, an inhibitor of M13 family activity, prevents the production of the putative phosphaturic factor by PHEX-deficient osteoblasts (21). In addition, PHEX deficiency is accompanied by decreased casein kinase II activity (59). These observations suggest that PHEX-dependent regulation of other enzymes, rather than direct action of PHEX to degrade phosphatonin or minibin, might be responsible for genesis of phosphaturic and mineralization-inhibitory factors.

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**Fig. 2.** Model explaining the pathogenesis of X-linked hypophosphatemia (XLH). In this model, yet-to-be-identified oligopeptide substrates link PHEX to abnormalities of FGF23 and matrix extracellular phosphoglycoprotein (MEPE). Increased FGF23, acting through FGF receptors (FGFRs) 2C and 3C in kidney and skeleton, and MEPE and/or its fragments, acting to modulate mineralization of extracellular matrix, are responsible for the phosphaturia and rickets/osteomalacia.
The fourth reason to modify this model is that it does not account for additional complexities of XLH that include a potential role for bone marrow-derived factors (46). To date, bone marrow transplantation is the only intervention to improve both hypophosphatemia and defective mineralization in Hyp mice. In this regard, Miyamura et al. (46) showed that syngeneic bone marrow transplantation from wild mice to Hyp mice partially corrected the renal and skeletal abnormalities associated with Phex deficiency. A simple model of Phex-dependent metabolism of FGF23 is difficult to reconcile with the inability to detect FGF23 in bone marrow (65).

For these reasons, the single-enzyme/single-substrate model needs to be modified to include other effectors, such as MEPE, and intermediate steps linking Phex to alterations in FGF23 and MEPE metabolism.

A MORE COMPLEX MODEL, WITH INTERMEDIATE STEPS LINKING PHEX TO FGF23 AND MEPE TO EXPLAIN THE PATHOGENESIS OF XLH

An alternative model is depicted in Fig. 2. It presumably that FGF23 is the circulating phosphaturic factor phosphatonin and that MEPE is the bone-derived factor minhibin, responsible for the local effects of PHEx on the mineralization of extracellular matrix. In this model complex model, neither FGF23 nor MEPE is a direct PHEx substrate but is downstream of PHEx. This model also postulates the existence of yet-to-be-identified oligopeptide substrates that are intermediate steps linking abnormalities of PHEx to altered FGF23 and MEPE metabolism.

Support of this preliminary model requires additional studies. FGF23 as the sole factor mediating phosphaturia and its contributions to the phosphate-independent skeletal manifestations in XLH/Hyp need to be established. Mouse genetic studies that transfer Phex deficiency onto a FGF23-null background are the most direct way to prove a cause-effect relationship between FGF23 and hypophosphatemia in Hyp mice. In addition, studies are needed to identify the enzymes responsible for FGF23 metabolism and to determine the mechanisms whereby inactivating mutations of Phex modify degradative pathways and/or increase the expression of FGF23. Understanding these intermediate pathways will ultimately require the identification of the physiologically relevant Phex substrates and/or interacting proteins. With regard to the apparent intrinsic defect in mineralization, the mechanisms whereby Phex alters MEPE metabolism and the role of MEPE in mediating the Phex-dependent skeletal abnormalities need to be investigated. Also needed is a better grasp of the significance of other putative phosphatonin, such as FRP. Despite controversies, inconsistencies, and gaps in our knowledge about the function of Phex and the metabolism of FGF23 and MEPE, this more complex model provides a better framework to unravel the pathogenesis of these hypophosphatemic disorders and to identify systemic and local regulatory pathways that control phosphate homeostasis and mineralization.

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