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A unified model for bone–renal mineral and energy metabolism

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The beginning of the millennium saw the discovery of a new bone-matrix protein, Matrix Extracellular PhosphoglycoprotEin (MEPE) and an associated C-terminal motif called ASARM. This motif and other distinguishing features occur in a group of proteins called SIBLINGs. These proteins include dentin matrix protein 1 (DMP1), osteopontin, dentin-sialophosphoprotein (DSPP), statherin, bone sialoprotein (BSP) and MEPE. MEPE, DMP1 and ASARM-motifs regulate expression of a phosphate regulating cytokine FGF23. Further, a trimeric interaction between phosphate regulating endopeptidase homolog X-linked (PHEX), DMP1, and $\alpha_5\beta_3$-integrin that occurs on the plasma-membrane of the osteocyte mediates FGF23 regulation (FAP pathway). ASARM-peptides competitively inhibit the trimeric complex and increase FGF23. A second pathway involves specialized structures, matrix vesicles pathway (MVP). This review will discuss the FAP and MVP pathways and present a unified model for mineral and energy metabolism.

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Introduction

The endoskeleton is not just a lifeless frame designed to defeat gravity it is also a complex endocrine gland. Throughout life the hidden artisanal mining of the osteoblast and osteoclast constantly remodel this exquisite mineral structure. Hidden deep within mineral caves and communicating through a network of dendritic tunnels, a third cell-type the osteocyte regulates a continuing process of bone formation (osteoblasts) and resorption (osteoclasts). Unlike any other cell, the osteocyte, related to the osteoblast survives entombed within the hydroxyapatite matrix for decades [1••]. Recent discoveries have revealed an emerging cornucopia of growth factors, hormones, matrix molecules and neuronal outputs that interact with these cells [2]. These factors are also responsible for centrally and peripherally regulating energy metabolism and mineral balance in the skeleton, kidney, muscle and gut. The roles of these molecules have emerged by studying the effects caused by mutations in humans and in rodent models. At times, comparing rodents and humans has proved to be difficult. This is perhaps not surprising given the difference in size, life span and basal metabolism of humans and mice. The size difference for example heightens the structural design needed to oppose gravity and this indirectly impacts energy and mineral metabolism. Thus, when using rodent models to assess the usefulness of human drug targets, species differences may sometimes confound interpretation. Despite these difficulties, murine models have undoubtedly provided powerful tools for the study of bone-mineral metabolism [3]. Also, unraveling the evolutionary history of the skeleton and kidney from marine to freshwater to terrestrial environments is helping to close the gaps [4,5,6•]. It is clear the ancient evolutionary paths of bone and kidney have remarkable associations. This review discusses and provides evidence for: first, FAP and MVP pathways and the key players (PHEX, FGF23, DMP1, MEPE, ASARM, $\alpha_5\beta_3$-inegrin, family with sequence similarity 20 member C kinase (FAM20C), tissue non-specific alkaline phosphatase (TNAP), and ecto-nucleotide phosphodiesterase pyrophosphatase (ENPP1)); second, the mechanism(s) linking both MVP and FAP pathways; and third, a unified model for mineral and energy metabolism incorporating both pathways. An understanding of these pathways will increase our knowledge and potential treatments for inherited forms of hypophosphatemic bone mineral loss disorders, chronic and end stage kidney diseases, cardiovascular soft tissue calcification diseases, obesity and diabetes.

The FGF23, PHEX, DMP1, ASARM and $\alpha_5\beta_3$-integrin (FAP) pathway

A detailed review describing this pathway has been published [4]. The following discussion therefore summarizes the pathway and presents new findings. Matrix Extracellular PhosphoglycoprotEin (MEPE) was cloned in 2000 from the resected intracranial tumor of a patient suffering with tumor induced osteomalcia (OHO) [7]. Patients with OHO present with pathophysiologies that overlap with X-linked (HYP) and autosomal forms of hypophosphatemic rickets (ARH) [4]. Later research showed MEPE is a substrate and ligand for PHEX, a Zn metalloendopeptidase that when mutated results in

X-linked hypophosphatemic rickets [4]. The earlier MEPE paper also characterized a conserved MEPE C-terminal ASARM motif (Acidic Serine Aspartate Rich MEPE Associated Motif). This motif with other distinguishing features occurs in a group of proteins now classed as a single family called SIBLINGs (Short Integrin Binding Ligand Interacting Glycoproteins) [4,7]. These proteins include DMP1, Osteopontin, DSPP, Statherin, BSP and MEPE. MEPE, DMP1 and associated ASARM-motifs regulate expression of a preeminent phosphate regulating cytokine FGF23 [4,8–10]. Further, a trimeric interaction between PHEX, DMP1, and $\alpha_5\beta_3$ integrin that occurs on the plasma membrane of the osteocyte mediates FGF23 regulation [4]. ASARM-peptides competitively inhibit the trimeric complex and increase FGF23 expression. ASARM-peptides and motifs (MEPE, DMP1 and osteopontin derived) are the only known biological substrates and or ligands for PHEX [4,8–14]. Compelling evidence suggests the ratio of ASARM-peptide to SIBLING-protein plays a role in regulating the mineral matrix and FGF23 production that then moderates systemic phosphate and vitamin-D metabolism [9,15–18]. ASARM-peptides are also responsible for the mineralization defect and component to the hypophosphatemia in HYP and ARHR [4,8–10,13,14,19–21]. Recent *in vivo* and *in vitro* experiments using a bio-engineered synthetic PHEX related peptide (SPR4; 4.2 kDa) that sequesters DMP1 and MEPE ASARM-motifs delivered additional support [10,13,14,22]. Administration of SPR4-peptide to wild type (WT) and HYP mice validated the ASARM-model and provided a new promising treatment strategy. Strikingly, this peptide suppresses bone, renal and serum sclerostin (SOST), increases active β-catenin and corrects energy metabolism defects in the HYP mouse. Figure 1 illustrates the FAP pathway and both *in vitro* and *in vivo* pharmacologic effects of SPR4-peptide have been reported [10,13,14].

The discovery of the central portal of the FAP pathway, the PHEX gene in 1995 and its role in HYP was instrumental in advancing the field of bone-mineral hypophosphatemic disorders [23]. Since 1995, several new hypophosphatemic bone-mineral loss disorders and their associated primary gene defects have surfaced [24]. A common denominator of these diseases is increased levels or increased half-life of FGF23, a cytokine and phosphatonin. The FAP pathway provides a model for the regulation of this important cytokine. FGF23 regulates vitamin D metabolism, renal phosphate homeostasis and plays an indirect role in the mineralization defects [25]. Despite intense research and the discovery of several genes responsible for X-linked and autosomal forms of hypophosphatemic rickets, effective treatments for these diseases are elusive. Classic treatments involve combined high phosphate and calcitriol supplements that partially correct the growth plate abnormalities but are ineffective at resolving the endochondral mineralization defects. For example, high calcitriol supplements lead to increased FGF23 levels and an exacerbation of the bone disease (vicious cycle) [26]. Also, high phosphate diets and supplements do not correct the hyperosteoidosis and the systemic hypophosphatemia *per se* is not the sole reason for the mineralization defect [27,28]. Recent research using HYP mice has raised optimism for possible treatment by targeting PC2 proprotein-convertase processing of FGF23. Specifically, Hexa-D-Arginine treatment is reported to enhance PC2 activity, normalize FGF23 levels and rescue the HYP-mice phenotype [29••]. Remarkably, although Hexa-L-Arginine (levorotary enantiomer) enhances PC2 activity 1.4 fold, Hexa-D-Arginine (dextrorotary enantiomer) is reported to have no stimulatory or inhibitory effect on PC2 activity *in vitro* [30]. Thus, by implication the Hexa-D-Arginine form used in the HYP mice study [29••] must either behave differently *in vivo* or affect the HYP phenotype via a PC2 independent-mechanism. Relevant to this, although a partial reduction in serum full-length active FGF23 levels occurred with HYP mice treated with Hexa-D-Arginine, the levels were still very high compared to WT mice (HYP non-treated = 2300 pg/mL, HYP treated = 1800 pg/mL and WT mice = 70 pg/mL). Also, since both enantiomers (L and D) are potent 'inhibitors' of related proprotein-convertases (Furin, PACE4 and PC1/3 for example) [30••] careful toxicity evaluations and further studies are required before Hexa-D-Arginine is used clinically for long-term treatment. Two recent reviews provide a more detailed discussion and provide a model involving BMP1, PC2, 7B2 in the context of inherited hypophosphatemic rickets [4,31]. Finally, several studies have shown encouraging results using a different approach. Notably, FGF23 neutralizing antibodies were used with some success to treat X-linked hypophosphatemic rickets (HYP) patients and mice [32••,33,34]. There are however concerns that FGF23 neutralizing antibody treatment may also have adverse outcomes [35,36••].

The matrix vesicle pathway (MVP)

A second pathway involving specialized matrix vesicles (MVs) is also clearly involved in bone-mineralization, arterial calcification, phosphate regulation and energy metabolism [37••]. The MVP pathway involves the microcrystalline formation of nascent mineral that occurs inside the MV structure. Key to this process is the generation of inorganic phosphate (P_i) by hydrolysis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) by PHOSPHO1 [37••,38,39]. The cataclysmic eruption of the MV marks the geniture of the nascent hydroxyapatite crystals into the extracellular matrix and the resulting binding of these crystals with collagen fibers. The growth of the incipient mineralized bone matrix is dependent on hydrolysis of ATP and nucleotide phosphates by TNAP and ENNP1 [37••]. The link between the DMP1–PHEX–ASARM–FGF23 (FAP) and MVP pathways remains uncharacterized. Of relevance, a common denominator of the matrix vesicle and FAP pathways