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A Unified Model for Bone-Renal Mineral and Energy Metabolism

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Abstract

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 (**MVP pathway**). This review will discuss the FAP and MVP pathways and present a unified model for mineral and energy metabolism.

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 has 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

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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 [4-6]. It is clear the ancient evolutionary paths of bone and kidney have remarkable associations. This review discusses and provides evidence for: (1). 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 ectonucleotide phosphodiesterase pyrophosphatase (ENPP1)); (2). The mechanism (s) linking both MVP and FAP pathways; and (3). 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 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 wild type (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 [32-34]. There are however concerns that FGF23 neutralizing antibody treatment may also have adverse outcomes [35,36].

The Matrix Vesicle (MVP) pathway

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 [37-39]. 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 Tissue Non-Specific Alkaline Phosphatase (TNAP) and Ectonucleotide Phosphodiesterase Pyrophosphatase (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 is ATP; the energy currency of the cell. ATP is also the chief source of PO_4 substrate used by the matrix vesicle pathway (MVP). A recent new discovery promises to provide the nexus to the MVP and FAP pathways. Specifically, FAM20C, an ATP-dependent osteocyte kinase that specifically phosphorylates ASARM-motifs [40-42] and FGF23 [43,44] may provide a link. Targeted bone deletion of FAM20C in mice results in autosomal hypophosphatemic rickets [45,46]. This adds to the growing number of X-linked and autosomal forms of rickets consequent to mutations in genes involved in both MVP and FAP pathways. The FAM20C link is discussed in more detail in the next section and Figure 2 sketches the MVP pathway and the proposed connections through the FAP pathway, ATP and FAM20C.

FAM20C Kinase: A Nexus to the FAP/MVP pathways and Energy Metabolism through ATP

Mineralization is a time and spatially coordinated process involving dispositional-crystallization of hydroxyapatite (HA) mineral. This process not only requires cytokines and hormones but also downstream structurally important matrix-proteins. These matrix-proteins are integral biophysical components needed to bioengineer the growing HA lattice by systematic sequestration and deposition of phosphate and calcium [4]. The specific phosphorylation of these proteins plays an important role. For example, murine bone FAM20C-kinase inactivation causes autosomal hypophosphatemic rickets 2 (ARHR 2) [40,45-47]. Also, FAM20C-kinase specifically phosphorylates SIBLING-protein ASARM-motifs and ASARM-peptides (MEPE, DMP1, osteopontin) [40,41]. Also, a reduced DMP1 expression occurs with FAM20C-kinase null mice [45,47]. Of relevance, phosphorylation of the DMP1 ASARM-motif is important for binding and substrate hydrolysis by PHEX (PHEX mutations cause X-linked hypophosphatemic rickets). Formation of the [PHEX-DMP1-$\alpha_5\beta_3$-integrin] trimeric complex on the surface of the osteocyte signals suppression of FGF23 expression [4], see also Figure 1 (FAP pathway). So, defective phosphorylation of the DMP1 C-terminal ASARM-motif results in compromised [(PHEX)-(DMP1-ASARM)-($\alpha_5\beta_3$-integrin)] binding, increased FGF23 mRNA expression and increased circulating FGF23 protein (as seen in ARHR2 and HYP mice). Recent research suggests FAM20C also controls FGF23 levels by directly affecting FGF23 protein stability. Specifically, FAM20C phosphorylates FGF23 and this inhibits FGF23 O-glycosylation by N-acetylgalactosaminyltransferase (GalNac-T3) [43,44]. Normal O-glycosylation by GalNac-T3 is needed to protect FGF23 from proteolytic-cleavage by Furin(s) thereby increasing circulating half-life [48]. Mutations in GalNac-T3 impair O-glycosylation of FGF23 and cause tumoral calcinosis, hyperphosphatemia with reduced FGF23 half-life [48]. In

summary, loss of bone FAM20C in ARHR2 mice has two effects on FGF23: (1). Reduced expression because of impaired [PHEX-DMP1-$\alpha_5\beta_3$-integrin] formation; and (2). Reduced FGF23 stability and half-life because of impaired O-glycosylation of FGF23 (Figure 1). This is consistent with the mineralization defects (rickets) and hypophosphatemia noted with these mice. Also, WT and HYP mice infused with SPR4-peptide show marked suppression of bone FAM20C kinase with improved energy metabolism [13,14]. This suggests a link with phosphate, FAM20C, ATP, energy and mineral metabolism. Figure 2 explains the FAM20C nexus between the FAP and MVP pathways and the proposed connections through ATP, ENPP1 and TNAP.

The powerful new approach of integrative physiology and the use of murine models has shown a clear link with energy and bone-kidney mineral metabolism [49]. Peripherally, insulin and osteocalcin a bone protein regulate bone turnover and glucose metabolism. The adipokine leptin mediates central control and does this by crossing the blood brain barrier to regulate the biosynthesis of serotonin, a neurotransmitter. Leptin suppresses synthesis of serotonin in the brain stem and this reduces serotonergic signaling and sympathetic-tone in the hypothalamic arcuate nucleus (ARC) and ventrolateral medial nucleus (VLM). Serotonergic signaling in the VLM nucleus increases bone-formation and decreases bone-resorption by activating β-adrenergic osteoblast receptors. Serotonergic signaling in ARC ganglion affects appetite [50]. More recent research has shown peripheral circulating serotonin has an opposite effect on bone turnover. This effect is mediated by Low-density lipoprotein receptor-related protein 5 (Lrp5) regulation of serotonin synthesis in the gut [51,52]. LRP5 also plays a major role in directly regulating bone homeostasis through the Wnt/β-catenin pathway [53]. Since serotonin does not cross the blood-brain barrier the peripheral and central serotonin pools have distinct catabolic and anabolic effects respectively on bone turnover. The new serotonin gut findings have major medical implications for treating osteoporosis, obesity and diabetes. However, the pathways and models proposed are currently controversial since recent studies contradict key findings likely because of strain and murine model differences [54-58].

The discovery of several new genes responsible for inherited hypophosphatemic rickets diseases are beginning to reveal additional players [59]. For example mice and humans with inherited hypophosphatemic rickets diseases show accompanying defects in insulin sensitivity, glucose and fat metabolism [13,14,60]. Also, obesity, insulin resistance and cardiovascular disease (CVD) show correlations with FGF23 a major hormone regulating phosphate and mineralization [61,62]. In obese patients with non-insulin-dependent diabetes mellitus (NIDDM) a causal link with low serum phosphate has been suggested [63]. Specifically, the hypophosphatemia is proposed to affect ATP production and thus glucose metabolism. This results in hyperglycemia with increased risk of NIDDM, hypertension and stroke [63]. Also, hyperphosphatemia plays an important physiological role in obesity by impairing thermogenesis and the basal metabolic rate [63,64]. Consistent with this, major increases in red blood cell oxygen affinity with changes in oxygen transport and glycolytic intermediates occur in hypophosphatemic subjects [65]. These changes are accompanied by reduced renal cortical ribonucleoside triphosphate pools and defective ATP synthesis in hypophosphatemic rickets mice models and humans [66,67]. Defective thermoregulatory control, defective ATP synthesis with increased metabolic rate and oxygen consumption are

key features of X-linked hypophosphatemic rickets mice (HYP) [67,68]. This is accompanied by increased osteoblastic pH, increased gluconeogenesis, increased glucose-6-phosphatase activity and decreased renal and bone GAPDH expression [13,14,60]. Oxygen tension and thus normal ATP synthesis is also required for osteoblast to osteocyte transformation [69]. The osteocyte, embedded within the lacuno-canalicular complex is subject to an environment with low partial pressures of oxygen (pO_2) [70]. Cell culture studies have shown hypoxia has major influence on the expression of osteocyte expressed proteins DMP1, MEPE, FGF23 Cx43 [69]. Also, osteoblast to osteocyte transformation was positively affected under hypoxic conditions and this was associated with changes in osteocalcin and alkaline phosphatase [69].

Phosphorylation also directly impacts glucose metabolism by influencing the activity of a key bone matrix protein osteocalcin and thus insulin secretion and sensitivity. Specifically, osteotesticular protein tyrosine phosphatase (OST-PTP) dephosphorylates and inactivates the osteoblast insulin receptor and this increases γ-carboxylation of osteocalcin. The γ-carboxylation of osteocalcin reduces bioactivity and suppresses osteocalcin mediated insulin secretion. This results in hyperglycemia, glucose intolerance and reduced insulin sensitivity [71,72]. In counterbalance, osteoclastic bone resorption and the accompanying increased acidity of the bone milieu is responsible for the γ-*de*carboxylation of osteocalcin. The active decarboxylated osteocalcin then increases insulin secretion and improves glucose tolerance and insulin sensitivity [71,72]. An important component of the MVP-pathway discussed earlier, Ectonucleotide Phosphodiesterase Pyrophosphatase (ENNP1) also plays a major role in hyperglycemia, insulin resistance and diabetes [73,74]. ENNP regulates matrix vesicle mineralization and mutations in this gene cause autosomal recessive hypophosphatemic rickets (ARHR2; MIM 173335) in mice and man [37,75,76]. ENPP1 like OST-PTP interacts with the insulin receptor (IR) and decreases IR β-subunit auto-phosphorylation [74,77]. Over expression of ENPP1 induces hyperglycemia, insulin resistance and diabetes [74]. Thus, energy metabolism, mineralization and bone mass are tightly linked to mineral phosphate regulation with FAM20C and ATP central players linking the different facets (see Figures 1 and 2).

Conclusion

In conclusion, DMP1 interacts with PHEX via an ASARM-motif and $\alpha_5\beta_3$-integrin on the surface of the osteocyte. This trimeric complex then suppresses FGF23 expression through the FAP pathway. Bone-derived ASARM-peptides control these interactions and thus regulate mineralization, bone turnover and phosphate. Indeed, ASARM-peptides are the only known physiological substrate(s) and ligand(s) for PHEX [10-12,15,20]. Formation of the trimeric complex and consequent suppression of FGF23 gene expression needs FAM20C kinase phosphorylation of ASARM-motif and free ASARM-peptide. FAM20C also phosphorylates FGF23 protein and decreases protein stability by preventing FGF23 O-glycosylation. A second pathway involves matrix vesicles, ATP and specific phosphatases (MVP pathway). FAM20C kinase and ATP provide the nexus between the FAP, MVP and energy metabolism pathways. There is a compelling medical need to study the functional implications of these associations in inherited bone-kidney mineral diseases, chronic kidney disease and mineral bone disorder (CKD-MBD), obesity, diabetes and osteoporosis.

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* of special interest

** of outstanding interest

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Highlights

- FGF23 is an important regulator of Vitamin D, phosphate and mineral metabolism.
- An osteocyte membrane complex of PHEX, DMP1 $\alpha_5\beta_3$-integrin suppresses FGF23.
- SIBLING ASARM-peptides disrupts this complex and increases FGF23 (FAP pathway).
- Phosphorylation of ASARM and FGF23 by FAM20C-Kinase also regulates this pathway.
- FAM20C-Kinase and ATP provide a nexus to matrix vesicle mineralization the FAP pathway and energy metabolism.

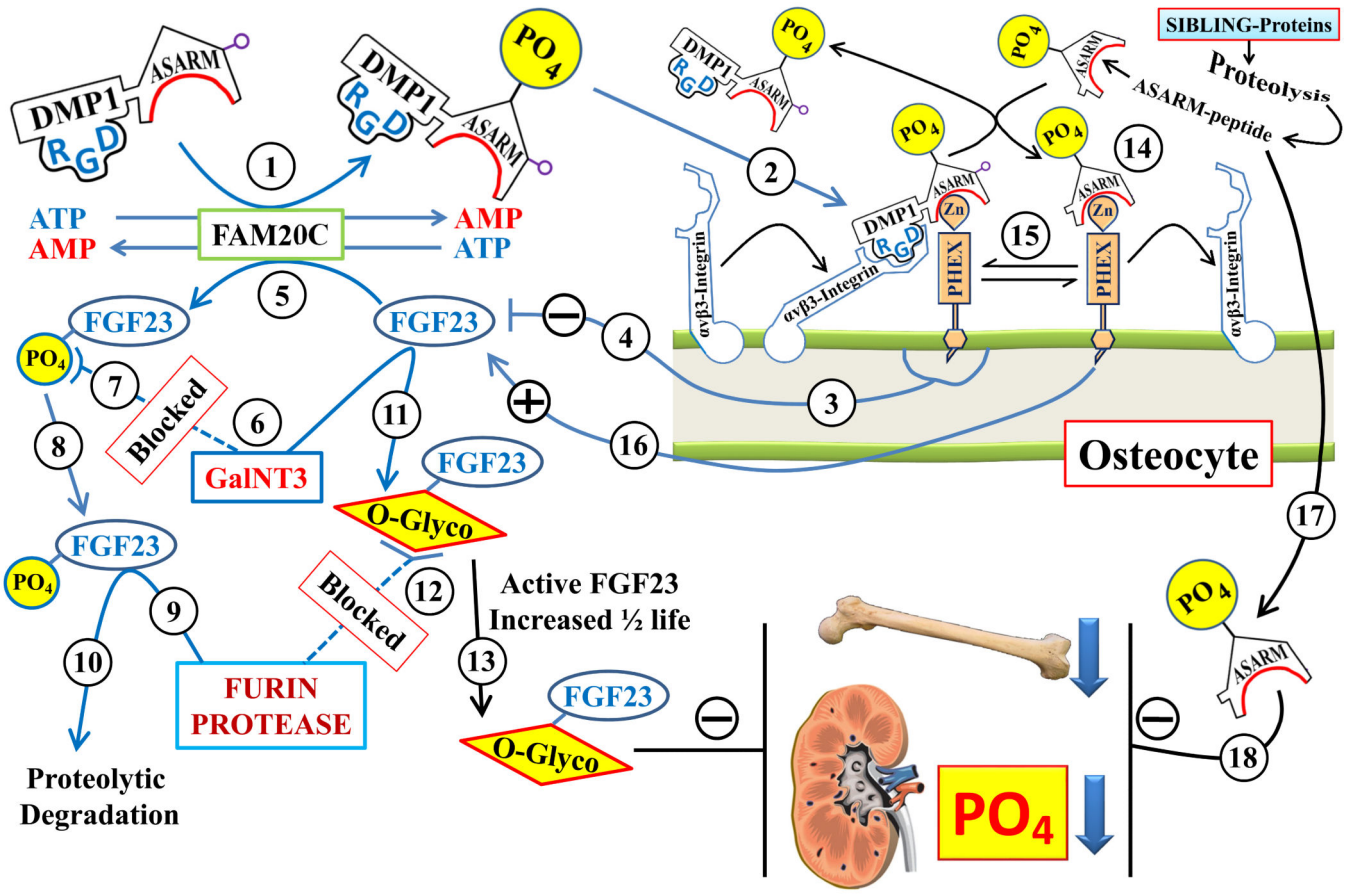

Figure 1.

Scheme illustrating the ASARM-model and the FAM20C kinase link to the FAP pathway: The numbers highlighted in the circles refer to the explanations in the following text. The interactions depicted on the osteocyte cell-surface between DMP1, PHEX, integrin and ASARM-peptides are dynamic and competitive occurring on the extracellular cell-surface. Arrows linking other factors represented in the cartoon illustrate positive and negative effector relationships (paracrine, autocrine, allosteric, signal transduction or gene expression): (**1**) FAM20C kinase phosphorylates the DMP1 C-terminal ASARM-motif; (**2**) Phosphorylation of the DMP1-ASARM motif is required for binding to PHEX and the RGD motif of DMP1 binds to $\alpha_5\beta_3$ integrin to form a [PHEX-DMP1-$\alpha_5\beta_3$-integrin] trimeric complex. This interaction occurs on the cell surface of the osteocyte where PHEX and $\alpha_5\beta_3$-integrin have an intramembranous, domain a short intracellular domain and a large extracellular domain; (**3 & 4**) Formation of the [PHEX-DMP1-$\alpha_5\beta_3$ integrin] trimeric complex initiates a signaling pathway (MAPK/Erk) that suppresses FGF23 expression; (**5**) FAM20C kinase also phosphorylates FGF23 (Ser^{180}); (**6 & 7**) FAM20C phosphorylation of Ser^{180} inhibits O-glycosylation of FGF23 by polypeptide N-acetylgalactosaminyltransferase 3 (GalNT3); (**8, 9 & 10**) The under-glycosylated and phosphorylated FGF23 is targeted for Furin degradation and proteolysis; (**6 & 11**) In contrast, non-phosphorylated FGF23 is O-glycosylated by GalNT3; (**12 & 13**) O-Glycosylation of FGF23 increases resistance to Furin degradation and increases ½ life of full length active FGF23. Of relevance, mutations in

GalNT3 are responsible for reduced circulating full-length FGF23 resulting in hyperphosphatemia and tumoral calcinosis [48,78,79]. This is the opposite phenotype to high FGF23 or hypophosphatemia. In summary FAM20C is responsible for suppressing FGF23 expression via the [PHEX-DMP1-$\alpha_5\beta_3$-integrin] trimeric complex and decreasing full-length active FGF23 by targeting the hormone for furin degradation. This is consistent with the high circulating active FGF23, increased FGF23 mRNA expression, rickets and hypophosphatemia (ARHR 2) reported in mice that are null for bone expressed FAM20C [45,46]; (**14 & 15**) The binding of PHEX to the DMP1-ASARM-motif is also competitively regulated by free ASARM-peptide. Specifically, free ASARM-peptide can directly bind to PHEX preventing the binding to DMP1 and thereby disrupting the [PHEX-DMP1-$\alpha_5\beta_3$-integrin] trimeric complex; (**16**) This in turn results in increased expression of FGF23; (**17 & 18**) Also, free ASARM-peptide inhibits mineralization and renal phosphate uptake. In X-linked and autosomal recessive rickets there are high levels of circulating ASARM-peptides. Both *in vitro* and *in vivo* (bolus and infusion) administration of ASARM-peptides and transgenic mice over expressing ASARM-peptides causes mineralization defects and hypophosphatemia [4,8-10,16-18,22,80]. The bimodal effects of SPR4-peptide administration are not shown in the scheme but are discussed in detail in recent publications [10,13,14]. The dual pharmacological effects depend on phosphate diet and mode of administration (bolus or continuous fusion). Briefly, SPR4-peptide binds to either the DMP1-ASARM motif or free ASARM-peptide. Binding to the DMP1-ASARM motif results in increased expression of FGF23 whereas binding to ASARM-peptides decreased FGF23 expression.

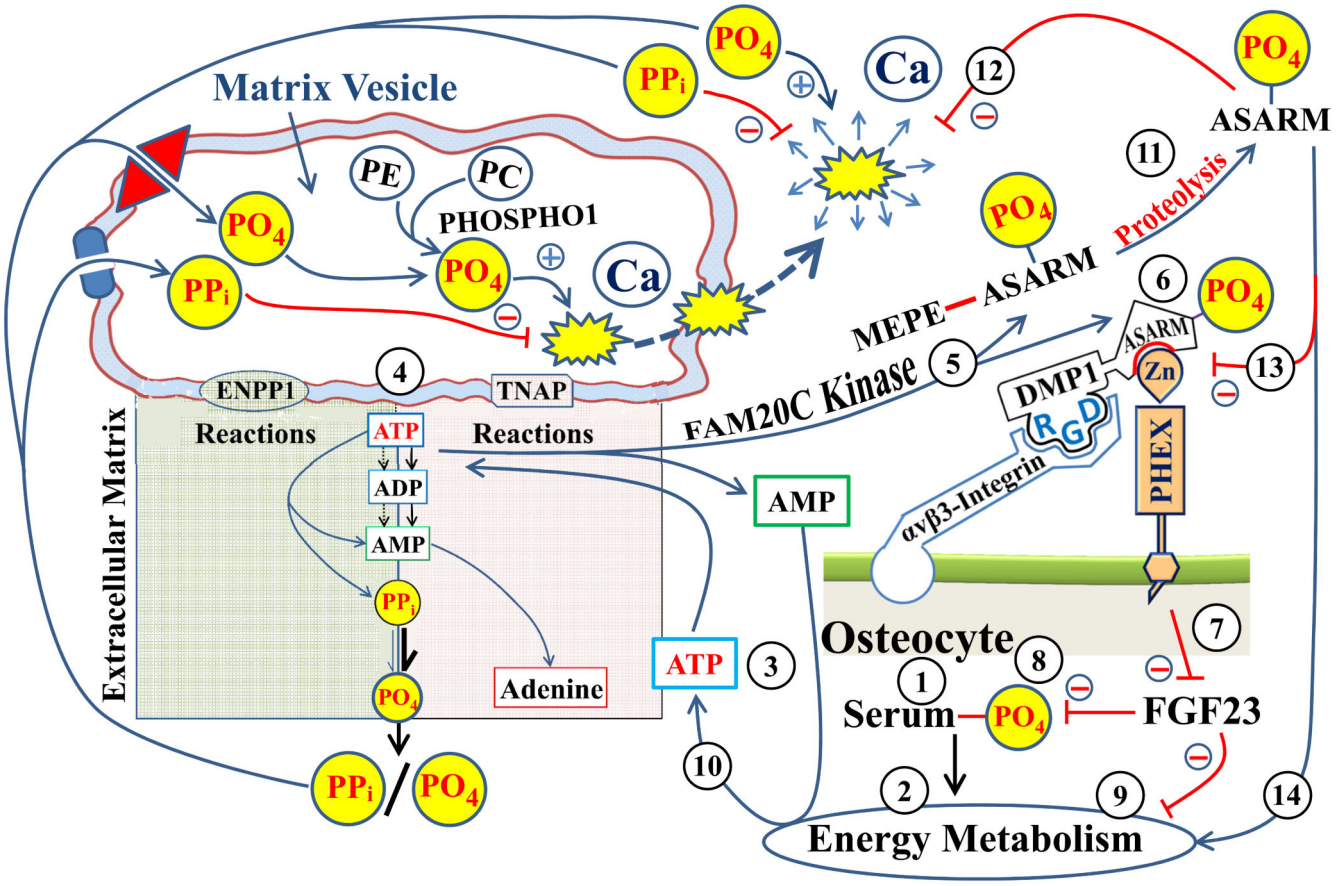

Figure 2.

Scheme illustrating the matrix vesicle (MVP) mineralization pathway and the PHEX/FGF23/DMP1 & ASARM extracellular matrix mineralization pathway (FAP). The encircled numbers refer to the following explanation of the model: (**1**) changes in serum phosphate (PO_4) will impact energy metabolism (**2**) and ATP production (**3 & 10**). ATP levels are pivotal for the generation of pyrophosphate (PPi) and PO_4 by two key matrix vesicle (MV) membrane bound phosphatases (ENPP1 and TNAP; see text and **4**). PPi inhibits mineralization and PO_4 is a required substrate both are transported into the MV by specific transporters (Pit and ANK; depicted by the blue cartouche and red diamond). The ratio of PPi/PO_4 is key for mineralization to proceed normally. Within the MV, PHOSPHO1 also generates PO_4 from phosphatidylethanolamine (PE) and phosphatidylcholine (PC) providing additional substrate for the growth of nascent MV hydroxyapaptite (depicted by yellow explosion cartoon). FAM20C kinase (**5**) also utilizes ATP to phosphorylate (**6**) ASARM-motif (DMP1) and ASARM-peptide (MEPE). Phosphorylation of DMP1 ASARM motif is requisite for the formation of a PHEX/DMP1/$\alpha_5\beta_3$-integrin complex on the surface of osteocytes (**6**). This results in down regulation of FGF23 (**7**). This in turn affects renal PO_4 handling and energy metabolism (**8 & 9**). A feedback is provided by proteolysis and release of ASARM peptides (**11**) that inhibits the PHEX/DMP1/$\alpha_5\beta_3$-integrin complex (**13**). FGF23 increases and ASARM-peptides inhibit mineralization and energy metabolism (**12 & 14**).

The ATP/FAM20C component (**3, 5 & 10**) provides a nexus between the MV, FAP and energy metabolism pathways.