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The importance of the SIBLING family of proteins on skeletal mineralisation and bone remodelling

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Abstract

The small integrin-binding ligand N-linked glycoprotein (SIBLING) family consists of osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). These proteins share many structural characteristics and are primarily located in bone and dentin. Accumulating evidence has implicated the SIBLING proteins in matrix mineralization. Therefore, in this review we discuss the individual role that each of the SIBLING proteins has in this highly orchestrated process. In particular, we will emphasise how the nature and extent of their proteolytic processing and post-translational modification affects their functional role. Finally we establish what role the SIBLING proteins have in clinical disorders of hypophosphatemia and their potential therapeutic use.
Introduction

The skeleton is a highly intricate and complex organ that has a range of functions spanning from locomotion to ion homeostasis. It is structurally adapted to suit its function; strong and stiff to withstand loading, and yet light for movement and flexible to prevent fracture. The organic component of bone, termed the osteoid, comprises of an extracellular matrix (ECM) composed primarily of collagen type I together with several non-collagenous proteins (NCPs).

One such family of NCPs is the small integrin-binding ligand N-linked glycoprotein (SIBLING) family. This consists of osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). It is likely that this protein family arose from the sectrotry calcium-binding phosphoprotein (SCPP) family by gene duplication, due to their similar structural features (Kawasaki & Weiss 2006). Despite having little intrinsic sequence homology, the SIBLING family of proteins share some common characteristics (Fisher et al. 2001). All are located to a 375kb region on the human chromosome 4q21, and 5q in mouse, and have similar exon structures (Huq et al. 2005). The SIBLING proteins are principally expressed in bone and dentin, and are secreted into the ECM during osteoid formation and subsequent mineralisation.

All SIBLING proteins undergo similar post translational modifications (PTMs) such as phosphorylation and glycosylation, the extent of which is crucial in determining their function (Boskey et al. 2009). It has long been known that the SIBLING proteins have an Arg-Gly-Asp (RGD) sequence which facilitates cell attachment signaling by binding to cell surface integrins (Fisher et al. 2001). More recently, work by Peter Rowe and colleagues, primarily focused upon MEPE, has identified a new functional domain termed the ASARM peptide (acidic serine- and aspirate-rich motif) which is highly conserved between the SIBLING family members (Rowe et al. 2000; Rowe et al. 2004). This peptide is proving critical in the functional activity of the SIBLING proteins. The SIBLING proteins and their ASRAM motifs have individually been the subject of several excellent reviews, as are detailed later. In this review we focus upon the role in which each of the SIBLING
proteins have on skeletal matrix mineralisation and bone remodelling, as well as their clinical relevance in disorders of bone mineralisation.

**Matrix mineralisation and bone remodelling**

Endochondral ossification is a carefully orchestrated process attributed to the formation and postnatal linear growth of the long bones. It involves the replacement of a cartilage scaffold by mineralised bone. Integral to this process is the epiphyseal growth plate, a highly specialised cartilaginous structure derived from a mesenchyme precursor that is located between the head and the shaft of the bone. The growth plate consists of chondrocytes arranged in columns that parallel the axis of the bone surrounded by their extracellular matrix (ECM) (Mackie et al. 2008; Ballock & O'Keefe 2003) which is rich in collagens, proteoglycans and numerous other NCPs (Gentili & Cancedda 2009; Heinegard 2009). The chondrocytes of the growth plate sit in distinct cellular zones of maturation as are clearly visible (Fig. 1), and proceed through various stages of differentiation whilst maintaining their spatially fixed locations (Hunziker et al. 1987). It is the terminally differentiated hypertrophic chondrocyte that mineralizes its surrounding ECM, localized to the longitudinal septa of the growth plate (Castagnola et al. 1988).

Chondrocyte, as well as osteoblast, mineralization of the ECM is widely accepted to involve membrane-limited matrix vesicles (MVs) within which calcium (Ca\(^{2+}\)) and inorganic phosphate (P\(_i\)) accumulate to initiate the bi-phasic process of mineralization (Anderson 2003). When sufficient concentrations of both exist, calcium phosphate begins to precipitate to form HA crystals. This initial stage of mineralization is followed by the penetration of the MV trilaminar membrane by the HA crystals and the modulation of ECM composition, promoting the propagation of hydroxyapatite (HA) outside of the MVs (Anderson 2003; Anderson 1995; Wu et al. 2002; Golub 2011). Mineralization of the ECM is a tightly regulated process such that concentrations of Ca\(^{2+}\) and P\(_i\) are permissive for effective mineralization, and that the levels of mineralization inhibitors such as inorganic pyrophosphate (PP\(_i\)) are balanced. Extracellular PP\(_i\) is a well recognised and potent inhibitor of mineralization (Meyer 1984) that is regulated by alkaline phosphatase (ALP). In bone, ALP is a
ectoenzyme enzyme located on the cell membrane’s outer surface of osteoblasts and chondrocytes, as well as on the membrane of their MVs (Anderson 1995). Classically, ALP was thought to generate the P\textsubscript{i} required for HA formation, however it has since been shown to also hydrolyse PP\textsubscript{i} thus achieving a ratio of P\textsubscript{i}/PP\textsubscript{i} permissive for HA crystal formation and growth (Fig. 2) (Hessle et al. 2002; Anderson 2003; Moss et al. 1967; Majeska & Wuthier 1975). PP\textsubscript{i} inhibits the enzymatic activity of ALP (Addison et al. 2007) offering a feedback loop by which mineralization is mediated. Other regulators of ECM biomineralization include nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and the Ankylosis protein (ANK) which work in synergy to increase extracellular PP\textsubscript{i} levels (Fig. 2). Whilst NPP1 ectoplasmically generates PP\textsubscript{i} from nucleoside triphosphates, ANK mediates its intracellular to extracellular channelling (Ho et al. 2000; Hakim et al. 1984; Terkeltaub et al. 1994). Analysis of mice deficient in ALP function (\textit{Akp2}\textsuperscript{-/-}) which are normally mineralized at birth has lead to the identification of other phosphatases such as PHOSPHO1. Since its discovery and characterisation, PHOSPHO1 has been proposed to play a crucial role in the accumulation of P\textsubscript{i} within the MV and bone mineralisation (Stewart et al. 2006; Roberts et al. 2008; Roberts et al. 2007; MacRae et al. 2010; Houston et al. 2002). PHOSPHO1 has a non-redundant functional role during bone mineralisation and the ablation of both PHOSPHO1 and ALP results in the complete lack of bone mineralisation throughout the whole skeleton (Yadav et al. 2011).

Mineralization of the ECM not only facilitates the deposition of HA, but also enables vascular invasion; a significant phase in the development of the skeleton. Vascular invasion allows the infiltration of osteoclasts and differentiating osteoblasts which absorb the cartilaginous mineralized matrix and replace it with trabecular bone respectively. This synergistic process of resorption and formation continues throughout life, replacing approximately 10% of the adult skeleton every year (Frost 1990). Despite this, the net effect of osteoblasts and osteoclasts on bone mass is zero to ensure disorders such as osteoporosis or osteopetrosis do not occur. During bone resorption, osteoclasts adhere to the bone surface forming a tight connection and allowing efficient resorption through the release of acid and proteinases. Like bone formation, this is under tight control by a variety of
autocrine, paracrine and endocrine factors, and is thought to be primarily regulated by the terminally
differentiated osteoblast, the osteocyte (Henriksen et al. 2009; Hill 1998; Manolagas 2000).

The SIBLING family of proteins

(1) MEPE

Matrix extracellular phosphoglycoprotein (MEPE) was originally identified as a substrate for PHEX
(Phosphate regulating endopeptidase homolog, X-linked), a cell membrane associated glycoprotein. In
bone, MEPE is primarily expressed by osteocytes, but Mepe mRNA expression has also been
observed by chondrocytes and osteoblasts (Nampei et al. 2004)(REF). Mepe mRNA is detected as
early as 2-days postpartum in the mouse skeleton (Lu et al. 2004). Several regulators of Mepe mRNA
expression are documented in the literature. The addition of fibroblast growth factor 2 (FGF2) to
osteoblasts down regulates Mepe mRNA levels in a dose dependent manner. The mechanism of action
is part through the mitogen-activated protein kinase (MAPK) pathway (Zhang et al. 2004). Furthermore,
osteoblasts stimulated with bone morphogenetic protein 2 (BMP2) also display a
decreased Mepe mRNA expression level (Siggelkow et al. 2004).

The first evidence for a direct role of MEPE in bone mineralization came from the increased mRNA
expression levels of Mepe seen during osteoblast matrix mineralization (Argiro et al. 2001; Petersen
et al. 2000). The development of a MEPE null mouse further fuelled the proposed role for MEPE in
mineralization. This mouse model had increased bone mass, with associated increased numbers and
thickness of trabeculae. The mineral apposition rate (MAR) was dramatically increased as was the
activity of MEPE null osteoblasts in culture (Gowen et al. 2003). Conversely, the overexpression of
MEPE in mice, under the control of the coll1a1 promotor, leads to a growth and mineralization defect,
due to a decrease in bone remodelling. The MEPE transgenic mice displayed wider epiphyseal growth
plates, and expanded primary spongiosa and a significant decrease in the MAR (David et al. 2009).

Like the other SIBLING proteins, the activity of MEPE is dependent upon its state of cleavage and its
phosphorylation. Recent work has identified the 2.2kDa ASARM peptide of MEPE as the central
provider for the mineralization activity of MEPE. This ASARM peptide is highly conserved across
the SIBLING proteins and in MEPE, is located immediately downstream of a cathepsin B cleavage site (Rowe et al. 2000). The administration of the MEPE-ASARM peptide in vitro and in vivo can inhibit the uptake of P. This is likely through a decreased expression of the NPT2a renal phosphate transporter, or through the promotion of FGF23 expression, a potent inhibitor of Pi reabsorption (David et al. 2010; Dobbie et al. 2008; Shirley et al. 2010; Marks et al. 2008; Liu et al. 2007b; Martin et al. 2008). It has however been suggested that MEPE may have a direct effect on matrix mineralization outwith the supply and demand of Pi. The ASARM peptide of MEPE inhibits mineralization in osteoblasts by directly binding to HA crystals (Martin et al. 2008; Addison et al. 2008). This inhibitory effect has also been shown in growth plate chondrocytes (REF). Integral to this inhibitory effect is the post translational phosphorylation of the ASARM peptide at three serine residues. In osteoblasts it appears that without this phosphorylation, the ASARM peptide has no effect on mineralization (Martin et al. 2008; Addison et al. 2008) however in chondrocytes, it appears the non-phosphorylated ASARM peptide can promote matrix mineralization (REF). This is not the only evidence for a role for MEPE in the promotion of mineralization. Recently it has been shown that a truncated form of MEPE which has the ASARM peptide removed, can promote bone mineralization in culture and in mice (Sprowson et al. 2008). Furthermore, a mid-terminal fragment of MEPE (termed ‘AC100’) has been shown to enhance cell binding, through the stimulation of focal adhesion kinase and extracellular signal-regulated kinases (ERK) (Hayashibara et al. 2004). Taken together, these results highlight the importance of post-translational processing in determining the functional role of MEPE.

The interaction between MEPE and PHEX is well documented in the literature. PHEX plays a central role in the protection of MEPE from proteolytic cleavage by cathepsin B; it can bind to MEPE and prevent the release of the ASARM peptide (Guo et al. 2002). The Hyp mouse, a spontaneous Phex knockout model, has an increased expression of cathepsin D, an upstream activator of cathepsin B (Rowe et al. 2006). This therefore suggests that PHEX can alter the activation of cathepsin B, and therefore the cleavage of MEPE to the ASARM peptide. Furthermore, PHEX can bind to free
ASARM peptides therefore neutralizing their activity by sequestration and hydrolysis (Addison et al. 2008; Martin et al. 2008; Liu et al. 2007a).

MEPE transgenic mice display a decrease in ALP enzyme activity in both the growth plate and the primary spongiosa (David et al. 2009). In vivo, the addition of the phosphorylated ASARM peptide also reduced the number of ALP-positive cells in an osteoblast cell culture model (Martin et al. 2008). However, this remains controversial as normal ALP activity has been reported in osteoblasts treated with phosphorylated ASARM peptide (Addison et al. 2008). No difference was also observed in the metatarsal organ culture used to examine the effects of the ASARM peptide on chondrocyte mineralization. Intriguingly, this metatarsal model also showed that in chondrocytes, the phosphorylated ASARM peptide directly inhibits angiogenesis through an inhibition of VEGF and other markers associated with vascularisation (REF). Contradictory to this, in the MEPE overexpressing mouse vascularisation was increased, as was VEGF expression (David et al. 2009). Consonant with angiogenesis is the infiltration of osteoclasts for bone resorption. Interestingly, in mice administered with recombinant MEPE or transgenic for MEPE had a significant decrease in the numbers and activity of osteoclasts (David et al. 2009; Hayashibara et al. 2007).

(2) OPN

Osteoponin (OPN), also known as secreted phosphoprotein I (SPI), is a 34kDa protein, originally identified as the bridge between the cells and HA in the ECM of bone (Sodek et al. 2000). The protein and gene structure, as well as the localisation, of OPN are well described in several excellent reviews (Fisher et al. 2001; Sodek et al. 2000; Denhardt & Guo 1993). In bone, OPN is produced by osteoblasts and osteocytes (Zohar et al. 1997; Sodek et al. 1995), as well as osteoclasts (Dodds et al. 1995). It has also been localized to hypertrophic cartilage of the growth plate (Landis et al. 2003). This broad expression of OPN reflects its vast hormonal, cytokine and growth factor regulation.

Several studies have documented the inhibitory role of OPN in HA formation and growth (Hunter et al. 1994; Boskey et al. 1993). It has also been shown to inhibit mineralization in vascular smooth muscle cells (Jono et al. 2000; Wada et al. 1999). This inhibitory role for OPN is confirmed further by
analysis of the OPN knockout mouse which has increased mineral content and size, as shown by FT-IR analysis in two different lines of Opn"mice at two different ages (Boskey et al. 2002). More specifically, it has recently been shown that the ASARM peptide of OPN inhibits ECM matrix mineralization by binding to HA crystals (Addison et al. 2010). This study by Addison et al also showed that, like MEPE, the ability of the OPN-ASARM to inhibit mineralization is dependent upon its phosphorylation at specific serine residues (Addison et al. 2010). However, the phosphorylation of other serine residues outwith the ASARM motif can also inhibit HA formation (Pampena et al. 2004). This therefore emphasises the essentiality of phosphorylation in the functional capability of OPN to inhibit ECM mineralisation. This is further confirmed when examining the interaction between OPN, ALP and PPi. Several studies have shown that ALP dephosphorylates OPN, thus preventing much of its inhibitory activity on HA formation and growth (Boskey et al. 1993; Jono et al. 2000; Hunter et al. 1994). Furthermore, PPi directly upregulates OPN mRNA expression in osteoblasts, therefore the hydrolysis of PPi by ALP will have a significant effect on the expression levels of OPN (Addison et al. 2007). This is in concordance with the Enpp1 deficient mouse in which the PPi deficiency brings about a deficiency in OPN (Johnson et al. 2003). This is also seen in the Akp2 deficient mouse, and is further confirmed by the Akp2"/Opn" mouse in which the hypomineralization seen in the Akp2" mouse alone is partially rescued (Harmey et al. 2006).

Although previous studies have implicated a phosphate dependent mechanism (Beck, Jr. et al. 2000; Beck, Jr. & Knecht 2003), work by Addison and colleagues has implicated the MAPK signaling pathways as responsible for the regulation of OPN by PPi. Analysis of the Opn" mouse has also indicated a role for OPN in the function and activity of osteoclasts. An increase in osteoclast production by spleen cells from mice lacking OPN was observed (Rittling et al. 1998). This could be a compensatory mechanisms for the disabled motility and resorption activity in osteoclasts deficient in OPN (Chellaiah et al. 2003). Further studies have attempted to elucidate the precise role of OPN in bone resorption and have implicated CD44, a major cell-surface receptor for hyaluronate (Aruffo et al. 1990) and a receptor for OPN (Suzuki et al. 2002; Chellaiah et al. 2003).
Bone sialoprotein (BSP) is a 70-80kDa protein for which its gene and protein structure have been extensively reviewed (Ganss et al. 1999). The localisation of BSP is unique to the SIBLING family of proteins as it is exclusively located to mineralized tissues such as bone, dentin and mineralizing cartilage (Chen et al. 1991; Bianco et al. 1991). In bone, it is expressed in abundance by osteoblasts, as well as by osteoclasts, osteocytes and chondrocytes (Gordon et al. 2007; Fisher & Fedarko 2003; Gordon et al. 2007).

During embryogenesis, BSP is first expressed at the onset of bone formation thus suggesting BSP to be a strong candidate for a role in HA nucleation (Chen et al. 1992). This certainly seems convincing as numerous studies have documented BSP to be involved in the initial formation of HA (Fisher et al. 2001; Harris et al. 2000; Tye et al. 2003; Wang et al. 2006). Indeed it has been shown that as little as 9nM BSP is required to nucleate HA and recently the overexpression of BSP in osteoblasts has been shown to enhance mineralization (Gordon et al. 2007; Hunter et al. 1996). Similarly, osteoblast cultures grown in the presence of an anti-BSP antibody exhibit reduced mineralization (Cooper et al. 1998; Mizuno et al. 2000). This nucleation potency is increased upon BSP binding to ????? suggesting a co-operative relationship between the two (Baht et al. 2008).

This nucleation has been reported to involve the membrane bound enzyme, ALP, where high levels of ALP activity can promote the initiation of mineral deposition in the presence of BSP (Wang et al. 2006). This is further confirmed in BSP overexpressing cell cultures which have a higher ALP activity (Valverde et al. 2008). It is likely that, like the other SIBLING proteins, the function of BSP is highly dependent upon its PTM (Stubbs, III et al. 1997). In bone remodelling, BSP is crucial for the maintenance of osteoblast and osteoclast homeostasis coupling. BSP increases osteoclastogenesis and therefore bone resorption (Valverde et al. 2005; Ross et al. 1993; Raynal et al. 1996). Furthermore, BSP expression in bone diseases characterised by excessive bone resorption, e.g. Pagets disease, is abnormally high (Valverde et al. 2008). This has been further examined in BSP transgenic
mice in which an uncoupling of bone formation and resorption resulted in an osteopenia-like phenotype (Valverde et al. 2008).

(4) DSPP

The role of dentin sialophosphoprotein (DSPP) in biomineralization has recently been reviewed (Prasad et al. 2010). Although originally thought to be exclusively expressed by dentin, DSPP is also expressed in bone, cementum and in non-mineralizing tissues including the lung and kidney (Verdelis et al. 2008; Alvares et al. 2006; Ogbureke & Fisher 2007; Qin et al. 2002; Baba et al. 2004).

DSPP is proteolytically processed to two fragments, dentin phosphoprotein (DPP) and dentin sialoprotein (DSP); both of which have important functions in mineralization. Interestingly, a third fragment called dentin glycoprotein (DGP) has been identified as being cleaved from the C-terminal end of DSP by MMP-2 and MMP-20 (Yamakoshi et al. 2005). It has been suggested that the proteolytic processing of DSPP to DPP, DSP and DGP is the activating stage in the mechanism of DSPP function (Prasad et al. 2010; Qin et al. 2004; Zhang et al. 2001). Analysis of the DSPP knockout mouse reveals defects in both dentin mineralization (Sreenath et al. 2003), as well as bone mineralization (Verdelis et al. 2008). In humans, a mutation in the DSPP gene results in dentinogenesis imperfecta characterized by dentin hypomineralization and significant tooth decay (Kim et al. 2005).

Of particular interest are the variations in the mineralization properties observed at different ages in the Dspp<sup>−/−</sup> mouse. This suggests that DSPP has roles in not only the initial mineralization of bone, but also in the remodelling of the skeleton. Various studies have shown DPP to be important in the formation and growth of HA as it has a strong affinity to Ca<sup>2+</sup> when bound to collagen fibrils (Boskey et al. 1990; Saito et al. 1997; He et al. 2005). The phosphorylation of DPP is believed to be crucial to its function since removal of the phosphate groups results in a loss of its role in HA promotion (Saito et al. 1997). On the other hand, although DSP has been shown to be involved in the initiation of mineralization, it appears not to have a functional role in the maturation of the tissue (Suzuki et al. 2009). The mechanism by which DSPP regulates HA formation is thought to involve the canonical
BMP2 signaling pathway as BMP2 has been shown to increase DSPP mRNA expression via BMP-R Smads, Runx2 and Dlx5 (Cho et al. 2010; Iohara et al. 2004; Chen et al. 2008).

(5) DMP1

Dentin matrix protein 1 (DMP1) was first cloned from dentin (George et al. 1993) and has since been identified in dentin, bone, and cementum (MacDougall et al. 1998), as well as other non-mineralized tissues (Sun et al. 2011). In bone, DMP1 is primarily expressed by osteocytes (Toyosawa et al. 2001) but also by osteoblasts (Feng et al. 2003) and hypertrophic chondrocytes (Fen et al. 2002). The first evidence for a role for DMP1 in biomineralization was its promotion of ECM mineralization in MC3T3 cells overexpressing DMP1 (Narayanan et al. 2001). The generation of a Dmp1-null mouse has further fuelled the potential role for DMP1 in bone mineralization. The mineral-matrix properties of the knockout mice were significantly lower when compared to their control counterparts (Ling et al. 2005). Furthermore, the DMP1 deficient mice displayed a severe defect in cartilage formation with a highly widened growth plate suggesting an impairment of mineralisation at the chondro-osseous junction. Indeed, this cartilage defect results in a phenotype resembling dwarfism with chondrodysplasia (Ye et al. 2005) and highlights the role of DMP1 in chondrocyte matrix mineralization. It has since been shown that the distorted growth plates seen in the DMP1 null mouse are in fact due to disorganisation as opposed to enlargement of what?? (Sun et al. 2010). Furthermore, this disorganisation can be recovered by crossing the DMP1 null mouse with transgenic mice expressing a mutant form of DMP1, which blocks the processing of mouse DMP1 in vivo (Sun et al. 2011). Like other SIBLING proteins, the proteolytically processing of DMP1 appears essential to its function and localization. In bone and dentin, DMP1 is processed to two fragments; one 37kDa fragment originating from the NH2-terminal, and one 57kDa fragment originating from the COOH-terminal (Qin et al. 2003). In DMP1, it is the COOH-terminal fragment that contains the ASARM motif (Martin et al. 2008). The full length DMP1 is expressed at much lower levels (Huang et al. 2008) than its fragments which themselves have different localization patterns in bone (Maciejewska et al. 2008). In the growth plate, whilst the NH2-terminal fragment is localized to the resting, proliferation and pre-hypertrophic zones, the COOH-terminal fragment is found in the calcification
front and ossification zone (Maciejewska et al. 2008). The localization of the COOH-terminal fragment is consistent with areas that are targets for the vascular invasion of the cartilage, a significant phase in matrix mineralization. DMP1 has been postulated to play a role in angiogenesis as treatment with DMP1 induced vascular endothelial cadherin (VE-cadherin), and inhibited the VEGFR-2 activity, therefore suggesting DMP1 to be an inhibitor of VEGF-induced angiogenesis (Pirotte et al. 2011). The direct role of DMP1 on HA formation is highly dependent upon its processing, and it’s PTM. When phosphorylated, full length DMP1 has been shown to inhibit the formation and growth of HA (Tartaix et al. 2004), however, its dephosphorylated form and its two fragments are well established nucleators of HA formation (Tartaix et al. 2004; He et al. 2003; Gericke et al. 2010). Thus native DMP1 inhibits mineralization unless it becomes cleaved or dephosphorylated, in which case it initiates mineralization (Tartaix et al. 2004). The signaling pathways involved in DMP1 function have recently been investigated in osteoblasts. Wu et al., 2011 showed DMP1, through the activation of the αvβ3 integrin, to activate the downstream effectors of the MAPK pathway, ERK and JNK (Wu et al. 2011). Concomitant to this is the stimulation of phosphorylated JNK translocation coupled with an up-regulation of phosphorylated c-jun activation (Wu et al. 2011). Furthermore, it has been shown that the internalization of DMP1 not only results in a release of stored Ca2+, but also activates p38 MAP kinase (Eapen et al. 2011). DMP1 null mice have distinct abnormalities in the morphology and maturation of their osteocytes (Feng et al. 2006). The two DMP1 fragments also display differing localization patterns in osteocytes (Maciejewska et al. 2009), suggesting that osteocytes may play a critical role in ECM mineralization that involves DMP1. This is further supported by the stimulation of DMP1 expression in response to mechanical loading (Gluhak-Heinrich et al. 2007). Furthermore, the deletion of DMP1 leads to a dramatic increase in Fgf23 mRNA expression in the osteocytes, likely due to the defects seen in osteoblast-osteocyte transition (Feng et al. 2006; Qin et al. 2007). FGF23, a hormone produced by osteoblasts and osteocytes, has allowed the definition of bone as an endocrine organ as it targets the kidney to regulate Pi homeostasis. This therefore suggests that DMP1 can control Pi levels, as is in concurrence with the hypophosphatemia observed in the DMP1 null mouse (Feng et al. 2006; Ye et al. 2005).
SIBLING ASARM peptides in mineralization diseases

Accumulating evidence has implicated the members of the SIBLING family of proteins in bone and mineralization diseases. Their varying involvements in the process of matrix mineralization make them potentially attractive candidates for therapeutic targets and therapies.

X-linked hypophosphatemic rickets (XLH) is the most common form of inherited rickets, characterised by defective bone and tooth mineralization, growth retardation, and defective renal re-absorption of P_i (Carpenter et al. 2011). Mutations in PHEX, have been associated with XLH in humans, and have led to the development of the Hyp mouse (Holm et al. 1997). Hypophosphatemia alone is insufficient to explain the bone defect seen in the Hyp mouse as correction of the hypophosphatemia failed to correct the mineralization defect observed (Ecarot et al. 1992; Rowe et al. 2006). Furthermore, when osteoblast cells from the Hyp mouse are grown in culture, they have a defective ECM production and thus reduced mineralization (Xiao et al. 1998). This therefore suggests that PHEX has multiple substrates which are involved in regulating mineralization directly. MEPE was first identified as a potential substrate for PHEX however in vitro studies have failed to demonstrate PHEX-dependent hydrolysis of MEPE (Guo et al. 2002). It has also been suggested that PHEX is likely responsible for the cleavage of DMP1 and DSPP, as it has a strong preference for cleaving bonds at the N-terminal of aspartyl residues found in these two SIBLING proteins (Qin et al. 2004). However analysis of the Hyp mouse indicated no differences in DMP1 and DSPP mRNA expression in comparison to their wild-type controls, suggesting that DMP1 and DSPP are in fact properly processed in the PHEX-deficient mouse (Zhang et al. 2010). In addition to this, there is an accumulation of SIBLING ASARM peptides in the Hyp mouse and patients with XLH (Bresler et al. 2004; Martin et al. 2008; Boukpessi et al. 2010) thus challenging the hypothesis that the SIBLING proteins are substrates for PHEX. Instead it appears that it is the ASARM peptide which PHEX digests (Addison et al. 200, 2010). This therefore provides compelling evidence for the ASARM hypothesis, as proposed by Peter Rowe, which describes the role of the SIBLING ASARM peptides, PHEX, and FGF23 in bone renal phosphate homeostasis, and therefore mineralization (Fig. 2) (Rowe 2004; David et al. 2010). This hypothesis can be used to explain numerous disorders of mineralization
including tumor-induced osteomalacia, autosomal-dominant hypophosphatemic rickets, and XLH. The potential for a therapeutic treatment is therefore high however further elucidation of the exact mechanisms involved is required.

Conclusions

The aim of this review is to present an overview of the role that each member of the SIBLING family of proteins has in matrix mineralization. The SIBLING proteins are principally found in bone and dentin, and are secreted into the ECM during its formation and subsequent mineralization. It is apparent that the functional role of the SIBLING proteins is highly dependent upon their state of cleavage and their post translational modification (Table 1). Furthermore, the identification of the ASARM motif which is present across the SIBLING proteins is proving critical in the functional activity of the SIBLING proteins. Much remains to be learnt regarding the in vivo role of the SIBLING protein and the ASARM peptide in bone disease. Future investigations should focus upon determining the interactions between the SIBLING proteins and the potential therapeutic use that they may have.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution

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