REVIEW

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

Katherine A Staines, Vicky E MacRae and Colin Farquharson

The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Edinburgh, Midlothian EH25 9RG, UK

(Correspondence should be addressed to K A Staines; Email: katherine.staines@roslin.ed.ac.uk)

Abstract

The small integrin-binding ligand N-linked glycoprotein (SIBLING) family consists of osteopontin, bone sialoprotein, dentin matrix protein 1, dentin sialophosphoprotein and matrix extracellular phosphoglycoprotein. These proteins share many structural characteristics and are primarily located in bone and dentin. Accumulating evidence has implicated the SIBLING proteins in matrix mineralisation. Therefore, in this review, we discuss the individual role that each of

the SIBLING proteins has in this highly orchestrated process. In particular, we emphasise how the nature and extent of their proteolytic processing and post-translational modification affect their functional role. Finally, we describe the likely roles of the SIBLING proteins in clinical disorders of hypophosphataemia and their potential therapeutic use.

Journal of Endocrinology (2012) **214**, 241–255

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 an extracellular matrix (ECM) primarily composed 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 (IBSP)), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). It is likely that this protein family arose from the secretory calcium-binding phosphoprotein family by gene duplication due to their apparent common evolutionary heritage, as is elegantly reviewed by Kawasaki & Weiss (2006), Kawasaki *et al.* (2007), Kawasaki (2011) and Rowe (2012). It is therefore somewhat surprising that the SIBLING proteins have little intrinsic sequence homology and yet they share the following characteristics: i) all are located to a 375 kb region on the human chromosome 4q21 and mouse chromosome 5q, ii) display similar exon structures, iii) display an Arg-Gly-Asp (RGD) motif that mediates cell attachment/signalling and iv) are principally expressed in

bone and dentin and are secreted into the ECM during osteoid formation and subsequent mineralisation. These similarities in SIBLING gene and protein structure have been well illustrated in other reviews (Rowe *et al.* 2000, Fisher *et al.* 2001, Fisher & Fedarko 2003, Qin *et al.* 2004, Rowe 2004, 2012, Huq *et al.* 2005, Bellahcene *et al.* 2008).

All SIBLING proteins undergo similar post-translational modifications 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 RGD sequence that facilitates cell attachment and cell signalling by binding to cell surface integrins (Fisher *et al.* 2001). More recently, work by Rowe *et al.* (2000, 2004), primarily focused on MEPE, has identified a new functional domain termed the acidic serine- and aspartate-rich motif (ASARM) peptide, which is highly conserved across species. This peptide is proving critical in the functional activity of the SIBLING proteins, as is evidenced by the ASARM hypothesis proposed by Peter Rowe (Rowe 2004, David *et al.* 2010). This hypothesis describes the role of the SIBLING ASARM peptides, the cell membrane-associated glycoprotein phosphate-regulating endopeptidase homologue, X-linked (PHEX) and fibroblast growth factor 23 (FGF23) in bone renal phosphate (P_i) homeostasis and mineralisation. This hypothesis can be used to explain numerous disorders of mineralisation including tumour-induced osteomalacia, autosomal-dominant

hypophosphataemic rickets (ADHR) and X-linked hypophosphataemic rickets (XLH) and will be discussed in more detail in this review.

The SIBLING proteins have been extensively reviewed individually; however, in the present review, we focus on the role that each of the SIBLING proteins has on skeletal matrix mineralisation and bone remodelling, as well as their clinical relevance in disorders of bone matrix mineralisation and bone remodelling (Denhardt & Guo 1993, Ganss *et al.* 1999, Sodek *et al.* 2000, Fisher *et al.* 2001, Prasad *et al.* 2010).

Matrix mineralisation and bone remodelling

Endochondral ossification is a carefully orchestrated process responsible for 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 ECM that is rich in collagens, proteoglycans and numerous other NCPs (Ballock & O'Keefe 2003, Mackie *et al.* 2008, 2011, Gentili & Cancedda 2009, Heinegard 2009). The chondrocytes of the growth plate sit in distinct cellular zones of maturation and proceed through various stages of differentiation while maintaining their spatially fixed locations (Hunziker *et al.* 1987). It is the terminally differentiated hypertrophic chondrocyte that mineralises its surrounding ECM, localised to the longitudinal septa of the growth plate (Castagnola *et al.* 1988).

Chondrocyte, as well as osteoblast, mineralisation of the ECM is widely accepted to involve membrane-limited matrix vesicles (MVs) within which calcium (Ca^{2+}) and inorganic P_i accumulate to initiate the biphasic process of mineralisation (Anderson 2003). When sufficient concentrations of both exist, Ca^{2+} and P_i begins to precipitate to form hydroxyapatite (HA) crystals. This initial stage of mineralisation is followed by the penetration of HA crystals through the MV trilaminar membrane and the modulation of ECM composition, promoting the propagation of HA outside of the MVs (Anderson 1995, 2003, Wu *et al.* 2002, Golub 2011).

Mineralisation of the ECM is a tightly regulated process such that concentrations of Ca^{2+} and P_i are permissive for effective mineralisation and that the levels of mineralisation inhibitors such as inorganic pyrophosphate (PP_i) and matrix gla protein are balanced. Extracellular PP_i is a well-recognised and potent inhibitor of mineralisation that is regulated by ALP (Meyer 1984). In bone, ALP is an ectoenzyme 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_i required for HA formation; however, it has since been shown to also hydrolyse PP_i , thus achieving a ratio of P_i/PP_i

permissive for HA crystal formation and growth (Moss *et al.* 1967, Majeska & Wuthier 1975, Hessle *et al.* 2002, Anderson 2003). PP_i inhibits the enzymatic activity of ALP, offering a feedback loop by which mineralisation is regulated (Addison *et al.* 2007).

Other regulators of ECM biomineralisation include nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and the ankylosis protein (ANK) that work in synergy to increase extracellular PP_i levels. While NPP1 ectoplasmically generates PP_i from nucleoside triphosphates, ANK mediates its intracellular to extracellular channelling (Hakim *et al.* 1984, Terkeltaub *et al.* 1994, Ho *et al.* 2000). Analysis of mutant mice deficient in ALP function (*Akp2*^{-/-} (*Alpl*^{-/-})), which were surprisingly found to exhibit normal levels of bone mineralisation at birth, led us to search for other phosphatases that might also contribute to bone mineralisation, and this led to our description of PHOSPHO1 (Houston *et al.* 2002). As its discovery and characterisation, PHOSPHO1 has been proposed to play a crucial role in the accumulation of P_i within the MV and bone mineralisation (Houston *et al.* 2002, Stewart *et al.* 2006, Roberts *et al.* 2007, 2008, MacRae *et al.* 2010, Huesa *et al.* 2011). 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).

Mineralisation of the ECM not only facilitates the deposition of HA but also enables vascular invasion, a significant phase in endochondral ossification and the development of the skeleton. Hypertrophic chondrocytes express factors such as vascular endothelial growth factor (VEGF) that induce vascular invasion, allowing the infiltration of osteoclasts and differentiating osteoblasts that resorb the cartilaginous mineralised matrix and replace it with trabecular bone respectively (Zelzer *et al.* 2002). This process of bone remodelling continues throughout life and is responsible for the annual replacement of ~10% of the adult skeleton (Frost 1990). Tight regulation of this process maintains an equilibrium such that disorders of bone mass, such as osteoporosis or osteopetrosis, do not occur (Manolagas 2000). During bone resorption, osteoclasts adhere to the bone surface forming a tight connection and allowing efficient resorption through extracellular acidification (Palokangas *et al.* 1997, Mellis *et al.* 2011). 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 (Hill 1998, Manolagas 2000, Henriksen *et al.* 2009).

The SIBLING family of proteins

The SIBLING family of proteins consists of OPN, BSP, DMP1, DSPP and MEPE, all of which share common characteristics. Despite this, they display differential tissue distributions and functions that are highly dependent on their

post-translational modifications. The key role that each of the SIBLING proteins plays in biomineralisation is described in detail below (Fig. 1).

Matrix extracellular phosphoglycoprotein

MEPE, originally identified as a substrate for PHEX, is primarily expressed by osteocytes as well as by osteoblasts (Nampei *et al.* 2004). In the mouse skeleton, *Mepe* is detected as early as 2 days *post partum*, and several regulators of this expression have been documented in the literature (Lu *et al.* 2004). The addition of FGF2 to osteoblasts downregulates *Mepe* levels in a dose-dependent manner. The mechanism of action is part through the MAPK pathway (Zhang *et al.* 2004). Furthermore, osteoblasts stimulated by bone morphogenetic protein 2 (BMP2) also display a decreased *Mepe* expression level (Siggekkow *et al.* 2004). Recently, it has been shown that Wnt3a, a canonical Wnt signalling stimulator, induces this BMP2 signal and also as has its own direct stimulatory effects on *Mepe* expression through β -catenin and LEF1 (Cho *et al.* 2011).

The first evidence for a direct role of MEPE in bone mineralisation came from the increased mRNA expression levels of *Mepe* seen during osteoblast matrix mineralisation (Petersen *et al.* 2000, Argiro *et al.* 2001). The development of a *Mepe* null mouse further fuelled the proposed role of MEPE in mineralisation. 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* promoter, leads to a growth and mineralisation defect due to a decrease in bone remodelling. *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 on its state of cleavage and its phosphorylation. Recent work has identified the 2.2 kDa ASARM peptide of MEPE as the functional component of MEPE. This ASARM peptide is highly conserved across the SIBLING proteins, and in MEPE it is located immediately downstream of a

Figure 1 A schematic figure detailing the (A) expression and (B) function of the SIBLING family of proteins: dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), matrix extracellular phosphoglycoprotein (MEPE) and osteopontin (OPN). MEPE is expressed by osteoblasts and the terminally differentiated osteoblast (indicated by the dashed arrow), the osteocyte. MEPE directly inhibits hydroxyapatite (HA) formation in bone through its cleavage product, a small acidic serine- and aspartate-rich motif (ASARM) that undergoes post-translational phosphorylation. MEPE also inhibits the numbers and activities of osteoclasts. OPN has similar functional effects to MEPE in bone mineralisation; however, along with BSP, it is also expressed by osteoclasts. BSP is well established as a HA nucleator and is proving pivotal in diseases of increased bone formation as it increases osteoclastogenesis. DMP1 and DSPP are both expressed by bone and both are processed into numerous fragments. While DSPP promotes biomineralisation in both bones and teeth, DMP1 inhibits it. The full details of the cleavage products of the SIBLING proteins and their roles in biomineralisation are detailed in Table 1.

Table 1 The functional role of each of the SIBLING proteins: dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), matrix extracellular phosphoglycoprotein (MEPE) and osteopontin (OPN), in biomineralisation and phosphate homeostasis, as is dependent on their cleavage and post-translational modification. Detailed are i) the cellular expression pattern, ii) the phenotype of the knockout mouse, iii) the phenotype of transgenic mice, iv) clinical conditions associated with mutation in this gene, v) cleavage product and post-translational modification, vi) known role of each cleavage product in ECM mineralisation, vii) role of cleavage product mineral metabolism, and viii) list of relevant references

Protein	Mouse bone phenotype			Clinical condition of gene mutation	Cleavage product and post-translational modification	Role of cleavage products in mineralisation	Role of cleavage products in mineral metabolism	References
	Cellular expression pattern	Knockout	Overexpression					
MEPE	Osteoblasts and osteocytes	Increased bone mass, MAR, trabecular number and thickness	Decreased MAR, bone remodelling, bone mass and increased growth plate widths	Unknown	> ASARM peptide-3 serine phosphorylation > AC100	Inhibition Promotion	ASARM peptide inhibits phosphate uptake in the kidney and increases FGF23	Gowen <i>et al.</i> (2003), Hayashibara <i>et al.</i> (2004), Nampai <i>et al.</i> (2004), Addison <i>et al.</i> (2008), Marks <i>et al.</i> (2008), Martin <i>et al.</i> (2008) and David <i>et al.</i> (2009)
DMP1	Osteoblasts, osteoclasts, osteocytes, hypertrophic chondrocytes and dentin	Lower mineral content, defective cartilage formation resembling dwarfism with chondrodysplasia. Hypophosphataemia and increased FGF23	Narrow growth plate with accelerated mineralisation and increased bone turnover	Autosomal recessive hypophosphataemic rickets	Full-length, unphosphorylated DMP1 Full-length, phosphorylated DMP1 N-terminal fragment C-terminal fragment ASARM peptide	Promotion Inhibition Promotion Promotion Inhibition	Interacts with PHFEX to orchestrate P_i homeostasis through decreasing FGF23 levels	Toyosawa <i>et al.</i> (2001), Fen <i>et al.</i> (2002), Feng <i>et al.</i> (2003), Qin <i>et al.</i> (2003), Tartaix <i>et al.</i> (2004), Ye <i>et al.</i> (2005), Feng <i>et al.</i> (2006) and Martin <i>et al.</i> (2008)
OPN	Osteoblasts, osteoclasts, osteocytes and hypertrophic chondrocytes	Increased mineral content and size Increased osteoclast production	Bone phenotype not defined	Unknown	> ASARM peptide-3 serine phosphorylation > N-terminal fragment > C-terminal fragment > Central fragment Unknown	Inhibition Promotion Promotion Inhibition	Opn knockout mice have no differences in serum P_i or Ca^{2+} ; however, known interactions with PTH suggest an indirect role	Dodds <i>et al.</i> (1995), Sodek <i>et al.</i> (1995), Boskey <i>et al.</i> (2002, 2012), Landis <i>et al.</i> (2003) and Addison <i>et al.</i> (2007)
BSP	Osteoblasts, osteoclasts, osteocytes, chondrocytes and dentin	Short hypomineralised bones with high trabecular bone mass and low bone turnover	Multi-dwarfism decreased BMD and decreased trabecular bone volume	Unknown	Unknown	Promoter	Bsp transgenic mice have increased Ca^{2+} levels but no difference in P_i levels compared with WT mice	Chen <i>et al.</i> (1992), Gordon <i>et al.</i> (2007), Malaval <i>et al.</i> (2008) and Valverde <i>et al.</i> (2008)

(continued)

Table 1 Continued

Cellular expression pattern	Mouse bone phenotype			Clinical condition of gene mutation	Cleavage product and post-translational modification	Role of cleavage products in mineralisation	Role of cleavage products in mineral metabolism	References
	Knockout	Overexpression						
DSPP	Dentin, bone and cementum Defect in dentin mineralisation. Bones display accelerated mineralisation and changes in structural properties	DSP-accelerated mineralisation in teeth yet DPP-deletious effects on enamel		Dentinogenesis imperfecta type II/III and dentine dysplasia	DPP, phosphorylated DPP, unphosphorylated DPP DGP	Promoter No effect Promoter Unknown	DPP fragment (ASARM containing) may competitively displace the DMP1–PHEX complex	Sreenath <i>et al.</i> (2003), Kim <i>et al.</i> (2005), Yamakoshi <i>et al.</i> (2005), Verdelis <i>et al.</i> (2008) and Prasad <i>et al.</i> (2010)

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_i . This is likely through a decreased expression of the type II sodium-dependent P_i cotransporter NPT2a, or through the promotion of FGF23 expression, a potent inhibitor of P_i (Liu *et al.* 2007, Dobbie *et al.* 2008, Marks *et al.* 2008, Martin *et al.* 2008, David *et al.* 2010, Shirley *et al.* 2010). It has, however, been suggested that MEPE may have a direct effect on matrix mineralisation outwith the supply and demand of P_i . The ASARM peptide of MEPE inhibits mineralisation by osteoblasts by directly binding to HA crystals (Addison *et al.* 2008, Martin *et al.* 2008). 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 mineralisation (Addison *et al.* 2008, Martin *et al.* 2008). This is not the only evidence for a role for MEPE in the promotion of mineralisation. Recently, it has been shown that a truncated form of MEPE, which has the ASARM peptide removed, can promote bone mineralisation 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 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 neutralising their activity by sequestration and hydrolysis (Liu *et al.* 2007, Addison *et al.* 2008, Martin *et al.* 2008). Recently, it has been shown that sclerostin (SCL), a potent inhibitor of the canonical Wnt signalling pathway, may act through the MEPE–PHEX axis, highlighting its significance in biomineralisation (Atkins *et al.* 2011).

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). In the MEPE-overexpressing mouse, vascularisation is increased, as is VEGF expression, highlighting a role for MEPE in angiogenesis, an important stage in endochondral ossification (David *et al.* 2009). Consonant with angiogenesis is the

infiltration of osteoclasts for bone resorption. Interestingly, mice administered with recombinant MEPE or transgenic for MEPE had a significant decrease in the numbers and activity of osteoclasts (Hayashibara *et al.* 2007, David *et al.* 2009). This therefore suggests that MEPE is highly relevant to both bone mineralisation and P_i homeostasis. Future studies should focus on the interactions between MEPE and the Wnt signalling pathway due to its known implications in bone and cartilage mechanobiology.

Osteopontin

OPN, also known as secreted phosphoprotein 1 (SPP1), is a 34 kDa protein, originally identified as the bridge between the cells and HA in the ECM of bone (Sodek *et al.* 2000). The protein and gene structures, as well as the localisation, of OPN are well described in several excellent reviews (Denhardt & Guo 1993, Sodek *et al.* 2000, Fisher *et al.* 2001). In bone, OPN is produced by osteoblasts and osteocytes, as well as osteoclasts (Dodds *et al.* 1995, Sodek *et al.* 1995, Zohar *et al.* 1997). It has also been localised to hypertrophic cartilage of the growth plate (Landis *et al.* 2003).

Several studies have documented the inhibitory role of OPN in HA formation and growth (Boskey *et al.* 1993, 2012, Hunter *et al.* 1994). It has also been shown to inhibit mineralisation in vascular smooth muscle cells (Wada *et al.* 1999, Jono *et al.* 2000). This inhibitory role of OPN is confirmed further by analysis of the *Opn* knockout mouse that has increased mineral content and size, as shown by Fourier transform infrared spectroscopy 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 mineralisation by binding to HA crystals (Addison *et al.* 2010, Boskey *et al.* 2012). Furthermore, a recent study by Boskey *et al.* showed the C- and N-terminal fragments of OPN, in this study, derived from milk OPN to promote *de novo* HA formation. Conversely, a central fragment inhibited it as is similar to bone OPN (Boskey *et al.* 2012). This highlights the importance of the post-translational fragmentation of OPN in determining its function. The study by Addison *et al.* (2010) also showed that, like MEPE, the ability of the OPN-ASARM to inhibit mineralisation is dependent on its phosphorylation at specific serine residues.

The importance of post-translational phosphorylation is further confirmed when examining the interaction between OPN, ALP and PP_i . Several studies have shown that ALP dephosphorylates OPN, thus preventing much of its inhibitory activity on HA formation and growth (Boskey *et al.* 1993, Hunter *et al.* 1994, Jono *et al.* 2000). Furthermore, PP_i directly upregulates *Opn* expression in osteoblasts, and therefore the hydrolysis of PP_i 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 PP_i deficiency brings about a deficiency in OPN (Johnson *et al.* 2003). The *Akp2*-deficient mouse

displays a similar decreased PP_i and OPN with an associated hypomineralisation. This hypomineralisation can be partially rescued by the double knockout: the *Akp2*^{-/-}/*Opn*^{-/-} mouse (Harmey *et al.* 2006). Although previous studies have implicated a P_i -dependent mechanism (Beck *et al.* 2000, Beck & Knecht 2003), work by Addison *et al.* has implicated the MAPK signalling pathways responsible for the regulation of OPN by PP_i .

Analysis of the *Opn*^{-/-} mouse has also indicated a role for OPN in the function and activity of osteoclasts. In these mice, there is an increase in osteoclast production, which could be a compensatory mechanism for the observed disabled motility and resorption activity of the osteoclast cells (Rittling *et al.* 1998, 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).

The loading of the skeleton in daily function results in the continuous modelling and remodelling of the skeleton (Frost 1990). This loading upregulates *OPN* expression in bone *in vivo*, and more recently it has been shown that the cyclical loading of rabbit joints has shown increased cellular *OPN* expression in the cartilage as well (Terai *et al.* 1999, Morinobu *et al.* 2003, Gross *et al.* 2005, King *et al.* 2005, Fujihara *et al.* 2006). This upregulation in response to loading has also been shown in *in vitro* cell cultures, and it is thought that MAPKs are involved in the transduction of the stimulus for *OPN* expression (Klein-Nulend *et al.* 1997, Owan *et al.* 1997, You *et al.* 2001). These intriguing results provide some clues into the molecular mechanisms underpinning adaptive bone remodelling.

Bone sialoprotein

BSP is a 70–80 kDa protein for which its gene and protein structures 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 the mineralised tissues such as bone, dentin and mineralising cartilage (Bianco *et al.* 1991, Chen *et al.* 1991). In bone, it is expressed in abundance by osteoblasts, as well as by osteoclasts, osteocytes and chondrocytes (Fisher & Fedarko 2003, Gordon *et al.* 2007).

During embryogenesis, BSP is first expressed at the onset of bone formation, thus suggesting it 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, which is localised to MVs, to be involved in the initial formation of HA (Harris *et al.* 2000, Fisher *et al.* 2001, Tye *et al.* 2003, Wang *et al.* 2006, Nahar *et al.* 2008). Indeed, the *Bsp* null mouse displays shorter, hypomineralised bones with associated higher trabecular bone mass with low bone turnover (Malaval *et al.* 2008). Moreover, it has been shown that as little as 9 nM BSP is required to nucleate HA, and recently the overexpression of BSP in osteoblasts has been shown to enhance mineralisation (Hunter *et al.* 1996,

Gordon *et al.* 2007). Similarly, osteoblast cultures grown in the presence of an anti-BSP antibody exhibit reduced mineralisation (Cooper *et al.* 1998, Mizuno *et al.* 2000). This nucleation potency is increased on BSP binding to collagen, suggesting a cooperative relationship (Baht *et al.* 2008).

The role of BSP as a HA nucleator is thought to involve the membrane-bound enzyme, ALP. Indeed, in the presence of BSP, high levels of ALP activity can promote the initiation of mineral deposition (Wang *et al.* 2006). This is further confirmed in BSP-overexpressing cell cultures that 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 on its post-translational modification (Stubbs *et al.* 1997).

BSP increases osteoclastogenesis and therefore bone resorption, making it crucial in the homeostasis of bone remodelling (Ross *et al.* 1993, Raynal *et al.* 1996, Malaval *et al.* 2008, 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). Furthermore, serum BSP expression in bone diseases characterised by excessive bone resorption, e.g. Paget's disease, is abnormally high (Valverde *et al.* 2008). This highlights the need to investigate whether antibodies to BSP could decrease the pathological bone loss observed in the *Bsp* transgenic mouse and as such be an important therapeutic target for patients with bone diseases characterised by high BSP.

Dentin sialophosphoprotein

The role of DSPP in biomineralisation 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-mineralising tissues including the lung and kidney (Qin *et al.* 2002, Baba *et al.* 2004, Alvares *et al.* 2006, Ogbureke & Fisher 2007, Verdelis *et al.* 2008).

Analysis of the *Dspp* knockout mouse reveals defects in dentin mineralisation (Sreenath *et al.* 2003), as well as bone hypomineralisation (Verdelis *et al.* 2008). In humans, a mutation in the *DSPP* gene results in dentinogenesis imperfecta, characterised by dentin hypomineralisation and significant tooth decay (Kim *et al.* 2005). Of particular interest are the variations in the mineralisation properties observed at different ages in the *Dspp*^{-/-} mouse. At 5 weeks of age, these mice displayed accelerated mineralisation, while at 9 months of age significant changes in bone structural properties were observed. This therefore suggests that DSPP has roles not only in the initial mineralisation of bone but also in the remodelling of the skeleton and therefore on bone turnover (Verdelis *et al.* 2008).

DSPP is proteolytically processed to two fragments: dentin phosphoprotein and dentin sialoprotein (DSP), both of which have important functions in mineralisation. Interestingly, a third fragment called dentin glycoprotein (DGP) has been identified as being cleaved from the C-terminal end of DSP

by matrix metalloproteinase 2 (MMP2) and MMP20 (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 (Zhang *et al.* 2001, Qin *et al.* 2004, Prasad *et al.* 2010). The cleavage of DPP from DSPP is catalysed by a group of zinc metalloproteinases that includes BMP1, and it is this fragment of DSPP that contains the ASARM peptide (Tsuchiya *et al.* 2011). Various studies have shown DPP to be important in the formation and growth of HA as it has a strong affinity to Ca²⁺ 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 as 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 mineralisation, 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 signalling pathway as BMP2 has been shown to increase *Dspp* expression via BMPR Smads, Runx2 and Dlx5 (Iohara *et al.* 2004, Chen *et al.* 2008, Cho *et al.* 2010).

The vast information obtained about the DPP and DSP fragments over the past few decades serves to strengthen knowledge on the role of DSPP in biomineralisation. Future studies should focus on the recently identified DGP fragment and its specific functional role, as well as further detailing the mechanisms of DSP and DPP functions.

Dentin matrix protein 1

DMP1 was first cloned from dentin and has since been identified in dentin, bone and cementum as well as in other non-mineralised tissues (George *et al.* 1993, MacDougall *et al.* 1998, Sun *et al.* 2011). In bone, DMP1 is primarily expressed not only by osteocytes but also by osteoblasts and hypertrophic chondrocytes (Toyosawa *et al.* 2001, Fen *et al.* 2002, Feng *et al.* 2003).

The first evidence of a role for DMP1 in biomineralisation was its promotion of ECM mineralisation in MC3T3 cells overexpressing DMP1 (Narayanan *et al.* 2001). The generation of a *Dmp1*-null mouse has further fuelled the potential role of DMP1 in bone mineralisation. The knockout mice have significantly lower mineral content when compared with their control counterparts (Ling *et al.* 2005). Interestingly, the re-expression of DMP1 in these *Dmp1* null mice rescues the skeletal defects seen (Lu *et al.* 2011).

Additionally, the *Dmp1*-deficient mice displayed a severe defect in cartilage formation as is similar to the human hereditary hypophosphatemic disease autosomal recessive hypophosphatemic rickets (ARHR) that is caused by mutations in *Dmp1* (Feng *et al.* 2006, Farrow *et al.* 2009). These mice display 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).

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 growth plate enlargement (Sun *et al.* 2010). Interestingly, the *Dmp1* null mouse displays increased serum FGF23 levels and associated hypophosphataemia (Feng *et al.* 2006). Correction of this hypophosphataemia, by a high P_i diet, restored the *Dmp1* null mouse growth plate defect (Feng *et al.* 2006). Furthermore, the *DMP1*^{-/-} and *FGF23*^{-/-} double knockout mice display growth plate widths similar to that seen in the single *Fgf23* null mouse (Liu *et al.* 2008). This therefore suggests that the defective cartilage mineralisation observed in the *Dmp1* null mouse is not simply a direct consequence of the lack of DMP1. More recently, a transgenic mouse has been developed that expresses a mutant form of *Dmp1*. The substitution of Asp213 with Ala213 blocks the processing of mouse *Dmp1*. Crossing this transgenic mouse with the *Dmp1* null mouse recovered the growth plate disorganisation seen in the null mouse alone (Sun *et al.* 2011).

Like other SIBLING proteins, the proteolytic processing of DMP1 appears essential to its function and localisation. In bone and dentin, DMP1 is processed to two fragments: one 37 kDa fragment originating from the NH₂-terminal and one 57 kDa fragment originating from the COOH-terminal (Qin *et al.* 2003). In DMP1, it is the COOH-terminal fragment that contains the ASARM peptide (Martin *et al.* 2008). The full-length DMP1 is expressed at much lower levels than its fragments, which themselves have different localisation patterns in bone (Huang *et al.* 2008, Maciejewska *et al.* 2008). In the growth plate, while the NH₂-terminal fragment is localised 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 localisation of the COOH-terminal fragment is consistent with areas that are targets for the vascular invasion of the cartilage, a significant phase in matrix mineralisation. DMP1 has been postulated to play a role in angiogenesis as treatment with DMP1-induced vascular endothelial cadherin (VE-cadherin) and inhibited the VEGFR2 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 on its processing and its post-translational modification. When phosphorylated, full-length DMP1 has been shown to inhibit the formation and growth of HA; however, its dephosphorylated form and its two fragments are well-established nucleators of HA formation (He *et al.* 2003, Tartaix *et al.* 2004, Gericke *et al.* 2010). Thus, native DMP1 inhibits mineralisation unless it becomes cleaved or dephosphorylated, in which case it initiates mineralisation (Tartaix *et al.* 2004).

In addition to the ASARM peptide, signalling pathways are involved in DMP1 function and have recently been investigated in osteoblasts. Wu *et al.* (2011) showed that DMP1, through the activation of the $\alpha v\beta 3$ integrin, activated 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

upregulation of phosphorylated c-jun activation (Wu *et al.* 2011). Furthermore, it has been shown that the internalisation 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 localisation patterns in osteocytes (Maciejewska *et al.* 2009), suggesting that osteocytes may play a critical role in ECM mineralisation 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* 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 P_i homeostasis. This therefore suggests that DMP1 can control P_i levels, as is consistent with the hypophosphataemia observed in the *Dmp1* null mouse (Ye *et al.* 2005, Feng *et al.* 2006). This important discovery has allowed the further development of the ASARM hypothesis and has implicated DMP1 as central to biomineralisation and P_i homeostasis.

The ASARM hypothesis and bone diseases

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

XLH is the most common form of inherited rickets, characterised by defective bone and tooth mineralisation, growth retardation and defective renal reabsorption 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). Hypophosphataemia alone is insufficient to explain the bone defect seen in the *Hyp* mouse as correction of the hypophosphataemia failed to correct the mineralisation defect observed (Ecarot *et al.* 1992, Rowe *et al.* 2006). Furthermore, when osteoblast cells from the *Hyp* mouse are grown in culture, they have defective ECM production and thus reduced mineralisation (Xiao *et al.* 1998). This therefore suggests that PHEX has multiple substrates that are involved in regulating mineralisation directly and this has allowed the creation of the ASARM hypothesis, as previously mentioned and as has recently been elegantly reviewed (Rowe 2004, 2012, David *et al.* 2010). The ASARM hypothesis is based on the concept of a minihibin, an unknown secreted factor that is a substrate for PHEX and therefore would accumulate in the *Hyp* mouse and in patients with XLH.

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 these two SIBLING proteins (Qin *et al.* 2004). However, analysis of the *Hyp* mouse indicated no differences in *Dmp1* and *Dspp* expression in comparison with their WT 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, thus challenging the hypothesis that the SIBLING proteins are substrates for PHEX. Instead, it appears that it is the ASARM peptide that PHEX digests (Addison *et al.* 2008, 2010), and the rise in SIBLING ASARM peptides in the *Hyp* mouse and XLH therefore further implicates them as substrates for PHEX (Bresler *et al.* 2004, Martin *et al.* 2008, Boukpepsi *et al.* 2010).

It also appears that PHEX regulates *Fgf23* expression as increased *Fgf23* expression is observed in the *Hyp* mouse and patients with XLH (Liu *et al.* 2006). Accordingly, *Fgf23* knockout reversed the hypophosphataemia observed in *Hyp* mice (Sitara *et al.* 2004). Although initial studies appeared to confirm FGF23 as a substrate for PHEX, this has not been shown since (Bowe *et al.* 2001). Interestingly, a similar increase in *FGF23* expression is observed in models of loss of DMP1, along with associated ARHR (Feng *et al.* 2006, Lorenz-Depiereux *et al.* 2006). This has led to the suggestion that a PHEX–DMP1 interaction is responsible for orchestrating mineralisation through decreasing *FGF23* expression. Furthermore, current paradigm suggests that ASARM peptides can competitively displace this PHEX complex and this would therefore increase FGF23 activity, as is seen in the *Hyp* mouse and in patients with XLH (David *et al.* 2010, Martin *et al.* 2011, Rowe 2012).

Additionally, the accumulation of ASARM peptides can directly inhibit Na⁺-dependent P_i uptake in the kidney, as has been shown both *in vivo* and *in vitro*, thus exacerbating the upregulation of *FGF23* expression, the downregulation of 1,25(OH)₂D₃ and the inhibition of hypophosphataemia observed in XLH, ARHR and ADHR (Rowe *et al.* 2004, Dobbie *et al.* 2008, Marks *et al.* 2008, David *et al.* 2010, Shirley *et al.* 2010). The decrease in 1,25(OH)₂D₃ provides a feedback loop for increased *PHEX* expression through the increased expression of a 100 kDa transcription factor, a requirement for this *PHEX* expression (Ecarot & Desbarats 1999).

This regulatory loop of *ASARM*, *PHEX* and *FGF23* expression and function highlights the multiple and complex functions of the SIBLING ASARM peptides in both P_i homeostasis and matrix mineralisation in disease and health. It is therefore vital that we endeavour to fully establish the interactions within this hypothesis to allow future therapeutic developments.

Certainly, much remains to be learnt regarding the *in vivo* role of the SIBLING proteins and the ASARM peptide in bone diseases. This is not just in disorders related to P_i

homeostasis but also to other bone diseases such as osteoporosis and osteoarthritis (OA). Indeed, there are close links between the SIBLING proteins and OA, with serum BSP and OPN levels significantly correlating with OA disease severity (Petersson *et al.* 1998, Hasegawa *et al.* 2011). Furthermore, microarray data and gene analysis studies have highlighted MEPE and DMP1 as being differentially expressed in OA tissues (Hopwood *et al.* 2007, Sanchez *et al.* 2008). The interaction between MEPE and SCL, as described previously, is an exciting development due to the known anabolic effects of the SCL-neutralising antibodies on osteoporosis (Li *et al.* 2009, 2010, Atkins *et al.* 2011). This could therefore warrant investigation into the potential therapeutic use of MEPE in osteoporosis and potentially in OA due to the ever emerging role of SCL in this debilitating disease (Power *et al.* 2010, Chan *et al.* 2011, Delgado-Calle *et al.* 2011).

Conclusions

The aim of this review is to present an overview of the role of each member of the SIBLING family of proteins in matrix mineralisation. The SIBLING proteins are principally found in bone and dentin and are secreted into the ECM during its formation and subsequent mineralisation. It is apparent that the functional role of the SIBLING proteins is highly dependent on their state of cleavage and their post-translational modification (Table 1). Furthermore, the identification of the ASARM peptide, which is present across the SIBLING proteins, is proving critical in the functional activity of the SIBLING proteins. Future investigations should focus on determining the underpinning interactions between the SIBLING proteins and their place within the current ASARM hypothesis. This will allow the investigation into their potential therapeutic application to disorders of mineralisation including disorders of hypophosphataemia, osteoporosis and OA.

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.

Funding

The authors acknowledge the Institute Strategic Programme Grant Funding from the Biotechnology and Biological Sciences Research Council (BBSRC) UK (C F, V M) and BBSRC studentship funding (K S) for support.

Author contribution statement

K S drafted the manuscript. K S, V M and C F revised the manuscript content. K S, V M and C F approved the final manuscript.

References

- Addison WN, Azari F, Sorensen ES, Kaartinen MT & McKee MD 2007 Pyrophosphate inhibits mineralization of osteoblast cultures by binding to mineral, up-regulating osteopontin, and inhibiting alkaline phosphatase activity. *Journal of Biological Chemistry* **282** 15872–15883. (doi:10.1074/jbc.M701116200)
- Addison WN, Nakano Y, Loisel T, Crine P & McKee MD 2008 MEPE–ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. *Journal of Bone and Mineral Research* **23** 1638–1649. (doi:10.1359/jbmr.080601)
- Addison WN, Masica DL, Gray JJ & McKee MD 2010 Phosphorylation-dependent inhibition of mineralization by osteopontin ASARM peptides is regulated by PHEX cleavage. *Journal of Bone and Mineral Research* **25** 695–705. (doi:10.1002/jbmr.110)
- Alvares K, Kanwar YS & Veis A 2006 Expression and potential role of dentin phosphophoryn (DPP) in mouse embryonic tissues involved in epithelial–mesenchymal interactions and branching morphogenesis. *Developmental Dynamics* **235** 2980–2990. (doi:10.1002/dvdy.20935)
- Anderson HC 1995 Molecular biology of matrix vesicles. *Clinical Orthopaedics and Related Research* **314** 266–280.
- Anderson HC 2003 Matrix vesicles and calcification. *Current Rheumatology Reports* **5** 222–226. (doi:10.1007/s11926-003-0071-z)
- Argio L, Desbarats M, Glorieux FH & Ecarot B 2001 Mepe, the gene encoding a tumor-secreted protein in oncogenic hypophosphatemic osteomalacia, is expressed in bone. *Genomics* **74** 342–351. (doi:10.1006/geno.2001.6553)
- Arufo A, Stamenkovic I, Melnick M, Underhill CB & Seed B 1990 CD44 is the principal cell surface receptor for hyaluronate. *Cell* **61** 1303–1313. (doi:10.1016/0092-8674(90)90694-A)
- Atkins GJ, Rowe PS, Lim HP, Wellton KJ, Ormsby R, Wijenayaka AR, Zelenchuk L, Evdokiou A & Findlay DM 2011 Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE–ASARM-dependent mechanism. *Journal of Bone and Mineral Research* **26** 1425–1436. (doi:10.1002/jbmr.345)
- Baba O, Qin C, Brunn JC, Wygant JN, McIntyre BW & Butler WT 2004 Colocalization of dentin matrix protein 1 and dentin sialoprotein at late stages of rat molar development. *Matrix Biology* **23** 371–379. (doi:10.1016/j.matbio.2004.07.008)
- Baht GS, Hunter GK & Goldberg HA 2008 Bone sialoprotein–collagen interaction promotes hydroxyapatite nucleation. *Matrix Biology* **27** 600–608. (doi:10.1016/j.matbio.2008.06.004)
- Ballock RT & O'Keefe RJ 2003 The biology of the growth plate. *Journal of Bone and Joint Surgery* **85-A** 715–726.
- Beck GR Jr & Knecht N 2003 Osteopontin regulation by inorganic phosphate is ERK1/2-, protein kinase C-, and proteasome-dependent. *Journal of Biological Chemistry* **278** 41921–41929. (doi:10.1074/jbc.M304470200)
- Beck GR Jr, Zerler B & Moran E 2000 Phosphate is a specific signal for induction of osteopontin gene expression. *PNAS* **97** 8352–8357. (doi:10.1073/pnas.140021997)
- Bellahcene A, Castronovo V, Ogbureke KU, Fisher LW & Fedarko NS 2008 Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nature Reviews. Cancer* **8** 212–226. (doi:10.1038/nrc2345)
- Bianco P, Fisher LW, Young MF, Termine JD & Robey PG 1991 Expression of bone sialoprotein (BSP) in developing human tissues. *Calcified Tissue International* **49** 421–426. (doi:10.1007/BF02555854)
- Boskey AL, Maresca M, Doty S, Sabsay B & Veis A 1990 Concentration-dependent effects of dentin phosphophoryn in the regulation of *in vitro* hydroxyapatite formation and growth. *Bone and Mineral* **11** 55–65. (doi:10.1016/0169-6009(90)90015-8)
- Boskey AL, Maresca M, Ullrich W, Doty SB, Butler WT & Prince CW 1993 Osteopontin–hydroxyapatite interactions *in vitro*: inhibition of hydroxyapatite formation and growth in a gelatin–gel. *Bone and Mineral* **22** 147–159. (doi:10.1016/S0169-6009(88)80225-5)
- Boskey AL, Spevak L, Paschalis E, Doty SB & McKee MD 2002 Osteopontin deficiency increases mineral content and mineral crystallinity in mouse bone. *Calcified Tissue International* **71** 145–154. (doi:10.1007/s00223-001-1121-z)
- Boskey AL, Chiang P, Fermanis A, Brown J, Taleb H, David V & Rowe PS 2009 MEPE's diverse effects on mineralization. *Calcified Tissue International* **86** 42–46. (doi:10.1007/s00223-009-9313-z)
- Boskey AL, Christensen B, Taleb H & Sorensen ES 2012 Post-translational modification of osteopontin: effects on *in vitro* hydroxyapatite formation and growth. *Biochemical and Biophysical Research Communications* **419** 333–338. (doi:10.1016/j.bbrc.2012.02.024)
- Boukpeissi T, Gaucher C, Leger T, Salmon B, Le FJ, Willig C, Rowe PS, Garabedian M, Meilhac O & Chaussain C 2010 Abnormal presence of the matrix extracellular phosphoglycoprotein-derived acidic serine- and aspartate-rich motif peptide in human hypophosphatemic dentin. *American Journal of Pathology* **177** 803–812. (doi:10.2353/ajpath.2010.091231)
- Bowe AE, Finnegan R, Jan de Beur SM, Cho J, Levine MA, Kumar R & Schiavi SC 2001 FGF-23 inhibits renal tubular phosphate transport and is a PHEX substrate. *Biochemical and Biophysical Research Communications* **284** 977–981. (doi:10.1006/bbrc.2001.5084)
- Bresler D, Bruder J, Mohnike K, Fraser WD & Rowe PS 2004 Serum MEPE–ASARM-peptides are elevated in X-linked rickets (HYP): implications for phosphaturia and rickets. *Journal of Endocrinology* **183** R1–R9. (doi:10.1677/joe.1.05989)
- Carpenter TO, Imel EA, Holm IA, Jan de Beur SM & Insogna KL 2011 A clinician's guide to X-linked hypophosphatemia. *Journal of Bone and Mineral Research* **26** 1381–1388. (doi:10.1002/jbmr.340)
- Castagnola P, Dozin B, Moro G & Cancedda R 1988 Changes in the expression of collagen genes show two stages in chondrocyte differentiation *in vitro*. *Journal of Cell Biology* **106** 461–467. (doi:10.1083/jcb.106.2.461)
- Chan BY, Fuller ES, Russell AK, Smith SM, Smith MM, Jackson MT, Cake MA, Read RA, Bateman JF, Sambrook PN *et al.* 2011 Increased chondrocyte sclerostin may protect against cartilage degradation in osteoarthritis. *Osteoarthritis and Cartilage* **19** 874–885. (doi:10.1016/j.joca.2011.04.014)
- Chellaiah MA, Kizer N, Biswas R, Alvarez U, Strauss-Schoenberger J, Rifas L, Rittling SR, Denhardt DT & Hruska KA 2003 Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. *Molecular Biology of the Cell* **14** 173–189. (doi:10.1091/mbc.E02-06-0354)
- Chen JK, Shapiro HS, Wrana JL, Reimers S, Heersche JN & Sodek J 1991 Localization of bone sialoprotein (BSP) expression to sites of mineralized tissue formation in fetal rat tissues by *in situ* hybridization. *Matrix* **11** 133–143. (doi:10.1016/S0934-8832(11)80217-9)
- Chen J, Shapiro HS & Sodek J 1992 Development expression of bone sialoprotein mRNA in rat mineralized connective tissues. *Journal of Bone and Mineral Research* **7** 987–997. (doi:10.1002/jbmr.5650070816)
- Chen S, Gluhak-Heinrich J, Martinez M, Li T, Wu Y, Chuang HH, Chen L, Dong J, Gay I & MacDougall M 2008 Bone morphogenetic protein 2 mediates dentin sialophosphoprotein expression and odontoblast differentiation via NF- κ B signaling. *Journal of Biological Chemistry* **283** 19359–19370. (doi:10.1074/jbc.M709492200)
- Cho YD, Yoon WJ, Woo KM, Baek JH, Park JC & Ryoo HM 2010 The canonical BMP signaling pathway plays a crucial part in stimulation of dentin sialophosphoprotein expression by BMP-2. *Journal of Biological Chemistry* **285** 36369–36376. (doi:10.1074/jbc.M110.103093)
- Cho YD, Kim WJ, Yoon WJ, Woo KM, Baek JH, Lee G, Kim GS & Ryoo HM 2011 Wnt3a stimulates Mepe, matrix extracellular phosphoglycoprotein, expression directly by the activation of the canonical Wnt signaling pathway and indirectly through the stimulation of autocrine Bmp-2 expression. *Journal of Cellular Physiology* **227** 2287–2296. (doi:10.1002/jcp.24038)
- Cooper LE, Yliheikkilä PK, Felton DA & Whitson SW 1998 Spatiotemporal assessment of fetal bovine osteoblast culture differentiation indicates a role for BSP in promoting differentiation. *Journal of Bone and Mineral Research* **13** 620–632. (doi:10.1359/jbmr.1998.13.4.620)

- David V, Martin A, Hedge AM & Rowe PS 2009 Matrix extracellular phosphoglycoprotein (MEPE) is a new bone renal hormone and vascularization modulator. *Endocrinology* **150** 4012–4023. (doi:10.1210/en.2009-0216)
- David V, Martin AC, Hedge AM, Drezner MK & Rowe PS 2010 ASARM peptides: PHEX-dependent, independent regulation of serum phosphate. *American Journal of Physiology - Renal Physiology* **300** F783–F791. (doi:10.1152/ajprenal.00304.2010)
- Delgado-Calle J, Arozamena J, Garcia-Renedo R, Garcia-Ibarbia C, Pascual-Carra MA, Gonzalez-Macias J & Riancho JA 2011 Osteocyte deficiency in hip fractures. *Calcified Tissue International* **89** 327–334. (doi:10.1007/s00223-011-9522-0)
- Denhardt DT & Guo X 1993 Osteopontin: a protein with diverse functions. *FASEB Journal* **7** 1475–1482.
- Dobbie H, Unwin RJ, Faria NJ & Shirley DG 2008 Matrix extracellular phosphoglycoprotein causes phosphaturia in rats by inhibiting tubular phosphate reabsorption. *Nephrology, Dialysis, Transplantation* **23** 730–733. (doi:10.1093/ndt/gfm535)
- Dodds RA, Connor JR, James IE, Rykaczewski EL, Appelbaum E, Dul E & Gowen M 1995 Human osteoclasts, not osteoblasts, deposit osteopontin onto resorption surfaces: an *in vitro* and *ex vivo* study of remodeling bone. *Journal of Bone and Mineral Research* **10** 1666–1680. (doi:10.1002/jbmr.5650101109)
- Eapen A, Ramachandran A, Pratap J & George A 2011 Activation of the ERK1/2 mitogen-activated protein kinase cascade by dentin matrix protein 1 promotes osteoblast differentiation. *Cells, Tissues, Organs* **194** 255–260. (doi:10.1159/000324258)
- Ecarot B & Desbarats M 1999 1,25-(OH)₂D₃ down-regulates expression of Phex, a marker of the mature osteoblast. *Endocrinology* **140** 1192–1199. (doi:10.1210/en.140.3.1192)
- Ecarot B, Glorieux FH, Desbarats M, Travers R & Labelle L 1992 Effect of dietary phosphate deprivation and supplementation of recipient mice on bone formation by transplanted cells from normal and X-linked hypophosphatemic mice. *Journal of Bone and Mineral Research* **7** 523–530. (doi:10.1002/jbmr.5650070508)
- Farrow EG, Davis SI, Ward LM, Summers LJ, Bubbear JS, Keen R, Stamp TC, Baker LR, Bonewald LF & White KE 2009 Molecular analysis of DMP1 mutants causing autosomal recessive hypophosphatemic rickets. *Bone* **44** 287–294. (doi:10.1016/j.bone.2008.10.040)
- Fen JQ, Zhang J, Dallas SL, Lu Y, Chen S, Tan X, Owen M, Harris SE & MacDougall M 2002 Dentin matrix protein 1, a target molecule for Cbfa1 in bone, is a unique bone marker gene. *Journal of Bone and Mineral Research* **17** 1822–1831. (doi:10.1359/jbmr.2002.17.10.1822)
- Feng JQ, Huang H, Lu Y, Ye L, Xie Y, Tsutsui TW, Kunieda T, Castranio T, Scott G, Bonewald LB *et al.* 2003 The dentin matrix protein 1 (Dmp1) is specifically expressed in mineralized, but not soft, tissues during development. *Journal of Dental Research* **82** 776–780. (doi:10.1177/154405910308201003)
- Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S *et al.* 2006 Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nature Genetics* **38** 1310–1315. (doi:10.1038/ng1905)
- Fisher LW & Fedarko NS 2003 Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connective Tissue Research* **44** (Suppl 1) 33–40. (doi:10.1080/713713644)
- Fisher LW, Torchia DA, Fohr B, Young MF & Fedarko NS 2001 Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. *Biochemical and Biophysical Research Communications* **280** 460–465. (doi:10.1006/bbrc.2000.4146)
- Frost HM 1990 Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. *Anatomical Record* **226** 403–413. (doi:10.1002/ar.1092260402)
- Fujihara S, Yokozeki M, Oba Y, Higashibata Y, Nomura S & Moriyama K 2006 Function and regulation of osteopontin in response to mechanical stress. *Journal of Bone and Mineral Research* **21** 956–964. (doi:10.1359/jbmr.060315)
- Ganss B, Kim RH & Sodek J 1999 Bone sialoprotein. *Critical Reviews in Oral Biology and Medicine* **10** 79–98. (doi:10.1177/10454411990100010401)
- Gentili C & Cancedda R 2009 Cartilage and bone extracellular matrix. *Current Pharmaceutical Design* **15** 1334–1348. (doi:10.2174/138161209787846739)
- George A, Sabsay B, Simonian PA & Veis A 1993 Characterization of a novel dentin matrix acidic phosphoprotein, Implications for induction of biomineralization. *Journal of Biological Chemistry* **268** 12624–12630.
- Gericke A, Qin C, Sun Y, Redfern R, Redfern D, Fujimoto Y, Taleb H, Butler WT & Boskey AL 2010 Different forms of DMP1 play distinct roles in mineralization. *Journal of Dental Research* **89** 355–359. (doi:10.1177/0022034510363250)
- Glühak-Heinrich J, Pavlin D, Yang W, MacDougall M & Harris SE 2007 MEPE expression in osteocytes during orthodontic tooth movement. *Archives of Oral Biology* **52** 684–690. (doi:10.1016/j.archoralbio.2006.12.010)
- Golub EE 2011 Biomineralization and matrix vesicles in biology and pathology. *Seminars in Immunopathology* **33** 409–417. (doi:10.1007/s00281-010-0230-z)
- Gordon JA, Tye CE, Sampaio AV, Underhill TM, Hunter GK & Goldberg HA 2007 Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization *in vitro*. *Bone* **41** 462–473. (doi:10.1016/j.bone.2007.04.191)
- Gowen LC, Petersen DN, Mansolf AL, Qi H, Stock JL, Tkalecic GT, Simmons HA, Crawford DT, Chidsey-Frink KL, Ke HZ *et al.* 2003 Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. *Journal of Biological Chemistry* **278** 1998–2007. (doi:10.1074/jbc.M203250200)
- Gross TS, King KA, Rabaia NA, Pathare P & Srinivasan S 2005 Upregulation of osteopontin by osteocytes deprived of mechanical loading or oxygen. *Journal of Bone and Mineral Research* **20** 250–256. (doi:10.1359/JBMR.041004)
- Guo R, Rowe PS, Liu S, Simpson LG, Xiao ZS & Quarles LD 2002 Inhibition of MEPE cleavage by Phex. *Biochemical and Biophysical Research Communications* **297** 38–45. (doi:10.1016/S0006-291X(02)02125-3)
- Hakim FT, Cranley R, Brown KS, Eanes ED, Harne L & Oppenheim JJ 1984 Hereditary joint disorder in progressive ankylosis (ank/ank) mice. I. Association of calcium hydroxyapatite deposition with inflammatory arthropathy. *Arthritis and Rheumatism* **27** 1411–1420. (doi:10.1002/art.1780271212)
- Harmey D, Johnson KA, Zelken J, Camacho NP, Hoylaerts MF, Noda M, Terkeltaub R & Millan JL 2006 Elevated skeletal osteopontin levels contribute to the hypophosphatasia phenotype in Akp2(–/–) mice. *Journal of Bone and Mineral Research* **21** 1377–1386. (doi:10.1359/jbmr.060619)
- Harris NL, Rattray KR, Tye CE, Underhill TM, Somerman MJ, D'Errico JA, Chambers AF, Hunter GK & Goldberg HA 2000 Functional analysis of bone sialoprotein: identification of the hydroxyapatite-nucleating and cell-binding domains by recombinant peptide expression and site-directed mutagenesis. *Bone* **27** 795–802. (doi:10.1016/S8756-3282(00)00392-6)
- Hasegawa M, Segawa T, Maeda M, Yoshida T & Sudo A 2011 Thrombin-cleaved osteopontin levels in synovial fluid correlate with disease severity of knee osteoarthritis. *Journal of Rheumatology* **38** 129–134. (doi:10.3899/jrheum.100637)
- Hayashibara T, Hiraga T, Yi B, Nomizu M, Kumagai Y, Nishimura R & Yoneda T 2004 A synthetic peptide fragment of human MEPE stimulates new bone formation *in vitro* and *in vivo*. *Journal of Bone and Mineral Research* **19** 455–462. (doi:10.1359/JBMR.0301263)
- Hayashibara T, Hiraga T, Sugita A, Wang L, Hata K, Ooshima T & Yoneda T 2007 Regulation of osteoclast differentiation and function by phosphate: potential role of osteoclasts in the skeletal abnormalities in hypophosphatemic conditions. *Journal of Bone and Mineral Research* **22** 1743–1751. (doi:10.1359/jbmr.070709)
- He G, Dahl T, Veis A & George A 2003 Dentin matrix protein 1 initiates hydroxyapatite formation *in vitro*. *Connective Tissue Research* **44** (Suppl 1) 240–245.
- He G, Ramachandran A, Dahl T, George S, Schultz D, Cookson D, Veis A & George A 2005 Phosphorylation of phosphophoryn is crucial for its function as a mediator of biomineralization. *Journal of Biological Chemistry* **280** 33109–33114. (doi:10.1074/jbc.M500159200)

- Heinegard D 2009 Proteoglycans and more – from molecules to biology. *International Journal of Experimental Pathology* **90** 575–586. (doi:10.1111/j.1365-2613.2009.00695.x)
- Henriksen K, Neutzsky-Wulff AV, Bonewald LF & Karsdal MA 2009 Local communication on and within bone controls bone remodeling. *Bone* **44** 1026–1033. (doi:10.1016/j.bone.2009.03.671)
- Hessle L, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R & Millan JL 2002 Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *PNAS* **99** 9445–9449. (doi:10.1073/pnas.142063399)
- Hill PA 1998 Bone remodelling. *British Journal of Orthodontics* **25** 101–107. (doi:10.1093/ortho/25.2.101)
- Ho AM, Johnson MD & Kingsley DM 2000 Role of the mouse ank gene in control of tissue calcification and arthritis. *Science* **289** 265–270. (doi:10.1126/science.289.5477.265)
- Holm IA, Huang X & Kunkel LM 1997 Mutational analysis of the PEX gene in patients with X-linked hypophosphatemic rickets. *American Journal of Human Genetics* **60** 790–797.
- Hopwood B, Tsykin A, Findlay DM & Fazzalari NL 2007 Microarray gene expression profiling of osteoarthritic bone suggests altered bone remodelling, WNT and transforming growth factor-beta/bone morphogenic protein signalling. *Arthritis Research and Therapy* **9** R100. (doi:10.1186/ar2301)
- Houston B, Paton IR, Burt DW & Farquharson C 2002 Chromosomal localization of the chicken and mammalian orthologues of the orphan phosphatase PHOSPHO1 gene. *Animal Genetics* **33** 451–454. (doi:10.1046/j.1365-2052.2002.00900.x)
- Huang B, Maciejewska I, Sun Y, Peng T, Qin D, Lu Y, Bonewald L, Butler WT, Feng J & Qin C 2008 Identification of full-length dentin matrix protein 1 in dentin and bone. *Calcified Tissue International* **82** 401–410. (doi:10.1007/s00223-008-9140-7)
- Huesa C, Yadav MC, Finnila MA, Goodyear SR, Robins SP, Tanner KE, Aspdren RM, Millan JL & Farquharson C 2011 PHOSPHO1 is essential for mechanically competent mineralization and the avoidance of spontaneous fractures. *Bone* **48** 1066–1074. (doi:10.1016/j.bone.2011.01.010)
- Hunter GK, Kyle CL & Goldberg HA 1994 Modulation of crystal formation by bone phosphoproteins: structural specificity of the osteopontin-mediated inhibition of hydroxyapatite formation. *Biochemical Journal* **300** (pt 3) 723–728.
- Hunter GK, Hauschka PV, Poole AR, Rosenberg LC & Goldberg HA 1996 Nucleation and inhibition of hydroxyapatite formation by mineralized tissue proteins. *Biochemical Journal* **317** 59–64.
- Hunziker EB, Schenk RK & Cruz-Orive LM 1987 Quantitation of chondrocyte performance in growth-plate cartilage during longitudinal bone growth. *Journal of Bone and Joint Surgery* **69** 162–173. (doi:10.1097/01241398-198707000-00027)
- Huq NL, Cross KJ, Ung M & Reynolds EC 2005 A review of protein structure and gene organisation for proteins associated with mineralised tissue and calcium phosphate stabilisation encoded on human chromosome 4. *Archives of Oral Biology* **50** 599–609. (doi:10.1016/j.archoralbio.2004.12.009)
- Iohara K, Nakashima M, Ito M, Ishikawa M, Nakasima A & Akamine A 2004 Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2. *Journal of Dental Research* **83** 590–595. (doi:10.1177/154405910408300802)
- Johnson K, Goding J, van Etten D, Sali A, Hu SI, Farley D, Krug H, Hessle L, Millan JL & Terkeltaub R 2003 Linked deficiencies in extracellular PP(i) and osteopontin mediate pathologic calcification associated with defective PC-1 and ANK expression. *Journal of Bone and Mineral Research* **18** 994–1004. (doi:10.1359/jbmr.2003.18.6.994)
- Jono S, Peinado C & Giachelli CM 2000 Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. *Journal of Biological Chemistry* **275** 20197–20203. (doi:10.1074/jbc.M909174199)
- Kawasaki K 2011 The SCPP gene family and the complexity of hard tissues in vertebrates. *Cells, Tissues, Organs* **194** 108–112. (doi:10.1159/000324225)
- Kawasaki K & Weiss KM 2006 Evolutionary genetics of vertebrate tissue mineralization: the origin and evolution of the secretory calcium-binding phosphoprotein family. *Journal of Experimental Zoology. Part B, Molecular Development and Evolution* **306** 295–316. (doi:10.1002/jez.b.21088)
- Kawasaki K, Buchanan AV & Weiss KM 2007 Gene duplication and the evolution of vertebrate skeletal mineralization. *Cells, Tissues, Organs* **186** 7–24. (doi:10.1159/000102678)
- Kim JW, Hu JC, Lee JI, Moon SK, Kim YJ, Jang KT, Lee SH, Kim CC, Hahn SH & Simmer JP 2005 Mutational hot spot in the DSPP gene causing dentinogenesis imperfecta type II. *Human Genetics* **116** 186–191. (doi:10.1007/s00439-004-1223-6)
- King KB, Opel CF & Rempel DM 2005 Cyclical articular joint loading leads to cartilage thinning and osteopontin production in a novel *in vivo* rabbit model of repetitive finger flexion. *Osteoarthritis and Cartilage* **13** 971–978. (doi:10.1016/j.joca.2005.06.015)
- Klein-Nulend J, Roelofs J, Semeins CM, Bronckers AL & Burger EH 1997 Mechanical stimulation of osteopontin mRNA expression and synthesis in bone cell cultures. *Journal of Cellular Physiology* **170** 174–181. (doi:10.1002/(SICI)1097-4652(199702)170:2<174::AID-JCP9>3.0.CO;2-L)
- Landis WJ, Jacquet R, Hillyer J & Zhang J 2003 Analysis of osteopontin in mouse growth plate cartilage by application of laser capture microdissection and RT-PCR. *Connective Tissue Research* **44** (Suppl 1) 28–32.
- Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, Gao Y, Shalhoub V, Tipton B, Haldankar R *et al.* 2009 Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. *Journal of Bone and Mineral Research* **24** 578–588. (doi:10.1359/jbmr.081206)
- Li X, Warmington KS, Niu QT, Asuncion FJ, Barrero M, Grisanti M, Dwyer D, Stouch B, Thway TM, Stolina M *et al.* 2010 Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats. *Journal of Bone and Mineral Research* **25** 2647–2656. (doi:10.1002/jbmr.182)
- Ling Y, Rios HF, Myers ER, Lu Y, Feng JQ & Boskey AL 2005 DMP1 depletion decreases bone mineralization *in vivo*: an FTIR imaging analysis. *Journal of Bone and Mineral Research* **20** 2169–2177. (doi:10.1359/JBMR.050815)
- Liu S, Zhou J, Tang W, Jiang X, Rowe DW & Quarles LD 2006 Pathogenic role of Fgf23 in Hyp mice. *American Journal of Physiology. Endocrinology and Metabolism* **291** E38–E49. (doi:10.1152/ajpendo.00008.2006)
- Liu S, Rowe PS, Vierthaler L, Zhou J & Quarles LD 2007 Phosphorylated acidic serine-aspartate-rich MEPE-associated motif peptide from matrix extracellular phosphoglycoprotein inhibits phosphate regulating gene with homologies to endopeptidases on the X-chromosome enzyme activity. *Journal of Endocrinology* **192** 261–267. (doi:10.1677/joe.1.07059)
- Liu S, Zhou J, Tang W, Menard R, Feng JQ & Quarles LD 2008 Pathogenic role of Fgf23 in Dmp1-null mice. *American Journal of Physiology. Endocrinology and Metabolism* **295** E254–E261. (doi:10.1152/ajpendo.90201.2008)
- Lorenz-Depiereux B, Bastepe M, Benet-Pages A, Amyere M, Wagenstaller J, Muller-Barth U, Badenhop K, Kaiser SM, Rittmaster RS, Shlossberg AH *et al.* 2006 DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *Nature Genetics* **38** 1248–1250. (doi:10.1038/ng1868)
- Lu C, Huang S, Miclau T, Helms JA & Colnot C 2004 Mepe is expressed during skeletal development and regeneration. *Histochemistry and Cell Biology* **121** 493–499. (doi:10.1007/s00418-004-0653-5)
- Lu Y, Yuan B, Qin C, Cao Z, Xie Y, Dallas SL, McKee MD, Drezner MK, Bonewald LF & Feng JQ 2011 The biological function of DMP-1 in osteocyte maturation is mediated by its 57-kDa C-terminal fragment. *Journal of Bone and Mineral Research* **26** 331–340. (doi:10.1002/jbmr.226)
- MacDougall M, Gu TT, Luan X, Simmons D & Chen J 1998 Identification of a novel isoform of mouse dentin matrix protein 1: spatial expression in mineralized tissues. *Journal of Bone and Mineral Research* **13** 422–431. (doi:10.1359/jbmr.1998.13.3.422)
- Maciejewska I, Cowan C, Svoboda K, Butler WT, D'Souza R & Qin C 2008 The NH₂-terminal and COOH-terminal fragments of dentin matrix

- protein 1 (DMP1) localize differently in the compartments of dentin and growth plate of bone. *Journal of Histochemistry and Cytochemistry* **57** 155–156. (doi:10.1369/jhc.2008.952630)
- Maciejewski I, Qin D, Huang B, Sun Y, Mues G, Svoboda K, Bonewald L, Butler WT, Feng JQ & Qin C 2009 Distinct compartmentalization of dentin matrix protein 1 fragments in mineralized tissues and cells. *Cells, Tissues, Organs* **189** 186–191. (doi:10.1159/000151372)
- Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS & Mirams M 2008 Endochondral ossification: how cartilage is converted into bone in the developing skeleton. *International Journal of Biochemistry and Cell Biology* **40** 46–62. (doi:10.1016/j.biocel.2007.06.009)
- Mackie EJ, Tatarczuch L & Mirams M 2011 The growth plate chondrocyte and endochondral ossification. *Journal of Endocrinology* **211** 109–121. (doi:10.1530/JOE-11-0048)
- MacRae VE, Davey MG, McTeir L, Narisawa S, Yadav MC, Millan JL & Farquharson C 2010 Inhibition of PHOSPHO1 activity results in impaired skeletal mineralization during limb development of the chick. *Bone* **46** 1146–1155. (doi:10.1016/j.bone.2009.12.018)
- Majeska RJ & Wuthier RE 1975 Studies on matrix vesicles isolated from chick epiphyseal cartilage. Association of pyrophosphatase and ATPase activities with alkaline phosphatase. *Biochimica et Biophysica Acta* **391** 51–60.
- Malaval L, Wade-Gueye NM, Boudiffa M, Fei J, Zirngibl R, Chen F, Laroche N, Roux JP, Burt-Pichat B, Duboeuf F *et al.* 2008 Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. *Journal of Experimental Medicine* **205** 1145–1153. (doi:10.1084/jem.20071294)
- Manolagas SC 2000 Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocrine Reviews* **21** 115–137. (doi:10.1210/er.21.2.115)
- Marks J, Churchill LJ, Debnam ES & Unwin RJ 2008 Matrix extracellular phosphoglycoprotein inhibits phosphate transport. *Journal of the American Society of Nephrology* **19** 2313–2320. (doi:10.1681/ASN.2008030315)
- Martin A, David V, Laurence JS, Schwarz PM, Lafer EM, Hedge AM & Rowe PS 2008 Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are directly responsible for defective mineralization in HYP. *Endocrinology* **149** 1757–1772. (doi:10.1210/en.2007-1205)
- Martin A, Liu S, David V, Li H, Karydis A, Feng JQ & Quarles LD 2011 Bone proteins PHEX and DMP1 regulate fibroblastic growth factor Fgf23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling. *FASEB Journal* **25** 2551–2562. (doi:10.1096/fj.10-177816)
- Mellis DJ, Itzstein C, Helfrich MH & Crockett JC 2011 The skeleton: a multifunctional complex organ: the role of key signalling pathways in osteoclast differentiation and in bone resorption. *Journal of Endocrinology* **211** 131–143. (doi:10.1530/JOE-11-0212)
- Meyer JL 1984 Can biological calcification occur in the presence of pyrophosphate? *Archives of Biochemistry and Biophysics* **231** 1–8. (doi:10.1016/0003-9861(84)90356-4)
- Mizuno M, Imai T, Fujisawa R, Tani H & Kuboki Y 2000 Bone sialoprotein (BSP) is a crucial factor for the expression of osteoblastic phenotypes of bone marrow cells cultured on type I collagen matrix. *Calcified Tissue International* **66** 388–396. (doi:10.1007/s002230010078)
- Morinobu M, Ishijima M, Rittling SR, Tsuji K, Yamamoto H, Nifuji A, Denhardt DT & Noda M 2003 Osteopontin expression in osteoblasts and osteocytes during bone formation under mechanical stress in the calvarial suture *in vivo*. *Journal of Bone and Mineral Research* **18** 1706–1715. (doi:10.1359/jbmr.2003.18.9.1706)
- Moss DW, Eaton RH, Smith JK & Whitby LG 1967 Association of inorganic-pyrophosphatase activity with human alkaline-phosphatase preparations. *Biochemical Journal* **102** 53–57.
- Nahar NN, Missana LR, Garimella R, Tague SE & Anderson HC 2008 Matrix vesicles are carriers of bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), and noncollagenous matrix proteins. *Journal of Bone and Mineral Metabolism* **26** 514–519. (doi:10.1007/s00774-008-0859-z)
- Nampei A, Hashimoto J, Hayashida K, Tsuboi H, Shi K, Tsuji I, Miyashita H, Yamada T, Matsukawa N, Matsumoto M *et al.* 2004 Matrix extracellular phosphoglycoprotein (MEPE) is highly expressed in osteocytes in human bone. *Journal of Bone Mineral and Metabolism* **22** 176–184. (doi:10.1007/s00774-003-0468-9)
- Narayanan K, Srinivas R, Ramachandran A, Hao J, Quinn B & George A 2001 Differentiation of embryonic mesenchymal cells to odontoblast-like cells by overexpression of dentin matrix protein 1. *PNAS* **98** 4516–4521. (doi:10.1073/pnas.081075198)
- Ogbureke KU & Fisher LW 2007 SIBLING expression patterns in duct epithelia reflect the degree of metabolic activity. *Journal of Histochemistry and Cytochemistry* **55** 403–409. (doi:10.1369/jhc.6A7075.2007)
- Owan I, Burr DB, Turner CH, Qiu J, Tu Y, Onyia JE & Duncan RL 1997 Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain. *American Journal of Physiology* **273** C810–C815.
- Palokangas H, Mulari M & Vaananen HK 1997 Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. *Journal of Cell Science* **110** 1767–1780.
- Petersen DN, Tkalecic GT, Mansolf AL, Rivera-Gonzalez R & Brown TA 2000 Identification of osteoblast/osteocyte factor 45 (OF45), a bone-specific cDNA encoding an RGD-containing protein that is highly expressed in osteoblasts and osteocytes. *Journal of Biological Chemistry* **275** 36172–36180. (doi:10.1074/jbc.M00362200)
- Pettersson IF, Boegard T, Svensson B, Heinegard D & Saxne T 1998 Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. *British Journal of Rheumatology* **37** 46–50. (doi:10.1093/rheumatology/37.1.46)
- Pirotte S, Lamour V, Lambert V, Alvarez Gonzalez ML, Ormenese S, Noel A, Mottet D, Castronovo V & Bellahcene A 2011 Dentin matrix protein 1 induces membrane expression of VE-cadherin on endothelial cells and inhibits VEGF-induced angiogenesis by blocking VEGFR-2 phosphorylation. *Blood* **117** 2515–2526. (doi:10.1182/blood-2010-08-298810)
- Power J, Poole KE, van Bezooijen R, Doube M, Caballero-Alias AM, Lowik C, Papapoulos S, Reeve J & Loveridge N 2010 Sclerostin and the regulation of bone formation: effects in hip osteoarthritis and femoral neck fracture. *Journal of Bone Mineral Research* **25** 1867–1876. (doi:10.1002/jbmr.70)
- Prasad M, Butler WT & Qin C 2010 Dentin sialophosphoprotein in biomineralization. *Connective Tissue Research* **51** 404–417. (doi:10.3109/03008200903329789)
- Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, Nagai N & Butler WT 2002 The expression of dentin sialophosphoprotein gene in bone. *Journal of Dental Research* **81** 392–394. (doi:10.1177/154405910208100607)
- Qin C, Brunn JC, Cook RG, Orkiszewski RS, Malone JP, Veis A & Butler WT 2003 Identification and characterization of processed fragments and cleavage sites. *Journal of Biological Chemistry* **278** 34700–34708. (doi:10.1074/jbc.M305315200)
- Qin C, Baba O & Butler WT 2004 Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Critical Reviews in Oral Biology and Medicine* **15** 126–136. (doi:10.1177/154411130401500302)
- Qin C, D'Souza R & Feng JQ 2007 Dentin matrix protein 1 (DMP1): new and important roles for biomineralization and phosphate homeostasis. *Journal of Dental Research* **86** 1134–1141. (doi:10.1177/154405910708601202)
- Raynal C, Delmas PD & Chenu C 1996 Bone sialoprotein stimulates *in vitro* bone resorption. *Endocrinology* **137** 2347–2354. (doi:10.1210/en.137.6.2347)
- Rittling SR, Matsumoto HN, McKee MD, Nanci A, An XR, Novick KE, Kowalski AJ, Noda M & Denhardt DT 1998 Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation *in vitro*. *Journal of Bone and Mineral Research* **13** 1101–1111. (doi:10.1359/jbmr.1998.13.7.1101)
- Roberts S, Narisawa S, Harmey D, Millan JL & Farquharson C 2007 Functional involvement of PHOSPHO1 in matrix vesicle-mediated skeletal mineralization. *Journal of Bone and Mineral Research* **22** 617–627. (doi:10.1359/jbmr.070108)

- Roberts SJ, Owen HC & Farquharson C 2008 Identification of a novel splice variant of the haloacid dehalogenase: PHOSPHO1. *Biochemical and Biophysical Research Communications* **371** 872–876. (doi:10.1016/j.bbrc.2008.04.163)
- Ross FP, Chappel J, Alvarez JI, Sander D, Butler WT, Farach-Carson MC, Mintz KA, Robey PG, Teitelbaum SL & Cheresch DA 1993 Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alpha v beta 3 potentiate bone resorption. *Journal of Biological Chemistry* **268** 9901–9907.
- Rowe PS 2004 The wrickken pathways of FGF23, MEPE and PHEX. *Critical Reviews in Oral Biology and Medicine* **15** 264–281. (doi:10.1177/154411130401500503)
- Rowe PS 2012 The chicken or the egg: PHEX, FGF23 and SIBLINGs unscrambled. *Cell Biochemistry and Function* (doi: 10.1002/cbf.2841).
- Rowe PS, de Zoysa PA, Dong R, Wang HR, White KE, Econs MJ & Oudet CL 2000 MEPE, a new gene expressed in bone marrow and tumors causing osteomalacia. *Genomics* **67** 54–68. (doi:10.1006/geno.2000.6235)
- Rowe PS, Kumagai Y, Gutierrez G, Garrett IR, Blacher R, Rosen D, Cundy J, Navvab S, Chen D, Drezner MK *et al.* 2004 MEPE has the properties of an osteoblastic phosphatonin and minihibin. *Bone* **34** 303–319. (doi:10.1016/j.bone.2003.10.005)
- Rowe PS, Matsumoto N, Jo OD, Shih RN, Oconnor J, Roudier MP, Bain S, Liu S, Harrison J & Yanagawa N 2006 Correction of the mineralization defect in hyp mice treated with protease inhibitors CA074 and pepstatin. *Bone* **39** 773–786. (doi:10.1016/j.bone.2006.04.012)
- Saito T, Arsenault AL, Yamauchi M, Kuboki Y & Crenshaw MA 1997 Mineral induction by immobilized phosphoproteins. *Bone* **21** 305–311. (doi:10.1016/S8756-3282(97)00149-X)
- Sanchez C, Deberg MA, Bellahcene A, Castronovo V, Msika P, Delcour JP, Crielaard JM & Henrotin YE 2008 Phenotypic characterization of osteoblasts from the sclerotic zones of osteoarthritic subchondral bone. *Arthritis and Rheumatism* **58** 442–455. (doi:10.1002/art.23159)
- Shirley DG, Faria NJ, Unwin RJ & Dobbie H 2010 Direct micropuncture evidence that matrix extracellular phosphoglycoprotein inhibits proximal tubular phosphate reabsorption. *Nephrology, Dialysis, Transplantation* **25** 3191–3195. (doi:10.1093/ndt/gfq263)
- Siggelkow H, Schmidt E, Hennies B & Hufner M 2004 Evidence of downregulation of matrix extracellular phosphoglycoprotein during terminal differentiation in human osteoblasts. *Bone* **35** 570–576. (doi:10.1016/j.bone.2004.03.033)
- Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, Juppner H & Lanske B 2004 Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in PheX-deficient mice. *Matrix Biology* **23** 421–432. (doi:10.1016/j.matbio.2004.09.007)
- Sodek J, Chen J, Nagata T, Kasugai S, Todescan R Jr, Li IW & Kim RH 1995 Regulation of osteopontin expression in osteoblasts. *Annals of the New York Academy of Sciences* **760** 223–241. (doi:10.1111/j.1749-6632.1995.tb44633.x)
- Sodek J, Ganss B & McKee MD 2000 Osteopontin. *Critical Reviews in Oral Biology and Medicine* **11** 279–303. (doi:10.1177/10454411000110030101)
- Sprowson AP, McCaskie AW & Birch MA 2008 ASARM-truncated MEPE and AC-100 enhance osteogenesis by promoting osteoprogenitor adhesion. *Journal of Orthopaedic Research* **26** 1256–1262. (doi:10.1002/jor.20606)
- Sreenath T, Thyagarajan T, Hall B, Longenecker G, D'Souza R, Hong S, Wright JT, MacDougall M, Sauk J & Kulkarni AB 2003 Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. *Journal of Biological Chemistry* **278** 24874–24880. (doi:10.1074/jbc.M303908200)
- Stewart AJ, Roberts SJ, Seawright E, Davey MG, Fleming RH & Farquharson C 2006 The presence of PHOSPHO1 in matrix vesicles and its developmental expression prior to skeletal mineralization. *Bone* **39** 1000–1007. (doi:10.1016/j.bone.2006.05.014)
- Stubbs JT III, Mintz KP, Eanes ED, Torchia DA & Fisher LW 1997 Characterization of native and recombinant bone sialoprotein: delineation of the mineral-binding and cell adhesion domains and structural analysis of the RGD domain. *Journal of Bone and Mineral Research* **12** 1210–1222. (doi:10.1359/jbmr.1997.12.8.1210)
- Sun Y, Prasad M, Gao T, Wang X, Zhu Q, D'Souza R, Feng JQ & Qin C 2010 Failure to process dentin matrix protein 1 (DMP1) into fragments leads to its loss of function in osteogenesis. *Journal of Biological Chemistry* **285** 31713–31722. (doi:10.1074/jbc.M110.137059)
- Sun Y, Chen L, Ma S, Zhou J, Zhang H, Feng JQ & Qin C 2011 Roles of DMP1 processing in osteogenesis, dentinogenesis and chondrogenesis. *Cells, Tissues, Organs* **194** 199–204. (doi:10.1159/000324672)
- Suzuki K, Zhu B, Rittling SR, Denhardt DT, Goldberg HA, McCulloch CA & Sodek J 2002 Colocalization of intracellular osteopontin with CD44 is associated with migration, cell fusion, and resorption in osteoclasts. *Journal of Bone and Mineral Research* **17** 1486–1497. (doi:10.1359/jbmr.2002.17.8.1486)
- Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Terse A, Cho A, Kohler T, Muller R, Goldberg M & Kulkarni AB 2009 Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biology* **28** 221–229. (doi:10.1016/j.matbio.2009.03.006)
- Tartais PH, Doulaverakis M, George A, Fisher LW, Butler WT, Qin C, Salih E, Tan M, Fujimoto Y, Spevak L *et al.* 2004 *In vitro* effects of dentin matrix protein-1 on hydroxyapatite formation provide insights into *in vivo* functions. *Journal of Biological Chemistry* **279** 18115–18120. (doi:10.1074/jbc.M314114200)
- Terai K, Takano-Yamamoto T, Ohba Y, Hiura K, Sugimoto M, Sato M, Kawahata H, Inaguma N, Kitamura Y & Nomura S 1999 Role of osteopontin in bone remodeling caused by mechanical stress. *Journal of Bone and Mineral Research* **14** 839–849. (doi:10.1359/jbmr.1999.14.6.839)
- Terkeltaub R, Rosenbach M, Fong F & Goding J 1994 Causal link between nucleotide pyrophosphohydrolase overactivity and increased intracellular inorganic pyrophosphate generation demonstrated by transfection of cultured fibroblasts and osteoblasts with plasma cell membrane glycoprotein-1. Relevance to calcium pyrophosphate dihydrate deposition disease. *Arthritis and Rheumatism* **37** 934–941. (doi:10.1002/art.1780370624)
- Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, Ijuhin N & Komori T 2001 Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. *Journal of Bone and Mineral Research* **16** 2017–2026. (doi:10.1359/jbmr.2001.16.11.2017)
- Tsuchiya S, Simmer JP, Hu JC, Richardson AS, Yamakoshi F & Yamakoshi Y 2011 Astacin proteases cleave dentin sialophosphoprotein (Dsp) to generate dentin phosphoprotein (Dpp). *Journal of Bone and Mineral Research* **26** 220–228. (doi:10.1002/jbmr.202)
- Tye CE, Rattray KR, Warner KJ, Gordon JA, Sodek J, Hunter GK & Goldberg HA 2003 Delineation of the hydroxyapatite-nucleating domains of bone sialoprotein. *Journal of Biological Chemistry* **278** 7949–7955. (doi:10.1074/jbc.M211915200)
- Valverde P, Zhang J, Fix A, Zhu J, Ma W, Tu Q & Chen J 2008 Overexpression of bone sialoprotein leads to an uncoupling of bone formation and bone resorption in mice. *Journal of Bone and Mineral Research* **23** 1775–1788. (doi:10.1359/jbmr.080605)
- Verdelis K, Ling Y, Sreenath T, Haruyama N, MacDougall M, van der Meulen MC, Lukashova L, Spevak L, Kulkarni AB & Boskey AL 2008 DSPP effects on *in vivo* bone mineralization. *Bone* **43** 983–990. (doi:10.1016/j.bone.2008.08.110)
- Wada T, McKee MD, Steitz S & Giachelli CM 1999 Calcification of vascular smooth muscle cell cultures: inhibition by osteopontin. *Circulation Research* **84** 166–178. (doi:10.1161/01.RES.84.2.166)
- Wang J, Zhou HY, Salih E, Xu L, Wunderlich L, Gu X, Hofstaetter JG, Torres M & Glimcher MJ 2006 Site-specific *in vivo* calcification and osteogenesis stimulated by bone sialoprotein. *Calcified Tissue International* **79** 179–189. (doi:10.1007/s00223-006-0018-2)
- Wu LN, Genge BR, Kang MW, Arsenault AL & Wuthier RE 2002 Changes in phospholipid extractability and composition accompany mineralization of chicken growth plate cartilage matrix vesicles. *Journal of Biological Chemistry* **277** 5126–5133. (doi:10.1074/jbc.M107899200)

- Wu H, Teng PN, Jayaraman T, Onishi S, Li J, Bannon L, Huang H, Close J & Sfeir C 2011 Dentin matrix protein 1 (DMP1) signals via cell surface integrin. *Journal of Biological Chemistry* **286** 29462–29469. (doi:10.1074/jbc.M110.194746)
- Xiao ZS, Crenshaw M, Guo R, Nesbitt T, Drezner MK & Quarles LD 1998 Intrinsic mineralization defect in Hyp mouse osteoblasts. *American Journal of Physiology* **275** E700–E708.
- Yadav MC, Simao AM, Narisawa S, Huesa C, McKee MD, Farquharson C & Millan JL 2011 Loss of skeletal mineralization by the simultaneous ablation of PHOSPHO1 and alkaline phosphatase function: a unified model of the mechanisms of initiation of skeletal calcification. *Journal of Bone and Mineral Research* **26** 286–297. (doi:10.1002/jbmr.195)
- Yamakoshi Y, Hu JC, Fukae M, Zhang H & Simmer JP 2005 Dentin glycoprotein: the protein in the middle of the dentin sialophosphoprotein chimera. *Journal of Biological Chemistry* **280** 17472–17479. (doi:10.1074/jbc.M413220200)
- Ye L, Mishina Y, Chen D, Huang H, Dallas SL, Dallas MR, Sivakumar P, Kunieda T, Tsutsui TW, Boskey A *et al.* 2005 Dmp1-deficient mice display severe defects in cartilage formation responsible for a chondrodysplasia-like phenotype. *Journal of Biological Chemistry* **280** 6197–6203. (doi:10.1074/jbc.M412911200)
- You J, Reilly GC, Zhen X, Yellowley CE, Chen Q, Donahue HJ & Jacobs CR 2001 Osteopontin gene regulation by oscillatory fluid flow via intracellular calcium mobilization and activation of mitogen-activated protein kinase in MC3T3-E1 osteoblasts. *Journal of Biological Chemistry* **276** 13365–13371. (doi:10.1074/jbc.M009846200)
- Zelzer E, McLean W, Ng YS, Fukai N, Reginato AM, Lovejoy S, D'Amore PA & Olsen BR 2002 Skeletal defects in VEGF(120/120) mice reveal multiple roles for VEGF in skeletogenesis. *Development* **129** 1893–1904.
- Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, Wu G, Qiang B, Lo WH & Shen Y 2001 DSPP mutation in dentinogenesis imperfecta Shields type II. *Nature Genetics* **27** 151–152. (doi:10.1038/84765)
- Zhang GX, Mizuno M, Tsuji K & Tamura M 2004 Regulation of mRNA expression of matrix extracellular phosphoglycoprotein (MEPE)/osteoblast/osteocyte factor 45 (OF45) by fibroblast growth factor 2 in cultures of rat bone marrow-derived osteoblastic cells. *Endocrine* **24** 15–24. (doi:10.1385/ENDO:24:1:015)
- Zhang B, Sun Y, Chen L, Guan C, Guo L & Qin C 2010 Expression and distribution of SIBLING proteins in the predentin/dentin and mandible of hyp mice. *Oral Diseases* **16** 453–464. (doi:10.1111/j.1601-0825.2010.01656.x)
- Zohar R, Lee W, Arora P, Cheifetz S, McCulloch C & Sodek J 1997 Single cell analysis of intracellular osteopontin in osteogenic cultures of fetal rat calvarial cells. *Journal of Cellular Physiology* **170** 88–100. (doi:10.1002/(SICI)1097-4652(199701)170:1<88::AID-JCP10>3.0.CO;2-K)

Received in final form 28 May 2012

Accepted 13 June 2012

Made available online as an Accepted Preprint
13 June 2012