Chapter 4
DMP-1 in Postnatal Bone Development

Shuxian Lin and Jerry Jian Q. Feng

Abstract Dentin matrix protein 1 (DMP1) was cloned from a rat dentin cDNA library 30 years ago. Initially, this non-collagenous matrix protein was thought to be dentin specific and attracted little interest in most scientific disciplines, except for the dental research area. In the last three decades, great progress has been made in the following four areas: (1) Gene expression studies show that DMP1 is widely expressed in both non-mineralized tissues (such as brain, kidney, salivary gland, and cancer tissues) and all mineralized tissues; it is highly expressed in osteocytes; (2) Protein chemistry studies confirm that full-length DMP1 is a precursor that is cleaved into two distinct forms: the C-terminal and N-terminal fragments; (3) Functional studies revealed that DMP1 is essential to the maturation of osteocytes and mineralization via local and systemic mechanisms; and (4) Genetic research identified DMP1 mutations in humans, leading to the discovery of a novel disease: autosomal recessive hypophosphatemic rickets. Importantly, the regulation of phosphate homeostasis by DMP1 through FGF23, a potent hormone that is released from bone and targeted in the kidneys, contributes to a new concept that connects bone with kidney and recognizes the osteocyte as an endocrine cell.

Keywords Dentin matrix protein 1 · Osteocyte · Bone · Phosphate homeostasis · Hypophosphatemic rickets

Introduction

The extracellular matrix (ECM) serves many functions including providing support, regulating intercellular communication, along with sequestering a wide range of cellular growth factors and acts as a local depot for them, etc. The ECM of bone

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J. J. Q. Feng (✉) · S. Lin
Biomedical Sciences, Baylor College of Dentistry, TX A&M, 3302 Gaston Ave,
Dallas, TX 75246, USA
e-mail: jfeng@bcd.tamhsc.edu

S. Lin
e-mail: shuxian.lin@hotmail.com

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and dentin contains a large amount of minerals and numerous non-collagenous proteins (NCPs). One of the categories of NCPs is termed the "Small Integrin-Binding Ligand, N-linked Glycoprotein (SIBLINGs) family" based on the similarities of the intron/exon properties, conserved protein biochemical properties (such as unstructured and acidic), and specific peptide motifs (e.g., phosphorylation and integrin-binding arginine-glycin-aspartic acid (RGD). There are five members in this SIBLINGs family: dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), osteopontin (OPN), enamelin, matrix extracellular phosphoglycoprotein (MEPE), and dentin sialophosphoprotein (DSPP)[1]. DMP1 was originally identified by cDNA cloning from the rat dentin matrix [2] and was initially believed to be dentin-specific but was later observed in bone matrices at a much higher level [3, 4]. Studies suggested that DMP1 begins its expression at E15.5 and resides mainly in osteoblasts during embryonic development. However, DMP1 is predominantly expressed in the osteocytes during postnatal development [4, 5].

The *Dmp1* gene is mapped to the long (q) arm of chromosome 4 at position 21 (4q21) in humans and to 5q21 in mice. *Dmp1* is encoded by 6 exons with 80 % of its coding information in exon 6. There are two promoter control domains: a proximal one located between the −2.4 kb and the +4 kb region, and a distal one between the −2.4 and −9.6 kb region. The proximal domain controls the early stages of DMP1 expression, and the distal domain controls the later stages [6]. AP −1, JunB, Runx2, Msx 1/2, Tcf/Lef, C/EBP, and YY −1 are essential transcription factors for bone and tooth tissue-specific regulation. The potential response elements for these transcription factors have been identified in the mouse *Dmp1* promoters [7].

DMP1 protein contains an unusually large number of acidic domains that are rich in Ser, Glu, and Asp; many of the Ser are in the consensus motif for potential phosphorylation via casein kinases I and II. Data obtained from protein chemistry studies on DMP1 isolated from rat bones showed that a full-length DMP1 molecule contains an average of 53 phosphate groups. This number indicates a potentially high calcium ion-binding capacity, a property considered necessary for a protein to participate in mineralization [8]. Studies suggested that the full-length DMP1 protein is likely a precursor that will be processed into 37-kDa N-terminal and 57-kDa C-terminal fragments (Fig. 4.1a). Extensive sequencing of tryptic peptides derived from DMP1 fragments and compared with the cDNA-deduced sequence has confirmed that rat DMP1 is proteolytically cleaved at four bonds: Phe^{173}-Asp^{174}, Ser^{180}-Asp^{181}, Ser^{217}-Asp^{218}, and Gln^{221}-Asp^{222}, among which Ser^{180}-Asp^{181} is the key cleavage site [8]. One group of candidate enzyme(s) responsible for DMP1 processing is bone morphogenetic protein 1 (BMP−1)/tolloid-like metalloproteinase [9], which is widely expressed in mesenchymal-derived tissues (including bone and cartilage) and has also been shown to cleave other protein precursors such as collagens [10]. Phosphate analysis indicated that DMP1 is highly phosphorylated: its C-terminal fragment contains 41 phosphate groups with a RGD tripeptide located in its central region, while the N-terminal fragment possesses only 12 phosphate groups. The C-terminal is currently considered to be the key functional domain, based on both *in vitro* and *in vivo* studies [11, 12] (Fig. 4.1a).

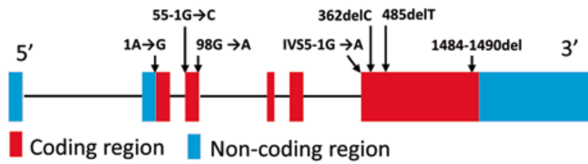

Fig. 4.1 Schematic structure of DMP1 protein, gene and ARHR mutrations. **a** Schematic structure of the human *DMP1* gene and ARHR mutations. *DMP1* is composed of six exons. Because of the recessive nature of this disease, there are only 7 variable mutations reported in the literature (see text for details). **b** Schematic structure of Full-length DMP1 protein:DMP1 signal peptide, MKTVILLVFLWGLSCAL, 37-kDa N-terminal fragment, a main cleavage site at peptide bond Ser196-Asp197, and the 57-kDa C-terminal fragment

Dmp1 Mutation/Deletion Leads to Autosomal Recessive Hypophosphatemia

DMP1 is mainly expressed in mineralized tissues and plays an important role during osteogenesis [13], although some *in vivo* animal studies showed that DMP1 may also have potential functions in non-mineralized tissues such as in brain [14], kidney [15] and salivary tissues [16]. For example, DMP1 is thought to actively promote and control the mineralization of collagen fibers and crystal growth within osteoids and predentin when these tissues are converted to mature bone and dentin [13]. In addition, DMP1 regulates and supports the maturation of osteoblasts to osteocytes and pre-odontoblasts to odontoblasts [17]. Importantly, *DMP1* mutations in humans or deletion in mice lead to autosomal recessive hypophosphatemic rickets (ARHR)/osteomalacia [18]. It is noteworthy that, unlike other genetic disease cases in which human mutations are identified first and then animal studies such as gene knockouts are performed, it is the *Dmp1* null mouse research results [19, 20] that triggered searches for *DMP1* mutations in humans.

Autosomal Recessive Hypophosphatemia

Rickets is the softening of bones in children because of a deficiency or impaired metabolism of vitamin D, phosphorus, or calcium. This disease not only interrupts child development but also can potentially lead to fractures and disturbance of normal ossification. Rickets due to nutrition deficiency is more common in children in developing countries. The adult equivalent of rickets is known as “osteomalacia”, a disease characterized by inadequate or delayed mineralization of osteoids in mature cortical and spongy bones. Hypophosphatemic rickets/osteomalacia is a group of disorders characterized by hypophosphatemia, which mainly results from disorders of renal phosphate wasting (Unlike the situation with calcium, there is no rickets due to phosphorus deficiency, since one’s daily diet is rich in this element). There are several inherited gene mutations responsible for deficiencies in reabsorption

in the renal tubule, which results in hypophosphatemia. For example, mutations in the *PHEX* gene (encoding phosphate regulating gene with homologies to endopeptidases on the X chromosome) lead to x-linked dominant hypophosphatemic (XLH) rickets [21], *FGF23* (encoding fibroblast growth factor 23) mutations result in autosomal dominant hypophosphatemic rickets (ADHR) [22], and homozygous inactivating mutations in *DMP1* cause autosomal recessive hypophosphatemic rickets (ARHR)/osteomalacia [18, 23 ,25]. Comparisons of patients having XLH, the most common hypophosphatemic rickets, there are fewer than ten cases of kindred ARHR have been identified worldwide due to the autosomal recessive nature of the *DMP1* mutations [18, 23–26]. Molecular genetic analysis of these cases revealed the following changes: (1) the deletion of nucleotides 1484–1490 in exon 6 (1484–1490del) resulting in a frameshift that replaced the 18 conserved C-terminal residues with 33 unrelated residues, or another deletion in the same exon leading to a frameshift replacing 335 conserved amino acids with 53 unrelated ones; (2) a mutation in the start codon (1A→G) causing a methionine to valine change (M1V); (3) a homozygous 1-bp deletion in exon 6 (362delC) led to the placement of a premature stop codon after 120 unrelated amino acids; (4) a biallelic nucleotide substitution at nucleotide 98 in exon 3 (98G→A) introduced a premature termination codon (PTC) at codon 33, which replaced the wild type tryptophan residue (W33X); and (5) a mutation at the splice acceptor junction of exon 6 (IVS5−1G→A) or intron 2 (55−1G→G) is predicted to alter pre-mRNA splicing, which results in a shift in the open-reading frame if the final message is stable (Fig. 4.1b).

Considering the reported cases, the primary clinical symptoms of ARHR include: lower limb deformities (bowed legs or knock-knees), waddling gait, short stature or stunted growth, tooth abscesses or early loss of teeth, bone and muscle pain, biochemical abnormalities (hypophosphatemia with normal levels of serum calcium and parathyroid hormone). More severely afflicted patients may also suffer from nerve deafness, facial and dental abnormalities, learning disabilities, joint pain, and contracture and immobilization of the spine. Patients diagnosed with ARHR display symptoms in their early childhood that are likely to have a wide spectrum of severity, depending on the site and size of the mutations and the severity and chronicity of the associated phosphate depletion [27] (see Table 4.1).

As with the human patients, *Dmp1* null mice do not have apparent abnormalities during prenatal bone development, but severely impaired after birth presented as a chondrodysplasia-like phenotype that characterized by short and widened long bones with flared and irregular metaphyses, and malformed ossification centers with delayed development during postnatal growth [20]. In accordance with human patients, *Dmp1* null animals display significantly lower serum phosphorus level than the wild type controls, which is due to the increased urinary phosphate excretion. In addition to human patients, *Dmp1* null mice also significantly increase their serum PTH level [12, 17, 18]. Bone histological studies demonstrated that the *Dmp1* null mice displayed severe osteomalacia, which show as a significant reduction in bone mineralization together with increased osteoid and more porous cortical bone, compared to wild type littermates. Under higher-magnification back-scattered scanning

Table 4.1 Characteristics of ARHP patients and mice models

Aspects	Characteristic features	
Clinical	Figure	Short stature or growth retardation
	Posture	Waddling gait, immobilization of the spine, kyphosis
	Craniofacial	Facial abnormalities, tooth abscesses or early loss
	Limb	Genu varum or knock-knees
	Symptom	Bone and muscle pain, joint pain, contracture, enthesopathy, nerve deafness, learning disabilities
Skeletal	Craniofacial	Enlarged dental pulp chambers and thin dentin, thick calvarium and skull base, skeletal malocclusion
	Spine	Disappearance of intervertebral disks and disk space, ossification of the longitudinal ligament
	Joint	Loss of articular cartilage, narrowed joint space, osteophytes
	Limb	Short, broad, bowing, ossification of tendon attachments, pathological bone fractures
	Chest	Rachitic rosaries, narrow chest with wide clavicles
Biochemistries	Increase	FGF23, ALP
	Decrease	Pi, TmP/GFR
	Normal	Ca

electron microscopic (SEM) images, the minerals, which are normally found to be evenly distributed around the osteocyte lacunae, were either absent or sparsely located in regions surrounding the *Dmp1* null osteocytes. Scanning transmission electron microscopic images indicated a much lower content of mineral, calcium, and phosphorus in the *Dmp1* null mineralized matrix (Fig. 4.2a, b, c) [12, 17, 18]. These observations suggested that genetic removal of *Dmp1* (in mice) and loss-function of *DMP1* mutation (in human ARHR kindreds) concurrently lead to independently altered skeletal mineralization and disturbed phosphate homeostasis.

Osteocyte Maturational Defects in Dmp1 Null Mice

An early transfection study of MC3T3-E1 cells that expressed excessive DMP1, displayed accelerated differentiation of osteoblasts and an earlier onset of mineralization, suggesting that DMP1 plays an important role during osteoblast differentiation, as well as osteocyte maturation and function [28]. It was later reported that patients with *DMP1* mutations or mice with *Dmp1* deletion did not display any gross abnormalities during the embryonic period or at birth [29]. However, they generally appeared to have the ARHP phenotype during their early postnatal development stage when DMP1 is highly produced by osteocytes and secreted into the mineral matrix. Further analysis indicated that *Dmp1* deletion leads to defects primarily in osteocytes [12, 17, 18], cells that account for more than 95 % of bone cells.

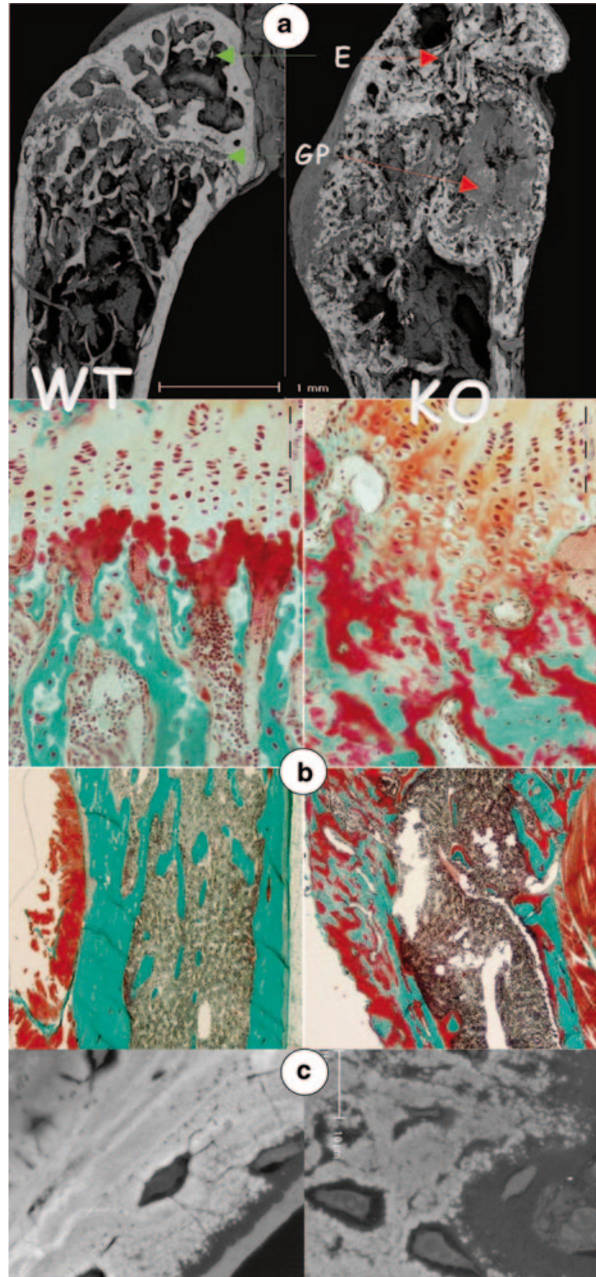

Fig. 4.2 Defective osteocytes are responsible for abnormal bone formation in the Dmp1 knockout (KO) mice (*right panels*). **a** Backscattered scanning electron microscopic (BSEM) images revealed the expanded and poorly organized growth plate (GP) in the Dmp1 KO femur, and severe defects in mineralization as reflected by discontinuous mineral content in the cortical bone region; **b** The Goldner stain showed sharp increases in osteoid areas (*red*) but greatly reduced mature bone (*green*) in the KO bone; and **c** BSEM images revealed that the mineral was evenly distributed surrounding the osteocyte lacunae in the control bone (*left, white*); however, the mineral content was either missing, or sparsely located in regions surrounding the *Dmp1* KO osteocytes

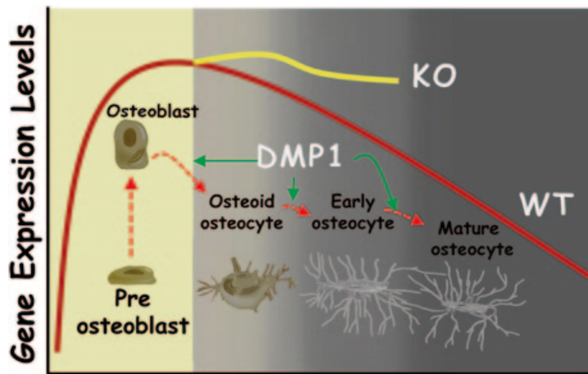

Fig. 4.3 Roles of DMP1 in control of osteocyte maturation. The gene expression levels are high during differentiation of osteoblasts from preosteoblasts, whereas the gene expression levels are gradually reduced during osteocyte maturation from osteoid osteocyte-early osteocyte and then mature osteocytes. DMP1 is one of key players during this process. The loss of *Dmp1* in mice or *DMP1* mutations in humans will disrupt normal osteoblast to osteocyte maturation. As a result, these immature osteoblast-like osteocytes express high levels of many osteoblast markers (RunX2, OSX, Col I, ALP, BSP, and OCN) and early osteocyte markers such as E11 in bone matrices

The osteocytes reside in lacunae within the mineralized bone matrix and send their dendritic processes (ranging from 40 to 100 per cell) through tiny tunnels called "canaliculi" to form the osteocyte lacunocanalicular network [30]. As the osteocytes mature, numerous cellular projections form and elongate. Next, their cell volume and ultrastructure, such as the endoplasmic reticulum and the Golgi apparatus, are reduced, and a well-organized lacunocanalicular system is built up [30]. Compared to the well-established smooth inner wall of the lacunae and canaliculi, *Dmp1* null osteocytes display bulky and coarse microstructural features, as well as abnormally enlarged and round-shaped osteocytes accompanied by a reduction in dendrite numbers [12, 17, 18]. A number of studies have consistently suggested the key pathological cause responsible for the ARHP phenotype in *Dmp1* null mice is the maturational and functional defects of the osteocytes [12, 17, 18].

There is general agreement that dramatic decreases in protein expression and metabolic activity occur during the maturation of osteoblasts into osteocytes [31]. However, the analysis of *Dmp1* null mice showed sharp increases of osteoblastic marker expression levels, such as runt-related transcription factor 2 (RunX2), osterix (OSX), Col I, alkaline phosphatase (ALP), bone sialoprotein (BSP), and osteocalcin (OCN). In addition, the early osteocyte marker E11/gp38 is widely and increasingly expressed throughout the whole cortical bone layer in the *Dmp1* null mice. In contrast, sclerostin (SOST), mainly expressed in mature osteocytes, is greatly reduced. These studies indicated that osteocyte differentiation is likely regulated by DMP1 [12, 18, 19]. In addition, there is strong evidence showing that the osteocytes, the terminal differentiated cells, regain the ability to divide and proliferate in the *Dmp1* mice [11, 12, 17, 18, 20] (see Fig. 4.3 for the working hypothesis).

Dmp1 Regulates Osteocyte Biology and Bone Development

As previously mentioned, DMP1 is essential for osteogenesis by regulating the maturational process of osteoblasts to osteocytes and bone mineralization during postnatal development. As a key regulator, DMP1 performs several functions in osteocyte biology and bone development as described below.

DMP1 Directly Promotes Hydroxyapatite Formation In Vitro

The initial finding linking DMP1 with mineralization was based on the close association of the DMP1 expression and the *in vitro* “bone nodule” formation in primary rat calvarial cell cultures [4]. Soon after, He and colleagues reported that the specific acidic clusters in DMP1 molecules can provide the molecular design necessary for controlling the formation of oriented calcium phosphate crystals, and that the self-assembly of acidic clusters into a beta-sheet template of DMP1 is likely required for the role of DMP1 in biomineral induction [32]. Gajjeraman et al. also showed that both full-length recombinant DMP1 and native DMP1 C-terminal fragments isolated from rat bone accelerated the nucleation of hydroxyapatite in the presence of type I collagen, whereas the N-terminal domain of DMP1 (amino acid residues 1–334) inhibited hydroxyapatite nucleation [33]. Further analysis of these three DMP1 fragments within the mineralized tissues [N-terminal, C-terminal, and a chondroitin-sulfate-linked N-terminal fragment (DMP1-PG)] implies that both the C-terminal and N-terminal fragments are promoters of hydroxyapatite formation and growth, while DMP1-PG is an inhibitor [34]. These findings appear controversial although they indicate that distinct forms of DMP1 may work collectively to control the mineralization process in a different manner. However, the direct role of DMP1 in mineralization described above is mainly based on *in vitro* culture studies with little *in vivo* evidence.

DMP1 Signaling

DMP1 Signals via Cell Surface Integrin

In vitro studies have shown that DMP1 promotes cell attachment through its RGD motif in a cell- and tissue-specific manner, suggesting a possible role for this protein in specific cell types to activate signaling pathways. This speculation is strengthened by the observation that exogenous DMP1 added to the exposed dental pulp may act as a morphogen trigger and/or promoter of the differentiation of undifferentiated ectomesenchymal cells in the pulp toward the odontoblast lineage. A further *in vitro* analysis demonstrated that either the full-length DMP1 or the 57-kDa fragment can activate phosphorylated extracellular signal-regulated kinase (p-ERK),

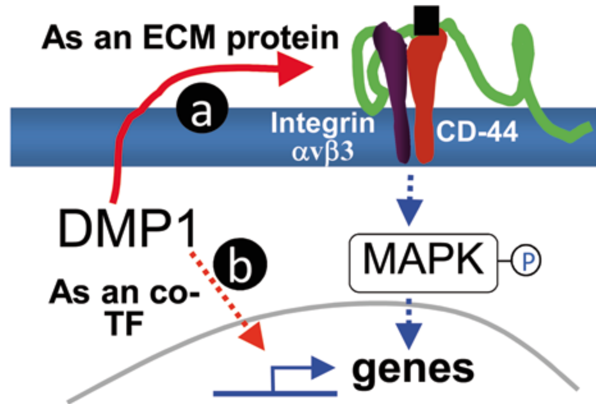

Fig. 4.4 Proposed Models for DMP1 control genes critical for osteogenesis. DMP1 either acts as an extracellular matrix (ECM) protein that binds to integrin avb3/CD −44 via its RGD domain and activates the MAPK signaling pathway (**a**), or as a co-transcriptional factor (TF) that targets the nucleus (**b**). The end results are that the genes critical for mineralization are activated

with the effect of the 57-kDa fragment lasting longer than that of the full-length protein [35]. More recently, two separate studies confirmed that matrix DMP1 has the ability to activate the mitogen activated protein kinase (MAPK) pathways via interaction with cell surface αvβ3 integrin. This interaction stimulates the activation of its downstream effectors, namely as extracellular signal-related kinases (ERK1/2) and Jun N-terminal kinases (JNK1/2). These kinases later lead to the nuclear phosphorylation of c-Jun and ATF2, which shown as transcriptional factors that involved in regulating osteoblastic gene expression. [36, 37] (Fig. 4.4a).

DMP1 Works as a Transcriptional Factor

As mentioned above, DMP1 is generally considered to be an extracellular matrix protein, but recently studies suggest there is a bi-functional role for DMP1 that it may also works as a transcriptional factor. *In vitro* studies showed the expression of DMP1 in the nuclei of several cell lines: MC3T3-E1, C3H10T1/2, and 17IIA11 [38, 39]. These studies suggest that DMP1 could function in the nucleus, possibly as a transcriptional factor. Moreover, Narayanan et al. reported a functional nuclear localization signal (NLS) peptide at the carboxyl terminal of DMP1 and proposed that this signal peptide could bring DMP1 into the nucleus [38]. They then found a specific domain in the N-terminal of DMP1 that interacted with the glucose-regulated protein −78 (GRP −78) receptor leading to the internalization and nuclear localization of full-length DMP1 [40]. Recently, Siyam et al. reported finding two DMP1 subpopulations in non-synchronized cells: one in the nucleus and one in the cytoplasm [39]. Nevertheless, this transcriptional factor theory is mainly supported by *in vitro* cell line studies with little *in vivo* evidence. Importantly, Lu and colleagues reported that the 57 kDa C-terminal fragment of DMP1 is sufficient to fully rescue the rachitic abnormalities found in the *Dmp1* null mice, suggesting that the putative N-terminal signal sequence is dispensable [12]. Thus, more *in vivo* studies are required to support this transcriptional factor theory (Fig. 4.4b).

Pathophysiologic Regulation

Recent findings showed that osteocytes embedded in the mineralized bone matrix are far more important than previously described in the literature. These studies imply that osteocytes are multifunctional cells that connect to themselves, to cells on the bone surface and to the vasculature, and they play a key regulatory role in bone and mineral homeostasis under normal and pathological conditions during postnatal development [31]. As a crucial protein mainly produced by osteocytes, DMP1 is responsible for the pathophysiologic development of the heritable disorders of rickets and osteomalacia by impacting the regulatory effect of osteocytes [27].

Regulating the Axis of FGF23-Renal Phosphorus Reabsorption-Serum Phosphorus Level

Prior studies demonstrated that serum FGF23, which is released from bone, targets the kidneys and decreases the expression of the sodium/phosphate cotransporters: NaPi-IIa and NaPi-IIc. These co-transporters are required for renal phosphate reabsorption, and a decrease in co-transporter expression will lead to an increase of the urinary excretion of phosphate and a decrease of serum phosphate levels [41]. Additionally, FGF23 also down-regulates the production of 1-ɑ hydroxylase, resulting in a decreased conversion of 25(OH)D to the active vitamin D metabolite, $1,25(OH)_2D$. This reduced production leads to a decreased expression of NaPi-IIb, a third sodium/phosphate co-transporter, and therefore affects phosphate absorption in the intestine [42]. It is generally agreed that the defect in functional axis of the FGF23 renal and intestinal phosphorus reabsorption is the key systematic pathological mechanism responsible for the development of inherited hypophosphatemic rickets/osteomalacia. Studies of *Dmp1* null mice and *DMP1* mutations in patients showed that FGF23 is significantly up-regulated in osteocytes, which is released into circulation through the connection between osteocytes and vessels, leading to an increase of FGF23 in serum and in subsequent hypophosphatemia [18]. Specifically, *in situ* hybridization (measuring mRNA levels) or immunohistochemistry (measuring protein levels), revealed that the FGF23 level in control osteocytes was much lower than that in osteoblasts, indicating differential regulatory mechanisms for FGF23 in bone cells of healthy versus disease conditions. The discovery of elevated level of FGF23 in the *Dmp1* null osteocytes indicates that bone is an endocrine organ regulating phosphate homeostasis [43] (Fig. 4.5).

Canonical Wnt/b-Catenin Pathway

In addition to controlling FGF23 levels, DMP1 may regulate hypophosphatemic rachitic/osteomalacic disorder via the canonical Wnt/β-catenin signaling pathway. In normal bone development, the canonical Wnt/β-catenin pathway is required for

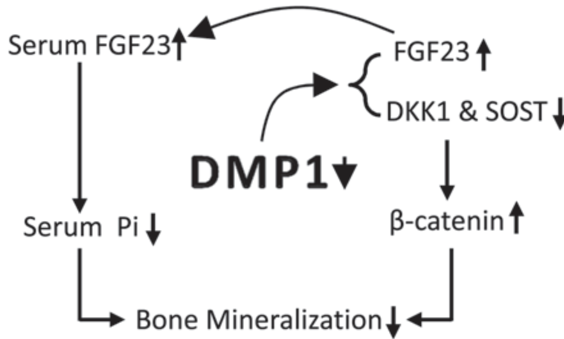

Fig. 4.5 Working hypothesis on mineralization defects in ARHR. In ARHR, the abnormal renal phosphate wasting and bone mineralization occur as a consequence of a DMP1 mutation that limits the production of the DMP1 protein. These phenotypic characteristics result from the same abnormal biomolecular events due to the deficient *Dmp1* KO mice, in which the ectopic expressed FGF23 from osteocytes targets the kidneys and leads to P waste. In addition, the immature osteocytes fail to produce enough DKK1 and SOST, resulting in increased activity of β-catenin. Both low Pi in circulates and high β-catenin in local cells are responsible for bone mineralization defect

osteogenesis and bone remodeling via regulation of early osteoblast lineage [44]. However, recent studies have indicated that a high level of β-catenin may cause bone defects in rickets [45]. This pathological change is likely due to a dramatic decrease in the expression levels of SOST (a potent inhibitor of the Wnt/β-catenin pathway[46]) in the *Dmp1*-KO osteocytes. In addition, the expression level of DKK1, another antagonist of the Wnt signaling pathway [47], is low in *Dmp1* null mice. As a result, the canonical Wnt/β-catenin signaling is abnormally up-regulated in *Dmp1* null mice. A pilot study by Lin et al. showed that normalization of the β-catenin level by overexpressing DKK1 significantly improved the osteomalacia phenotype of *Dmp1* null mice (Lin et al. unpublished) (Fig. 4.5).

Summary

DMP1 was cloned 20 years ago [2]. Since then, research has significantly advanced our understanding of this molecule, including gene regulation, biochemical characterization, cell/tissue localization and function, as well as identification of DMP1 mutations in ARHR patients. It is now clear that the full-length DMP1 is not biologically active and is cleaved into the 37 kDa N-terminal and the 57 kDa C-terminal fragments. The latter is the key functional form of DMP1. There is strong evidence that DMP1, highly expressed in dentin and bone, is critical for mineralization, although the direct role of DMP1 on the formation of hydroxyapatite is largely based on the *in vitro* non-cell model with little *in vivo* evidence. The development of different forms of *Dmp1* transgenic mouse models such as conventional and condi-

tional null mice, the full-length and the C-terminal forms of over-expressed *Dmp1* mice, and the *Dmp1* point mutation models have started to reveal the *in vivo* functions of DMP1 in bone and teeth during development [12, 29, 35, 48]. Because DMP1 controls the maturation of both odontoblasts and osteoblasts, defects in this process result in sharp changes in dentin morphologies (such as dentin tubules) and bone (especially the osteocyte-canalicular system). Finally, the observation of abnormal FGF23 production in *Dmp1* null osteocytes led directly to the discovery of DMP1 mutations in human patients with hypophosphatemia. Altogether, the accumulated data from different laboratories strongly support the notion that DMP1 is a key player in the control of osteocyte and bone biology.

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