Bone Matrix Proteins and Mineralization Process

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2.1 Bone Matrix Proteins

Bone proteins represent a complex structural entity of organic component of bone extracellular matrix. These biomolecules will be discussed below, starting from collagenic component focusing on collagen type I, the most dominant collagen in bone. Non-collagenous proteins which in turn are divided into gamma-carboxyglutamic acid-containing proteins, glycoprotein, small integrinbinding ligand N-glycosylated proteins and other adhesive proteins containing the Arg-Gly-Asp (RGD) domain recognized by the cells via integrin receptors (Table 2.1), will be then described.

Collagen type I (*COLL-I*) includes approximately 90 % of the entire collagen content of bone and about 80 % of the total proteins present in bone [1]. Other types of collagen, such as types III and V, are present at low levels in bone and appear to modulate the fibril diameter. COLL-I is a member of the wide collagen superfamily which serves as the main source of mechanical strength in connective tissue and the template for matrix deposition and mineralization in the bone [2]. Normal structural and functional COLL-I production and deposition in extracellular matrix need regulation at several steps, and defective synthesis in bone can cause human osteogenesis imperfecta [3]. The collagen molecules are derived from the aggregation of three polypeptide "α" chains, which have the characteristic Gly-XY repeating triplet (in which Gly is glycine, and X and Y are often proline and hydroxyproline) [4]. After their synthesis, each α chain becomes intertwined with two other chains in a triple left-handed helical structure. In particular, COLL-I, initially synthesized as procollagen-soluble precursor, is composed of two α1 chains and one α2 chain containing three distinct domains: a central triple-helical collagenous domain, and N- and C-terminal propeptides. Assembled procollagen trimers undergo several posttranslational modifications including hydroxylation, glycosylation, addition of mannose at the propeptide termini, and formation of intra- and intermolecular covalent cross-links. With the hydroxylation, the chains form a "nucleus" at the C-terminus where they assemble into a triple helix in a zipper-like fashion. This nucleation and "zippering" process must be highly orchestrated and depend on precise alignment of all the chains [5]. When procollagen molecules are secreted into the extracellular spaces, they are converted to collagen by proteolytic cleavage of the N- and C-terminal

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Table 2.1 Bone matrix proteins

Protein	Function
Collagen type I	Serves as template for matrix deposition and mineralization; binds and orients other proteins that nucleate hydroxyapatite deposition
Gamma-carboxyglutamic acid-containing proteins(Gla proteins)	
Osteocalcin	Role in osteoclast recruitment and maturation; it is often used as a marker for the bone formation process; considered as a core of the cross-talk between bone remodeling and glucose metabolism
Matrix Gla protein	Inhibitor of calcification
Glycoproteins	
Osteonectin	Regulates calcium-mediated processes, cell–matrix interactions, hydroxyapatite binding sites, and bone remodeling
Alkaline phosphatase	Decreases the concentration of pyrophosphates (mineralization inhibitor) and increases the concentration of the inorganic phosphate (mineralization promoter)
Small integrin-binding ligand N-glycosylated protein (SIBLINGs)	
Osteopontin	Mediates attachment of bone cells to mineral crystal structure; regulates bone resorption and bone calcification and is active in immunological reactions, tumorigenesis, and angiogenesis
Bone sialoprotein	Binds to cells; promotes the initial formation of hydroxyapatite crystal; stimulates bone resorption by inducing osteoclastogenesis and osteoclast survival and decreasing osteoclast apoptosis
Dentin matrix protein 1	Presents in three distinct segments that control the mineralization; acts as a transcriptional factor for activation of osteoblast-specific genes
Matrix extracellular phosphoprotein	Predominantly expressed by osteocytes; involved in the regulation of both osteoblast and osteoclast activities during bone remodeling, biomineralization, regulation of PHEX (phosphaturic hormone) activity
Other RGD-containing proteins	
Fibronectin	Regulates mineralization binding to other matrix proteins such as collagen
Thrombospondins	Modulate cell–matrix interactions, bind to types I and V collagens; they are potent anti-angiogenic factors; TSP2 expression increases in preosteoblasts and decreases in mature osteoblasts
Vitronectin	Binds to collagen and to heparin and promotes cell attachment, spreading, and migration
Fibrillins	Confer limited elasticity to the bone matrix

propeptides. The mechanism through which the collagen molecules assemble into fibrils is due to their spontaneous alignment. These fibrils provide tensile strength to bone and are composed of collagen helices that assemble parallel to each other in a regular quarter-staggered pattern, creating 68-nm gaps between adjacent collagen molecules. Hydroxyapatite crystals fill these gaps and are responsible for the compressive strength of bone [6].

Importantly, during the process of bone resorption, operated by osteoclasts, the release of small collagen fragments containing hydroxyproline occurs; thus, they can be detected in body fluids as a parameter of bone resorption activity.

2.1.1 Non-collagenous Proteins

Non-collagenous proteins represent 10–15 % of the total bone protein content. These proteins are multifunctional, having roles in organizing the extracellular matrix, coordinating cell–matrix and mineral–matrix interactions, and regulating the mineralization process. This large family of proteins will be discussed below and divided into gamma-carboxy glutamic acid-containing proteins, glycoproteins, small integrin-binding ligand N-glycosylated proteins, and other adhesive proteins containing RGD domain.

2.1.2 Gamma-Carboxy Glutamic Acid-Containing Proteins (Gla Proteins)

These proteins are named after the observation that the vitamin K-dependent, Ca^{2+}-binding, γ-carboxyglutamic acid (Gla) amino acid is a constituent of their molecule. The Gla residues enhance calcium binding so that Gla proteins may function in the control of mineral deposition and remodeling [7]. They include osteocalcin, which is bone specific, and matrix Gla protein, which is also found in many connective tissues [8].

Osteocalcin (*OSTC*), a 6-kDa protein, is synthesized by the cells of the osteoblastic lineage; indeed, its expression has been considered as a marker of osteoblast differentiation in vitro.

OSTC is the second most abundant protein in the bone matrix, and it is highly conserved among all vertebrate species. The biological function of OSTC is probably related to the regulation of bone turnover and/or mineralization [9]. Osteocalcin-deficient mice are reported to have increased bone mineral density compared with normal mice[9], but with age, the mineral properties did not show the changes that occurred in age-matched controls, suggesting a role of OSTC in osteoclast recruitment and maturation [10, 11].

OSTC embedded in bone matrix is released during bone resorption as intact carboxylated molecules and fragments. In fact, part of OSTC, synthesized or derived from resorption activity, could be found in the blood. This circulating OSTC has been widely used in clinical investigations as a marker of bone formation, whereas protein expression as index of osteoblastic phenotype and bone formation in vitro [12]. Furthermore, serum OSTC levels are also considered as a marker of bone turnover rather than bone formation.

Circulating OSTC exists in two forms: carboxylated on 3 glutamate residues and undercarboxylated; recently, it is emerged that the latter form is able to enhance insulin secretion by β-cells, insulin sensitivity, and energy expenditure [13], suggesting that bone cells regulate energy metabolism through an endocrine mechanism and leading to the identification of OSTC, as a core of the cross-talk between bone remodeling and glucose metabolism [14].

Matrix Gla protein (*MGP*) is a small ubiquitous matrix protein containing carboxyglutamic acid, initially isolated from bone [15] but also found in all soft tissues. MGP shares many features with OSTC, but MGP and OSTC do not cross-react and they must be kept distinct [16]. It has been thought that MGP affects differentiation in developing cartilage and bone. In fact, some authors found that MGP overexpression in developing limb leads delayed chondrocyte maturation and blocked endochondral ossification [17]. Its mechanism as inhibitor of calcification is not fully understood, but studies in MPG-deficient mice demonstrated that they develop vascular calcifications and die [18].

2.1.3 Glycoproteins

Many glycosylated proteins with diverse functions are present in bone, and the most well-known among these proteins are osteonectin and alkaline phosphatase which will be examined below.

Osteonectin (*OSN*), the most abundant non-collagenous glycoprotein in bone, can bind to both collagen fibrils and hydroxyapatite. OSN concentration in bone matrix is variable and appears to be inversely correlated with the degree of calcification [19]. Although the role of OSN in bone needs to be better clarified, currently it has been recognized as a protein with a general multifactorial function with particular reference to the regulation of calcium-mediated processes, cell–matrix interactions, hydroxyapatite binding sites, and regulation of bone remodeling [20].

Alkaline phosphatase (*ALP*) is a member of a family of membrane-bound zinc metalloprotein enzymes that catalyze the splitting off a terminal phosphate group from an organic phosphate ester in an alkaline environment (pH 10) [21]. ALP is present not only on the cell membrane and in matrix vesicles, but also in the mineralizing matrices of cartilage and bone [22]. The isoform found in bone, the tissue non-specific alkaline phosphatase (TNAP), is surely decisive for the occurrence of a normal mineralization process as shown by the skeletal defects that develop in congenital hypophosphatasia and TNAP knockout mice. In both cases, in fact, bone is characterized by excessive amounts of osteoid tissue [23]. ALP promotes mineralization by decreasing the concentration of inorganic pyrophosphate, known inhibitor of mineralization, and increasing the concentration of the mineralization promoter, the inorganic phosphate (Pi). Recently, it has become recognized that pathologic calcification of the cardiovascular system follows an osteogenic mechanism [24, 25]. In all these models, expression of ALP is a key part of the process, and presumably acts similarly to its action in hard tissues, i.e., by decreasing the inhibitor of mineralization and increasing Pi levels, which promote mineralization process. In this regard, it has been shown that polyphenols, which are reputed to be cardioprotective, inhibit ALP activity in vascular smooth muscle cells, perhaps inhibiting artery calcification [26].

2.1.4 Small Integrin-Binding Ligand *N*-Glycosylated Protein (SIBLINGs)

Bone cells synthesize different proteins that may mediate cell attachment grouped as members of the small integrin-binding ligand, N-glycosylated protein (SIBLING) family. This family is constituted by osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), and matrix extracellular phosphoprotein (MEPE). SIBLINGs are defined as small, soluble, and secreted proteins that can be localized through interactions with receptors either on the cell's own surface, enabling autocrine activities, or nearby in paracrine way. They display in their sequence a proline-rich stretch (basic), consensus sites for casein kinase, an arginine–glycine–aspartic acid (RGD) sequence, and (apart for BSP) one or several acidic serine–aspartate-rich MEPE-associated peptides (ASARM), which have a high affinity for hydroxyapatite and appear to be potent regulators of mineralization. RGD motif is the cell attachment consensus sequence that binds to the integrin class of cell surface molecules even if in some cases, cell attachment seems to be RGD independent. The SIBLINGs can engage a number of cell surface integrins. Although SIBLINGs were thought to be functionally restricted to mineralized tissues, recent results show that they are more widely distributed and expressed in non-mineralized normal tissues, upregulated in a number of tumors associated with pathological microcalcifications, and able to metastasize bone. Future investigations should validate the use of SIBLINGs as prominent molecular tools for diagnostic, prognostic, and therapeutic applications in cancer.

Osteopontin (*OPN*) is expressed in bone and bone marrow by mature osteoblasts and osteoclasts and their precursors, as well as by osteocytes. OPN expression is modulated during the various stages of differentiation. Relatively undifferentiated early-stage osteoblasts migrate to the resorbed bone surface and secrete matrix components such as OPN which is recognized

and bound by integrin on other activated osteoblasts (autocrine); here, OPN is immobilized onto preexisting mineral and/or organic moieties of the underlying bone and may act to initiate a new cycle of mineralization at this site. In this way, OPN has the function to mediate attachment of cells to mineral crystal structures; it has been demonstrated that OPN prevents both nucleation of mineral crystal formation and the growth of preexisting crystals [27, 28]. This inhibitory activity requires phosphorylated form of the protein. The production of the highly phosphorylated OPN is increased with osteoblast maturation. In addition to this, OPN may influence osteoclast activity during resorption [29]. The osteoclasts recognize OPN, adhere to the bone surface, and begin to resorb bone matrix. In many situations, OPN appears enriched in biological fluids, including blood, milk, urine, and seminal fluid having elevated levels of calcium; this is also indicative of a role in preventing spontaneous precipitation of calcium salts. Besides regulating bone resorption and bone calcification, OPN is active in diverse biological processes, such as wound healing, immunological reactions, tumorigenesis, atherosclerosis, and angiogenesis. In fact, some authors found that OPN acts as a potent constraining factor on hemopoietic stem cell proliferation, and its overexpression is a feature of hemopoietic malignancies, such as multiple myeloma and chronic myeloid leukemia, although its exact role in the aetiology and progression of these diseases remains unclear. Through osteoblasts and their cell surface, and expressed proteins, including OPN, bone is able to regulate the tissue that resides within it. In doing so, OPN can be considered a bridge between bone and blood [30].

Bone sialoprotein (*BSP*), which is a major non-collagenous extracellular matrix protein in bone, is produced by osteoclasts, osteoblasts, osteocytes, and hypertrophic chondrocytes and has long been identified as an early marker of osteogenic differentiation [31].

The Arg-Gly-Asp (*RGD*) sequence close to the carboxy-terminus of BSP protein is a cell attachment site recognized by $\alpha_v\beta_3$ integrin receptor, while polyglutamic acid regions in the amino-terminus mediate the binding of BSP to hydroxyapatite [32]. Although BSP expression has been shown to be coincident with de novo bone formation, substantial evidences suggested that BSP plays a role also in bone resorption. Some studies showed that BSP significantly stimulates bone resorption in a dose-dependent manner and this stimulation could be partially due to an increase in osteoclast adhesion to bone via its RGD sequence or its acidic sequences [33]. It is also conceivable that it is particularly involved in the process of osteoclastogenesis [34]. It has been suggested that BSP may contribute to the receptor activator of nuclear factor kappa-B ligand (RANKL)-mediated bone resorption by inducing osteoclastogenesis and osteoclast survival and decreasing osteoclast apoptosis. BSP levels are increased in patients affected by bone diseases with high bone remodeling, such as osteoporosis, hyperparathyroidism, Paget's disease, and rheumatoid arthritis, when compared with controls [35]. Furthermore, serum BSP concentrations are significantly higher in postmenopausal women than in premenopausal ones [36]. Numerous studies have suggested that BSP is also involved in the process of bone metastasis though the underlying mechanisms are not perfectly clear yet. There is a significant correlation between high BSP serum values and both the presence and the risk for the subsequent detection of bone metastases. Therefore, serum BSP could be considered as an early marker and a prognostic factor for the development of bone metastases.

Dental Matrix Protein 1 (*DMP1*) is a bone- and teeth-specific protein initially identified from mineralized dentin. Two proteolytic fragments, 37 kDa N-terminal and 57 kDa C-terminal, have been purified from rat long bone and dentin extracts, suggesting that these may be the bioactive forms in vivo. Additionally, the presence of a third chondroitin sulfate-linked N-terminal fragment (DMP1-PG) [37] has been demonstrated and recently correlated with an inhibition function in mineralization [38]. However, what is currently known is that both the highly phosphorylated 57-kDa and 37-kDa fragments could

act as hydroxyapatite nucleators [39]. In addition, the 57-kDa fragment contains almost all functional sequences and domains identified: the RGD motif, the nuclear localization signal, the ASARM peptide, and the peptide functioning as nucleator. DMP1 is a unique molecule that orchestrates mineralization of bone matrix and stimulates osteoblast differentiation during the later stages of their maturation [40]. DMP1 is primarily localized in the nuclear compartment of undifferentiated osteoblasts where it acts as a transcriptional factor for activation of osteoblast-specific genes, such as the osteocalcin gene. During the early phase of osteoblast maturation, Ca^{2+} surges into the nucleus from the cytoplasm, triggering the phosphorylation of DMP1 by a nuclear isoform of casein kinase II. This phosphorylated DMP1 is then exported into the extracellular matrix, where it regulates nucleation of hydroxyapatite. The importance of DMP1 in bone comes from studies in DMP1-null mice, in which it has been shown that vertebrae and long bones are shorter and wider with delayed and malformed secondary ossification centers and an irregular and highly expanded growth plate. Additionally, these mice unexpectedly develop a severe defect in cartilage formation during postnatal chondrogenesis and tooth mineralization defects [41, 42].

Matrix extracellular phosphoprotein (*MEPE*) is a protein predominantly expressed by osteocytes within mineralized bone. This protein is also expressed by differentiated osteoblasts, and its expression is markedly increased during matrix mineralization. Synthetic peptide fragment of MEPE, containing the RGD sequence and SGDG (Ser-Gly-Asp-Gly) glycosaminoglycan-attachment sequence, stimulates new bone formation in vitro and in vivo. MEPE is present in serum, and its levels correlate with bone mineral density and decrease in aged individuals when bone mineral density is low, suggesting a physiological role of MEPE in bone homeostasis [43]. MEPE protein is most likely cleaved by a cathepsin B-like protease to a highly phosphorylated C-terminal acidic serine and ASARM peptide in osteocytes. In vitro, it has been demonstrated that phosphorylated latter peptide inhibits osteoblast mineralization by binding to crystals in bone matrix, and the phosphorylation is necessary for the peptide affinity for crystals. ASARM peptides increase the expression of OPG, in bone marrow stromal cells [44]. MEPE might therefore be involved in the regulation of both osteoblast and osteoclast activities during bone remodeling. Furthermore, specific binding of MEPE to PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) regulates the degradation of MEPE and the release of ASARM peptides which play a role in phosphate regulation and inhibit mineralization [45] by binding to hydroxyapatite [46]. Because of these properties, MEPE has been considered a bone renal hormone [47].

2.1.5 Other Adhesive RGD-Containing Glycoproteins

Fibronectin (*FN*) is one of the most abundant glycoproteins produced during early stages of bone formation by osteoblasts and accumulated extracellularly at sites of osteogenesis. The molecule is characterized by specific binding sites for heparin and collagen, and FN-cell recognition occurs via integrin receptor.

In vitro studies have shown a key role for FN in the assembly of collagen [48], and it has been demonstrated that osteoblasts produce FN during their proliferation and differentiation at the same time of their collagen type I production [49]. It has also been shown that FN is not only needed during the initial steps of the collagen polymerization, but the continuous presence of FN is also required for matrix integrity [50], suggesting a complex role for this molecule in modulating bone matrix organization. Furthermore, FN may also regulate mineralization in bone [51] by binding to other matrix proteins and modulating their activities. Beside cell-derived FN, a soluble plasma FN has also been found in the bone matrix [52].

Thrombospondins (*TSPs*) are a small family of secreted modular glycoproteins constituted of five members. In bone is predominant TSP2 with

lower levels of other TSPs. In particular, TSP1 and TSP2 have the well-characterized ability to inhibit angiogenesis [53] in vivo, and the migration and proliferation of cultured microvascular endothelial cells (ECs), suggesting that they might regulate blood vessel formation in new bone.

TSP2 has been well investigated in bone, and it has been demonstrated that its expression increases in preosteoblasts when undergoing osteogenic differentiation and decreases as committed osteoblasts undergo terminal differentiation and mineralization. Studies on TSP2-null mice [54] showed increased cortical bone thickness due to enhanced endocortical bone formation. The increase in bone formation in TSP2-null mice is most likely due to higher number of osteoprogenitor cells in their bone marrow. In fact, when TSP2 is defective, proliferation of osteoprogenitor cells enhanced, resulting in an increased osteoblast numbers and bone formation [55]. The role of TSP1 in bone has not been examined as extensively as TSP2. TSP1 expression colocalizes with TSP2 in ossification centers of skull bones formed by intramembranous ossification. TSP1-null mice have no apparent defect in bone mass but show a mild lordotic curvature of the spine. Similar to TSP2, TSP1 expression is decreased in calvarial osteoblasts and long bones of mice overexpressing Fra-1, which is a critical gene for osteoblast differentiation. The role of TSP1 in osteogenic differentiation in vitro has been examined in preosteoblast cell lines and, rather surprisingly, shows the opposite effect of TSP2 [56].

Vitronectin (*VN*) is an RGD-containing protein found at low levels in mineralized matrix. VN is able to bind collagen by means of two functional groups: one adjacent to the RGD sequence and the other adjacent to the heparin-binding domain. It is anchored to the extracellular matrix and promotes cell adhesion, spreading, and migration by interaction with the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_v\beta_6$, and $\alpha_v\beta_8$ [57]. Upon binding of VN, these integrins activate signaling pathways and regulate cytoskeletal reorganization, intracellular ion transport, lipid metabolism, and gene expression. However, the major VN receptor appears to be $\alpha_v\beta_3$, which is distributed broadly throughout bone tissue and expressed on mature osteoclasts, thereby contributing to the regulation of bone resorption. However, VN-null mice were found to be normal, suggesting that this protein is not so critical and its function can be replaced by alternative constituents of the extracellular matrix [58].

Fibrillins are essential glycoproteins for the formation of elastic fibers found in connective tissue. They are secreted into the extracellular matrix and become incorporated into the insoluble microfibrils. Human osteoblast-like cells in culture were shown to synthesize and secrete fibrillin-1 and fibrillin-2 and to deposit the glycoproteins into the matrix, visualized as a fine fibrillar network. Fibrillin-1 is present in the haversian canals, in the osteocyte lacunae, and in adult human trabecular bone. Fibrillin-containing microfibrils play an ongoing, structural role in mature bone; it has been demonstrated that they possess intrinsic elastic properties, and it is possible that they confer limited elasticity to the bone matrix. Mutations in these genes lead to Marfan's syndrome, which exhibits abnormalities in bone growth [59].

2.2 Mineralization Process

Biomineralization is the process that consists in mineral deposition in extracellular spaces [60]. In bone tissue, minerals are composed of hydroxyapatite [$Ca_{10}(PO_4)_6.(OH)_2$] crystals, made of calcium and phosphate ions (Ca^{2+} and PO_4^{3-}). Osteoblasts, which line the unmineralized organic portion of the bone matrix, synthesize bone protein components and regulate matrix mineralization by releasing small spherical bodies membrane-bound matrix vesicles (MVs), which concentrate calcium and phosphate and destroy inhibitors of mineralization process via an enzymatic way. MVs serve as protected microenvironments in which Ca^{2+} and PO_4^{3-}concentrations can increase sufficiently to precipitate crystal formation. In particular, Ca^{2+} enters into the MVs via an annexin channel, and

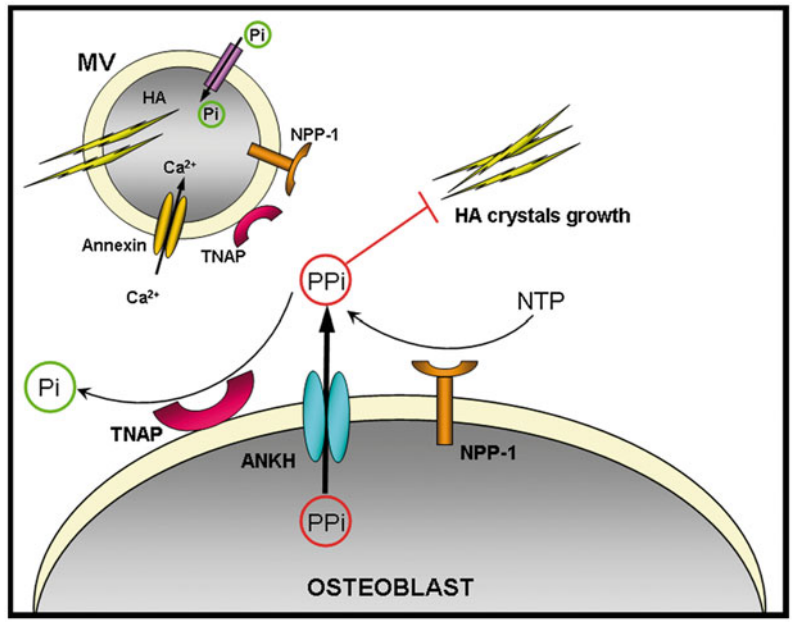

Fig. 2.1 Regulatory signaling pathways operated in controlling local Pi/PPi balance required for physiological bone mineralization. PPi: inorganic pyrophosphate; Pi: inorganic phosphate; NTP: nucleoside triphosphate; NPP1: nucleotide pyrophosphatase/phosphodiesterase 1; ANKH: pyrophosphate transporter; TNAP: tissue non-specific alkaline phosphatase: HA: hydroxyapatite; MV: matrix vesicle

phosphate enters via a Na^+-dependent phosphate transporter to form apatite crystals (Fig. 2.1). Acidic phospholipids and other MV components are thought to nucleate these intravesicular nanocrystals. It is currently believed that the role of MVs in initiating calcification is due to their capacity to regulate, via an enzymatic way, the ratio of inorganic phosphate (Pi) to pyrophosphate (PPi) in the extracellular fluid, while MV lipids and proteins constitute sites for apatite deposition. Inorganic pyrophosphate, which is the primary inhibitor of extracellular matrix mineralization, is formed by the enzymatic action of nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) and also transported through the membrane by a specific protein (ANKH) into extracellular space; NPP1 and ANKH are localized on the membrane of both MVs and osteoblasts. Moreover, MV membrane is enriched in tissue non-specific alkaline phosphatase (TNAP), which plays the crucial role of restricting the concentration of extracellular PPi to maintain a Pi/PPi ratio permissive for normal bone mineralization (Fig. 2.1). The biogenesis of MVs is still controversial. Some authors suppose that MVs arise by budding from specialized regions of osteoblast microvilli apical plasma membrane enriched in phosphatidyl serine that is important for calcium binding. MVs are surrounded by a lipid bilayer in association with hydroxyapatite crystals although the lipid composition is different from the plasma membrane which they originate. Once formed, MVs are released into extracellular matrix, and although the signals inducing this event are not completely clear, it seems that intracellular calcium and extracellular phosphate may be implied. Mineralization is believed to be a process characterized by an initial formation of hydroxyapatite crystals within MVs and their propagation through the membrane into the extracellular matrix [61]. In extracellular matrix, during early mineralization, apatite crystals become oriented so that their axes are parallel to the collagen fiber axis. It has been thought that small crystals can enter the fibrils probably via the “hole” zones and fibrillar pores, and they further propagate within the fibrils to fill all

available space, resulting in a flexing of collagen molecules away from the fiber axis [62]. Different bone matrix proteins, such as dentin matrix protein 1, bone sialoprotein, and type I collagen, critically contribute to mineralization process. In fact, they may facilitate initial crystal nucleation, sequester mineral ions to increase local concentrations of calcium and/or phosphorus, or facilitate heterogeneous nucleation. Macromolecules also bind to growing crystal surfaces to determine the size, shape, and number of crystals formed. In addition to local factors, systemic molecules play a critical role in regulating mineralization of bone matrix and principal among these is vitamin D important in coordinating calcium and phosphate homeostasis and stimulating osteoblast differentiation and activity [63]. Recently, a vitamin D counter-regulatory hormone, FGF23 [64], has been shown to affect mineralization. Studies in animal models and in cell cultures suggest that excess of FGF23 can negatively affect bone mineralization as a consequence of low phosphorus and vitamin D values (186-175), whereas FGF23 deficiency results in severe hyperphosphatemia, hypervitaminosis D, increased circulating calcium leading to a defective skeletal mineralization [65, 66].

Taking together all these data, we can argue that the process of bone matrix mineralization is very complex and under the control of many local and systemic players, many of which have been well studied, while others need to be better elucidated.

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