Journal of Cellular Biochemistry Supplements 30/31:83–91 (1998)

Biomineralization: Conflicts, Challenges, and Opportunities

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Abstract Biomineralization is the process by which mineral crystals are deposited in an organized fashion in the matrix (either cellular or extracellular) of living organisms. Over the past 25 years, new insights into the mechanisms that control these processes have been obtained, yet questions asked then still persist, especially in terms of vertebrate mineralization. Specifically, there are still debates concerning the chemical nature of the first mineral crystals formed in bone, dentin, and cementum; the factors leading to the initial deposition of these crystals; and the functions of macromolecules found associated with these crystals. In this review, emphasis is placed on the currently accepted answers to these questions, drawing insight from nonvertebrate systems. It is suggested that there are redundant calcification mechanisms and that, by taking advantage of our current knowledge of these mechanisms, opportunities will be provided for therapeutic manipulation of diseases in which biomineralization is impaired. J. Cell. Biochem. Suppls. 30/31:83–91, 1998. © 1998 Wiley-Liss, Inc.

Key words: biomineralization; calcification; bone; dentin; matrix proteins; matrix vesicles; collagen

Biomineralization is the process by which mineral crystals are deposited in an organized fashion in the matrix (either cellular or extracellular) of living organisms. The mineral phases deposited include iron oxides in magnetobacteria, magnesium silicates in some crustaceans, and more commonly, calcium carbonates and calcium phosphates in invertebrate shells, and vertebrate skeletons [Lowenstam and Weiner, 1989]. Despite numerous studies designed to elucidate the mechanisms that determine where, when, and how mineral crystals form in each of these organisms, the precise mechanisms of biomineralization remain uncertain. While some basic concepts are well established, several conflicting views persist in the literature. A number of these when examined in perspective can be resolved, but many questions still persist. The focus of this review is on the mechanism of formation of calcium phosphates, but information derived from other systems is used to resolve the conflicts.

Contract grant sponsor: National Institutes of Health; Contract grant number: DE04141; Contract grant number: AR037661; Contract grant number: AR041325

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Received 1 September 1998; Accepted 2 September 1998

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Thirty years ago, there was a great deal of descriptive information about calcium phosphate mineralization [Posner, 1969], but detailed information about mineralization processes was just starting to emerge. New analytical techniques, cell and organ culture systems, molecular biology, and nanotechnologies are now providing clues to the unsolved questions about biomineralization. These questions, listed in Table I, concern the chemical nature of the first mineral formed, the factors that control initial deposition and expansion of mineral crystals, and the function of proteins found associated with the mineral crystals. The answers to these questions should provide the basis for the design of new therapeutics and tissue engineered systems to correct defects in vertebrate biomineralization.

WHAT IS THE FIRST MINERAL DEPOSITED IN BONES AND TEETH?

During the 1920s, it was recognized that the mineral in bones and teeth was very similar in structure to the naturally occurring mineral, hydroxyapatite (Ca_{10} $(PO_4)_6$ $(OH)_2$). Chemical analysis of bone and dentin showed Ca:PO_4 ratios different from that of stoichiometric apatite, and their X-ray diffraction patterns were much more diffuse than those of geologic apa-

TABLE I. Unsolved Questions and Controversies About Vertebrate Mineralization

What is the nature of the first mineral deposited in bones and teeth?
What controls the initial deposition of mineral crystals?
Is there a common mechanism of biomineralization?
Does collagen or a noncollagenous proteins initiate mineralization?
How can the postulated functions of matrix proteins be verified?

tite. As reviewed elsewhere [Glimcher, 1998], such variations were attributed during the 1960s and early 1970s to the presence of a noncrystalline amorphous tricalcium phosphate (ACP). During the mid-1970s, it was suggested that bone mineral, in addition to apatite, contained an acidic calcium phosphate, brushite. Another acidic calcium phosphate phase, octacalcium phosphate, was also postulated to be a component of bone and dentin mineral. Each of these nonapatitic phases was proposed, on the basis of solution studies, to be precursors of apatite, the major component of all the samples analyzed. The nonstoichiometric $Ca:PO_4$ ratios were also explained in terms of "defects" in the lattice, inclusion or adsorption of HPO_4 in the crystal, and the presence of lattice vacancies [Posner, 1969]. Amorphous phases have been shown to be precursors of non calcium phosphate mineral in other species [Lowenstam and Weiner, 1989]; hence, the existence of an amorphous phase in bone was not ruled out. However, density fractionation of embryonic chick bones failed to show the presence of any nonapatitic phases even in the most recently mineralized, lowest-density, fractions. Two density fractionation studies of embryonic bone did show the presence of brushite, but these studies were not reproducible [Glimcher, 1998]. Thus, a few years ago, it was apparent to those of us investigating bone, cementum, and dentin, that the mineral phase was apatite, and if there were any precursor phases present, it accounted for less than 5% of the total mineral.

The development of sophisticated techniques of mineral analyses has slightly modified that view. ^{31}P-nuclear magnetic resonance (^{31}P-NMR) studies of bone [Glimcher, 1998] confirmed the presence of an acid phosphate-containing species distinct from both brushite and OCP. An acid phosphate species was also found based on Fourier transform infrared (IR) analysis of both bone and calcified turkey tendon [Gadaleta et al., 1996; Paschalis et al., 1997]. The content of this acid phosphate species is greatest in the youngest, most newly formed bone mineral [Boskey et al., 1998], decreasing as the mineral crystals mature. Most likely, the acid phosphate-containing species is adsorbed on the surface of the crystals, rather than representing a distinct precursor phase.

WHAT CONTROLS THE INITIAL DEPOSITION OF MINERAL CRYSTALS?

The question raised 30 years ago was whether the bulk of the mineral in bone was ACP or apatite, but even at that time it was recognized that physicochemical factors as well as biologic factors influenced the phases formed [Posner, 1969]. Physicochemical studies have since determined the solution conditions that favor the direct precipitation of different calcium phosphate and other calcific phases [Boskey, 1991]. Thus, it is not surprising that (1) in the acidic environment of the mouth, brushite may appear in dental calculus; (2) pancreatic stones, formed in a carbonate-rich environment are calcium carbonates; (3) patients with defective pyrophosphatase enzymes may form deposits of calcium pyrophosphate; and (4) patients with oxalosis deposit calcium oxalates in their tissues. The controversy today in the bone and dentin field concerns why the mineral forms at specific sites in the matrix. This is not a new question. There has been a long-standing debate as to whether mineralization starts associated with extracellular matrix vesicles or with collagen. More recently the controversy has expanded to include the noncollagenous proteins associated with the collagen. While it is known that mineral crystals deposit on collagen fibrils and that initial crystals deposit at discrete sites on these fibers, there is a good deal of evidence implicating lipid-rich membrane-bound bodies (the so-called extracellular matrix vesicles) with mineralization.

As reviewed elsewhere [Goldberg and Boskey, 1997], lipids were first associated with biomineralization in vertebrates during the early 1960s, and with biomineralization in nonvertebrates during the late 1970s. The lipids (phospholipids), in the form of extracellular organelles, were believed to provide a protected environment in which ions and clusters of ions

could associate, forming the initial crystalline deposits. Magnetobacteria provided an example of this mechanism. In calcium phosphate-forming species, the abundance of lipid staining at sites of new mineral deposition was associated with the presence of calcium–phosphate–phospholipid complexes, which in turn were shown to be the nucleational components of extracellular matrix vesicles [Wu et al., 1997]. Since these vesicles were located at a distance from the collagen fibers in the extracellular matrix of bones, calcifying cartilage, and dentin, the controversy that arose during the 1980s was whether mineralization started on/in matrix vesicles or on collagen. When all the evidence is examined, it appears that because this process is essential, the factors causing biomineralization may be redundant. Consequently, matrix vesicle based mineralization may occur along with collagen-based mineralization at sites where collagen-based mineralization is not favored (e.g., mantle dentin, calcifying cartilage, calcifying turkey tendon, and osteomalacic and other diseased bones), while in tissues such as circumpulpal dentin, healthy lamellar and osteonal bone, and intramembranous bone, vesicles are not required. This concept was recently verified in a histochemical study that used bisphosphonates to retard dentin mineralization in rats. The bisphosphonates blocked mineral crystal proliferation but left intact mineral deposits in vesicles within the mantle dentin, while vesicle formation was not noted within the circumpulpal dentin [Takano et al., 1998]. Lipid and matrix vesicle-associated calcification has not yet been proved to be an absolute requirement for vertebrate mineralization, but the data cited indicate that lipids may be among the redundant factors that control calcification.

As indicated above, it is now recognized that matrix vesicle and collagen mineralization may occur concurrently [Goldberg and Boskey, 1997]. In terms of collagen-based mineralization, the controversy today is related to whether the collagen itself causes the mineralization to start or whether noncollagenous proteins associated with the collagen are regulating vertebrate mineralization.

IS THERE A COMMON MECHANISM OF BIOMINERALIZATION?

There are several features common to biomineralization both in invertebrates and in vertebrates [Lowenstam and Weiner, 1989]. The way in which crystalline materials form in an oriented pattern on a cellular or extracellular matrix, be they calcium phosphate or calcium carbonate or iron oxide, is a multistep process. For crystal formation in general, the ions that will form the crystal lattice must come together with the right orientation to form a stable structure. This stable structure resembles the crystal phase being deposited. This "nucleation" process can be facilitated by increasing the local ion concentration, thereby increasing the probability of association, or by providing surfaces which in themselves resemble that of the nucleus crystal (heterogeneous nucleation). The organic matrices of shells, bones, and teeth are thought to contain such surfaces. Once the first crystals form on these surfaces, crystal expansion occurs as the ions add onto specific sites in the lattice and as crystals aggregate. Foreign molecules, in addition to facilitating the initial formation of crystals process by providing this surface, may retard mineral proliferation by blocking growth sites, thereby determining the shape and size of the crystals formed. Since each of these processes depends on stable interactions between the crystal and the protein, it is not unreasonable to expect that proteins that bind with high specificity and avidity to the crystal nucleus, stabilizing the nucleus and facilitating growth, can, in higher concentrations, bind to similar sites, and block growth.

In the case of apatite formation in bones and teeth (Fig. 1), the concentration of ions may be increased by the accumulation of calcium and phosphate inside matrix vesicles, by the chelation of calcium by anionic matrix molecules, and by the enzymatic hydrolysis of phosphate ions. A major issue in the calcium phosphate field is whether collagen or a specific noncollagenous proteins is the "nucleator." Both collagen and noncollagenous proteins regulate the extent to which the crystals can expand, while cells affect the maturation of the crystals.

DOES COLLAGEN OR A NONCOLLAGENOUS PROTEIN INITIATE MINERALIZATION?

Termine et al. [1987] reported several noncollagenous proteins associated with the mineralized matrices of bone and dentin. Currently, 17 such proteins (Table II) have been isolated and/or cloned or sequenced [for review, see Robey and Boskey, 1996]. Some of these proteins are not specific to the mineralized tissues;

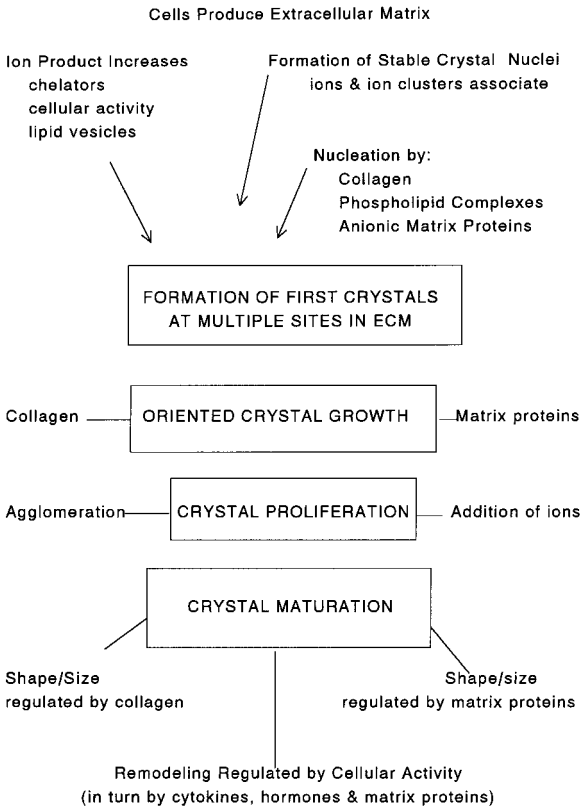

Fig. 1. Mechanism(s) of apatite formation in the collagenous matrix of bones and teeth. The cascade of events that lead from the formation of an extracellular matrix (ECM) to mature mineral are indicated in *boxes.* The factors and events that might be involved are linked to those boxes. In many cases, it is likely that all factors shown have some function. The noncollagenous matrix proteins that may be involved are indicated in Table II.

TABLE II. Extracellular Noncollagenous Proteins of Bone and Dentin and Their Effects on In Vitro Apatite Formation and Growth

Apatite nucleators
Bone sialoprotein[a]
Biglycan[a]
Dentin sialoprotein[a]
Osteonectin[a]
Dentin phosphophoryn[a]
Inhibitors of apatite formation and growth
Albumin
α-Fetuin (α2HS glycoprotein)
Aggrecan
Osteocalcin
Osteopontin
Osteonectin
Bone sialoprotein[a]
Biglycan[a]
Dentin phosphophoryn[a]
Dentin sialoprotein[a]
Matrix gla protein[b]
Effects on mineralization not yet studied
Bone acidic glycoprotein-75
Osteometrin
Dentin matrix protein I
Versican

[a]Inhibit at high concentrations and nucleate at lower concentrations.
[b]Effect established in knockout animals.

however, the localization of several of these proteins at the mineralization front suggested that they might be involved in regulation of biomineralization of bones and teeth. Proof of function cannot be established by localization alone, as most proteins can bind to apatite (hence the use of apatite columns for protein purification). Similarly, the demonstration that these proteins are expressed in culture before, or coincident with, the appearance of mineral (assessed by staining, electron microscopy, or Ca uptake) does not establish function. A variety of cell and organ culture systems, developed over the past 25 years have been used to determine how bone, dentin, and cartilage cells prepare a matrix for mineralization [Boskey et al., 1996]. Most of these investigations focused on protein production and gene expression, but a few have been concerned with biomineralization [Boskey et al., 1996; Ecarot-Charrier et al., 1983; Yaok et al., 1994; MacDougall et al., 1995; Cowles et al., 1998; Rey et al., 1995; Kasugai et al., 1991; Satoyoshi et al., 1995]. The results of these cell culture studies are somewhat contradictory, in part due to experimental variation. In many cases, β-glycerophosphate was used as a phosphate source. A substrate for alkaline phosphatase, this reagent increases the local phosphate concentration whenever the enzyme is present, in the presence or absence of cells or matrix. Thus, these studies actually monitor alkaline phosphatase expression, rather than mineralization per se. However, in some circumstances, when β-glycerophosphate supplements are kept at levels that produce inorganic phosphate concentrations in agreement with physiologic levels, its use may be viewed as acceptable. More credible studies, however, are those that do not depend on high concentrations of β-glycerophosphate. An additional source of variation in these cell culture studies lies in the cells used. Primary osteoblasts, isolated from fetal rat calvaria, are most frequently studied [Ecarot-Charrier et al., 1983]; however, bone marrow cells [Yaok et al., 1994], as well as

immortalized osteoblast, odontoblast [MacDougall et al., 1995], or cementoblast cell lines, have also been used. These cells do not always recapitulate the events that occur within the native tissue, although a recent study did demonstrate a comparable temporal pattern of gene expression in fetal rat calvarial cells and intact rat calvaria [Cowles et al., 1998]. An additional variable has been the addition of growth factors and steroids, each of which affects the differentiation and maturation of the cells being studied. A final difficulty with many other culture studies is that mineral analyses has been based on von Kossa or Alizarin red staining, which are known to give inconclusive results or by Ca uptake, when the matrix is known to uptake calcium independent of mineral deposition [Boskey et al., 1996], and not on electron microscopic, diffraction, or spectroscopic methods. In general, however, it can be stated that in cultures in which collagen-based apatite deposition has been demonstrated, type I collagen, osteonectin, alkaline phosphatase, and osteopontin appear before mineral deposition, bone sialoprotein expression concurrently, and osteocalcin appears afterward [Cowles et al., 1998; Kasugai et al., 1991]. Similarly, in odontoblast cultures, phosphophoryn formation precedes calcification of the collagenous matrix [Satoyoshi et al., 1995]. Unfortunately, the sequence in which these proteins are expressed in viable mineralizing cultures does not necessarily indicate that they are important for biomineralization.

Solution studies examining the ability of noncollagenous proteins to cause calcium phosphate deposition in the absence of cells have provided some clues. As summarized in a recent editorial [Boskey, 1998], proteins isolated from calcium carbonate-containing organisms can determine the phase (aragonite, vaterite, calcite), orientation, and size of mineral crystals formed in solution. The structural features that lead to crystal–matrix interactions have been established by a combination of crystallography and high-resolution electron microscopy [Addadi et al., 1989]. The apatite mineral crystals found in bone, cementum, and dentin are too small to use in such studies. Synthetic peptides that interact with mineral crystals have been identified [Robey and Boskey, 1996; Fujisawa et al., 1996], but, in general, the nature of the mineral–matrix interaction in the calcium phosphate field has been determined indirectly, either by using nonphysiologic models, e.g., apatites prepared at high temperatures, or octacalcium phosphate, or by molecular modeling [Dahlin et al., 1998]. These studies, similar to those that determined the mechanisms of actions of proteins in calcium carbonate-containing shells [Addadi et al., 1989; Belcher et al., 1996], have demonstrated that it is the anionic residues that interact with the mineral, and the interaction is generally with the c-axis (001) and a-axis (100) faces of the apatite crystals. Although it is not certain that the same types of interaction occur in the smaller physiologic apatites, it seems reasonable.

To determine which domains of the apatite-binding proteins interact with these smaller crystals, investigators have characterized the affinities for peptides, proteins, chemically modified and mutant proteins for poorly crystalline apatites [Robey and Boskey, 1996]. More recently, high-resolution NMR and conformational analyses using circular dichroism and Fourier transform infrared (FTIR) spectroscopy are being used to determine which residues interact directly with the mineral surface. In the future, combining these data with computer modeling should show which domains interact directly. Solution studies with modified proteins and representative peptides should then enable evaluation of which domains are involved in stabilizing apatite nuclei, and which interact with growing crystals to regulate their size and shape. Conducting these studies in the presence and absence of fibrillar collagen should also verify the effect of collagen. It is clear from data already available in these in vitro systems [Robey and Boskey, 1996; Hunter et al., 1996; Butler and Ritchie, 1995] that phosphorylated proteins are apt to be among the most important in influencing the biomineralization process in calcium phosphate systems.

There are at least seven different phosphorylated proteins in bone and dentin, and there may be more, since sequence analyses have shown sites for casein kinase activities in other matrix proteins as revised elsewhere [Robey and Boskey, 1996; Butler and Ritchie, 1995]. Some of these proteins, e.g., osteopontin, collagen (which is phosphorylated), and osteonectin, are not specific to the mineralized tissues, but differ in mineralized and nonmineralized tissues in their extent of phosphorylation. Their precise functions in mineralization are still being evaluated, and it is most likely that they

play some role in the sequence of events (mineralization cascade) that lead to a mature matrix (Fig. 1). Others, like bone sialoprotein, dentin sialoprotein, dentin matrix protein I, and dentin phosphophoryn, seem to be more specific in distribution [Butler and Ritchie, 1995]. The known effects of these proteins on mineralization in solution and in cell culture systems are indicated in Table II. Additional proteins, e.g., a novel-glycosylated phosphoprotein that is cross-linked to osteopontin [Zhou et al., 1998] and bone acidic protein 75 (BAG-75), have not yet been evaluated in mineralizing systems. What has been noted for those proteins analyzed to date is that the presence or absence of phosphate markedly alters their functions [Robey and Boskey, 1996; Hunter et al., 1996]. Although dephosphorylating the proteins, or studying nonphosphorylated recombinant proteins and contrasting their effects with those of proteins isolated with chaotropic solvents, certainly may result in comparison of proteins with altered conformations, some general trends can be noted. Proof of function will have to come from studies of proteins in their native conformation (interacting with other proteins present in the mineralizing systems). The first step in obtaining such proof has come from analyses of transgenic and knockout animals.

HOW CAN THE POSTULATED FUNCTIONS OF MATRIX PROTEINS BE VERIFIED?

Verification that some matrix proteins have an effect on mineral deposition has been provided by analysis of bone mineral crystals in naturally occurring animals that lack these proteins, and from newly developed transgenic and knockout animals that have altered proteins or that lack the protein entirely. While some transgenic and knockout animals in which bone proteins are altered develop visibly apparent bone and dentition defects at young ages (e.g., the type X collagen transgenic [Paschalis et al., 1996], the tissue-nonspecific alkaline phosphatase gene knockout [Narisawa et al., 1997] animals with type I and II collagen mutations, and the thrombospondin knockout [Kyriakides et al., 1998]), and others may fail to develop because the ablated protein is essential for life, most of those animals in which bone matrix proteins are not expressed or are modified require observation at older ages, as well as more detailed analyses.

The first analyses of the effects of genetic defects in matrix proteins on biomineral crystal structure was provided by X-ray diffraction line-broadening analysis of patients with osteogenesis imperfecta, a disease characterized by defects in the type I collagen gene. The mineral crystals were found to be smaller and less perfect than normal [Vetter et al., 1991], indicating the importance of the proper collagen matrix for mineral deposition. Such studies verified the concept that collagen was a required template but, because the matrix proteins associate with collagen, they did not provide insight into the necessity of specific matrix proteins in the mineralization process.

Similarly, Fourier transform infrared spectroscopy (FTIR) was used to characterize mineral crystal perfection and size in homogenized bones from animals with a different form of osteogenesis imperfecta [Casella et al., 1994] and from the bones of hypophosphatemic mice [Camacho et al., 1995]. Since hypophosphatemic mice have decreased amounts of phosphorylated matrix proteins in their bones, and show alterations in crystal size and mechanical strength relative to control animals, the importance of phosphorylated proteins was demonstrated. Unfortunately, such studies, which use homogenized tissues are limited because spatial information is lost.

The coupling of a light microscope with the FTIR spectrometer, enabled spatial characterization of the mineral quality and quantity in a variety of knockout and transgenic mice at 20-μm resolution. To use the FTIR microscope, sections of bone (0.5–5 μm thick) are placed on an IR window and examined under the microscope. An aperture limits the region seen by the IR radiation, and spectra are recorded at discrete sites using a mapping mode and computerized stage. By curve-fitting the phosphate region of the spectra (900–1,200 cm^{-1}), subbands characteristic of acid phosphate, phosphate in a nonstoichiometric apatite, phosphate in a stoichiometric apatite, and so forth, can be identified [Gadaleta et al., 1996; Paschalis et al., 1997; Boskey et al., 1998]. The percentage areas of these bands have been correlated, based on X-ray diffraction line-broadening analysis, with crystal size and perfection in the bone apatite. The integrated phosphate to amide I peak area ratio is linearly to the mineral content. Similarly, the carbonate vibration (860–890 cm^{-1}) is analyzed to determine the substitu-

tion by carbonate in phosphate lattice positions (type B substitution), in hydroxide lattice positions (type A substitution), and surface or labile substitution. Since the surface area is inversely related to crystal size (larger crystals have less surface area), the percentage labile carbonate is taken as an independent indication of apatite crystal size. The presence of any other calcium phosphate phases present can be determined, and the orientation of the vibrating bonds relative to the collagen axis can also be evaluated using polarized IR radiation [Gadaleta et al., 1996]. During the next few years, IR imaging, a high-tech version of FTIR microscopy that uses an array detector, will be used increasingly to decrease data acquisition time 1,000-fold and facilitate visualization of spatial variation in mineral and matrix properties [Marcott et al., 1998].

The first mutant animals studied using the technique of FTIR microspectroscopy were mice expressing a type X collagen mini-gene (transgenic mice) [Paschalis et al., 1996]. Type X collagen is a small, nonfibrillar collagen, expressed uniquely by hypertrophic chondrocytes. As such, it was believed to play an important role in cartilage calcification, as it is the lowermost hypertrophic chondrocytes that become surrounded by mineral. In cell culture studies, type X expression did not appear to be related to the mineralization process. Both in solution and in culture, type X collagen had little effect on apatite formation and proliferation. The type X transgenic animals expressed a mini-gene along with the native type X collagen. The mutant type X collagen could not properly assemble into normal triple-helical molecules, but rather had a "kink" that was postulated to disrupt their alignment. The type X transgenic animals were normal at birth but developed spinal curvatures as they became older. While the amount of mineral and crystal properties in 28- to 54-day old animals was not different from that in the control (wild-type) animals, the distribution of the mineral was altered. This finding was consistent with the hypothesis that the function of type X collagen was to provide an organized structure on which type I collagen fibrils became oriented and with the finding that type X collagen was not directly affecting mineralization.

Mice that did not express osteocalcin, a bone-specific γ-carboxylated small protein, were the next to be examined by FTIR microspectroscopy [Boskey et al., 1998]. These animals had thickened bones, contrasted with their age-matched controls, and formed bone at an increased rate. There were no visible differences (based on histochemical staining), in the mineralization in these animals. FTIR microspectroscopic analyses of the bones from 6-month-old and 9-month-old osteocalcin-knockout animals and their age-matched controls, and 6-month-old knockout and control animals in which bone turnover had been accelerated by removing their ovaries, showed significant alterations in mineral properties in osteocalcin-deficient animals. The amount of mineral at all bone sites in the osteocalcin-deficient bones was increased relative to the same sites in age-matched controls and the crystal size/perfection was decreased. There was no indication that the crystal size and perfection increased with age, as was seen in the control bones, indicating that osteocalcin plays a role in the regulation of bone crystal maturation. The teeth of these animals were not evaluated, but future studies should provide insight into osteocalcin's role in dentinogenesis.

Matrix-gla protein (MGP)-deficient animals had been reported to have massive calcification of the trachea and blood vessels [Luo et al., 1997]. FTIR microspectroscopic analysis of the bones of these animals showed no abnormalities, but the calcified cartilage was excessively mineralized, and the mineral-to-matrix ratio statistically elevated. These studies verified the histologic observation that MGP is a mineralization inhibitor/regulator. More detailed analyses of the bones of younger animals will be required to determine whether MGP has an effect on bone development.

FTIR microscopic studies of biglycan-deficient mice, confirmed the predictions from the solution studies [Xu et al., 1998]. Biglycan, a small bone proteoglycan, was shown in solution to be both an apatite nucleator, and at higher concentrations an inhibitor of apatite growth [Boskey et al., 1997]. The biglycan-deficient animals, similar to some human patients with Turner's syndrome, were shorter, smaller, and had thinner bones. Their bones were shown by FTIR microspectroscopy to contain less mineral, but the mineral crystals were larger [Xu et al., 1998]. This finding is consistent with biglycan as one of the nucleators controlling initial bone mineral deposition. Because there are fewer nucleation sites, the process of new bone

formation may be retarded, and the existing crystals will tend to grow larger.

Analyses of the other matrix protein-deficient animals, and those lacking more than one matrix protein, should provide confirmation of the importance of each of these proteins, and may help explain some disease processes.

THE FUTURE

Now that it is recognized that both matrix vesicles and collagen (in association with matrix proteins) are involved in apatite nucleation and that matrix proteins can and should be multifunctional, the research challenge has changed. We now need to determine which macromolecules are essential for biomineralization in each type of apatite-containing tissues and the temporal pattern of their activities. The opportunities in the future are to apply this information to the development of therapeutics—drug or gene—for the treatment of diseases in which mineralization is impaired or excessive and to develop new biologically based materials (tissue engineering) for repair of already damaged mineralized tissues.

REFERENCES

Addadi L, Berman A, Oldak JM, Weiner S (1989): Structural and stereochemical relations between acidic macromolecules of organic matrices of crystals. Connect Tissue Res 21:127–134.

Belcher AM, Wu HX, Christensen RJ, Hansma PK, Stucky GD, Morse DE (1996): Control of crystal phase switching and orientation by soluble mollusc-shell proteins. Nature 381:56–59.

Boskey AL (1991): Role of extracellular matrix components in dentin mineralization. Crit Rev Oral Biol Med 2:369–387.

Boskey AL (1998): Will biomimetics provide new answers to old problems of calcified tissues. Calcif Tissue Int 63:179–182.

Boskey AL, Doty SB, Stiner D, Binderman I (1996): Viable cells are a requirement for in vitro cartilage calcification. Calcif Tissue Int 58:177–185.

Boskey AL, Spevak M, Doty S, Rosenberg L (1997): Effects of bone proteoglycans, decorin and biglycan on hydroxyapatite formation in a gelatin gel. Calcif Tissue Int 61: 298–305.

Boskey AL, Gadaleta S, Gundberg C, Doty SB, Ducy P, Karsenty G (1998): FTIR microspectroscopic analysis of bones of osteocalcin deficient mice provides insight into the function of osteocalcin. Bone 23:187–196.

Butler WT, Ritchie H (1995): The nature and functional significance of dentin extracellular matrix proteins. Int J Dev Biol 39:169–179.

Camacho NP, Rimnac CM, Meyer RA Jr, Doty S, Boskey AL (1995): Effect of abnormal mineralization on the mechanical behavior of X-linked hypophosphatemic mice femora. Bone 17:271–278.

Casella JP, Pereira R, Khillan JS, Prockop DJ, Garrington N, Ali SY (1994): An ultrastructural, microanalytical, and spectroscopic study of bone from a transgenic mouse with a COLIA1 pro-alpha-1 mutation. Bone 15:611–619.

Cowles EA, DeRome ME, Pastizzo G, Brailey LL, Gronowicz GA (1998): Mineralization and the expression of matrix proteins during in vivo bone development. Calcif Tissue Int 62:74–82.

Dahlin S, Angstrom J, Linde A (1998): Dentin phosphoprotein sequence motifs and molecular modeling: Conformational adaptations to mineral crystals. Eur J Oral Sci Suppl 1:239–248.

Ecarot-Charrier B, Glorieux FH, van der Rest M, Pereira G (1983): Osteoblasts isolated from mouse calvaria initiate matrix mineralization in culture. J Cell Biol 96:639–643.

Fujisawa R, Wada Y, Nodasak Y, Kuboki Y (1996): Acidic amino acid-rich sequences as binding sites of osteonectin to hydroxyapatite crystals. Biochim Biophys Acta 1292: 53–60.

Gadaleta SJ, Camacho NP, Mendelsohn R, Boskey AL (1996): Fourier transform infrared microscopy of calcified turkey leg tendon. Calcif Tissue Int 58:17–23.

Glimcher MJ (1998): The nature of the mineral phase in bone: Biological and clinical implications. In Avioli LV, Krane SM (eds): "Metabolic Bone Disease and Clinically Related Disorders." 3rd Ed. San Diego: Academic Press, pp 23–51.

Goldberg M, Boskey AL (1997): Lipids and biomineralizations. Prog Histochem Cytochem 31:1–187.

Hunter GK, Hauschka PV, Poole AR, Rosenberg LC, Goldberg HA (1996): Nucleation and inhibition of hydroxyapatite formation by mineralized tissue proteins. Biochem J 317:59–64.

Kasugai S, Todescan R Jr, Nagata T, Yao KL, Butler WT, Sodek J (1991): Expression of bone matrix proteins associated with mineralized tissue formation by adult rat bone marrow cells in vitro: Inductive effects of dexamethasone on the osteoblastic phenotype. J Cell Physiol 147: 111–120.

Kyriakides TR, Zhu YH, Smith LT, Bain SD, Yang Z, Lin MT, Danielson KG, Iozzo RV, LaMarca M, McKinney CE, Ginns EI, Bornstein P (1998): Mice that lack thrombospondin 2 display connective tissue abnormalities that are associated with disordered collagen fibrillogenesis, an increased vascular density, and a bleeding diathesis. J Cell Biol 140:419–430.

Lowenstam HA, Weiner S (1989): "On Biomineralization." Oxford: Oxford University Press.

Luo G, Ducy P, McKee M, Pinero GL, Loyer G, Bensinger RB, Karsenty G (1997): Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 386:78–81.

MacDougall M, Thiemann F, Ta H, Hsu P, Chen LS, Snead ML (1995): Temperature sensitive simian virus 40 large T antigen immortalization of murine odontoblast cell cultures: Establishment of clonal odontoblast cell line. Connect Tissue Res 33:97–103.

Marcott C, Reeder RC, Paschalis EP, Tatakis DN, Boskey AL, Mendelsohn R (1998): Infrared microspectroscopic imaging of biomineralized tissues using a mercury-cadmium-telluride focal-plane array detector. Cell Mol Biol 44:109–115.

Narisawa S, Frohlander N, Millan JL (1997): Inactivation of two mouse alkaline phosphatase genes and establishment of a model of infantile hypophosphatasia. Dev Dynam 208:432–446.

Paschalis EP, Jacenko O, Olsen B, Mendelsohn R, Boskey AL (1996): FT-IR microscopic analysis identified alterations in mineral properties in bones from mice transgenic for type X collagen. Bone 18:151–156.

Paschalis EP, Betts F, DiCarlo E, Mendelsohn R, Boskey AL (1997): FTIR microspectroscopic analysis of human iliac crest biopsies from untreated osteoporotic bone. Calcif Tissue Int 61:487–492.

Posner AS (1969): Crystal chemistry of bone mineral. Physiol Rev 40:760–792.

Rey C, Kim HM, Gerstenfeld L, Glimcher MJ (1995): Structural and chemical characteristics and maturation of the calcium-phosphate crystals formed during the calcification of the organic matrix synthesized by chicken osteoblasts in cell culture. J Bone Miner Res 10:1577–1588.

Robey PG, Boskey AL (1996): Mineral and matrix. In Feldman D, Jowsey J (eds): "Osteoporosis." San Diego: Academic Press, pp 95–184.

Satoyoshi M, Koizumi T, Teranaka T, Iwamoto T, Takita H, Kuboki Y, Saito S, Mikuni-Takagaki Y (1995): Extracellular processing of dentin matrix protein in the mineralizing odontoblast culture. Calcif Tissue Int 57:237–241.

Takano Y, Sakai H, Baba O, Sakamoto Y, Terashima T, Ohya K, Kurosaki N (1998): Demonstration of putative Ca-binding domains in dentin matrix of rat incisors after daily injections of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP). Eur J Oral Sci 106:274–281.

Termine JD, Belcourt AB, Conn KM, Kleinman HK (1981): Mineral and collagen-binding proteins of fetal calf bone. J Biol Chem 256:10403–10408.

Vetter U, Eanes ED, Kopp JB, Termine JD, Robey PG (1991): Changes in apatite crystal size in bones of patients with osteogenesis imperfecta. Calcif Tissue Int 49:248–250.

Wu LN, Genge BR, Dunkelberger DG, LeGeros RZ, Concannon B, Wuthier RE (1997): Physicochemical characterization of the nucleational core of matrix vesicles. J Biol Chem 272:4404–4411.

Xu T, Fisher L, Bianco P, Longnecker G, Boskey AL, Smith E, Goldstein S, Bonadio J, Zhao C, Dominguez P, Heegaard A-M, Satomoura K, Gehron Robey P, Kulkarni A, Sommer B, Young M (1998): Targeted disruption of the Biglycan gene leads to an osteoporosis-like phenotype in mice. Nature Genet (in press).

Yaok L, Todescan R Jr, Sodek J (1994): Temporal changes in matrix protein synthesis and mRNA expression during mineralized tissue formation by adult rat bone marrow cells in culture. J Bone Miner Res 9:231–240.

Zhou HY, Salih E, Glimcher MJ (1998): Isolation of a novel bone glycosylated phosphoprotein with disulphide cross-links to osteonectin. Biochem J 330:1423–1431.