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Crystals and Life: An Introduction

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ABSTRACT: Living organisms of all sorts, whether single cell or complex multicellular, plant or animal, have the capacity to utilize metal ions obtained from their environment and form them into diverse structures with diverse uses. The formation of these mineral aggregates, generally considered as "biomineralization," requires the intervention of the host organism for the selection of the ions acquired, for the size, shape, crystal structure, and mechanical properties of the particular mineral formed. The biota, whether on land or in the waters, utilize macromolecules that they produce to mediate and regulate their biomineralization processes. The mineral-organic composites formed can thus be tuned to the purposes of the mineral, whether to form an exo- or endoskeletal structure, whether it is to be permanent or transient, whether it is to be high in tensile or compressive strength, or an extra- or intracellular storage depot for selected ions.

The interaction between the non-living rocks of the lithosphere and exposure to weather on the earth's surface over the vast extent of the geological time scale led to changes in the composition of the minerals as the mineral components have recycled from extrusion from the deep earth interior to the surface, and then, after weathering, returned as ocean sediments, different in composition from the initial minerals. Further diagenesis and metamorphosis during recycling continually alter the composition of the lithosphere.

The advent of life even in the most primitive forms began an immediate intervention into the rock cycle, adding to its complexity. The types of minerals recycled back into the deep earth interior changed in variety, and in amount, compressing the time scale for change. Living systems have become a major determinant in the chemistry of the earth atmosphere, oceans and land, and have altered the time scale on which changes occur. Thus, the geological and life cycle time scales and processes are intimately and irreversibly intertwined. Geologists must appreciate the role of biology; biologists must understand the ways in which the biota, in all its aspects, affect the earth itself.

KEYWORDS: biogenic minerals · carbonates · compartmentalization · phosphates · polymer matrix · silicates

1. INTRODUCTION

The title of this introductory chapter "Crystals and Life", assigned by the Editors, seemed simple enough when the task was accepted, but after reflection, it has several quite profound implicit and complex subtexts. In the present era of concern about global warming and how human activities may be affecting the environment in which we live it is easy to lose sight of the fact that long before the vertebrates arose, other organisms had an even greater impact on the composition and structure of the earth's crust. Today as well, organisms in the air, soil, and seas play overwhelming roles in governing the structural properties and regulatory mechanisms of the biosphere. Many of these processes that operate on a global scale take place over enormously long time scales, much longer than the life of any individual or even species. Thus, it is appropriate to consider these life-transcendent events within the biosphere that provide the environments in which living systems exist.

From a strictly chemical and biochemical perspective, a living organism is simply an endergonic, free energy requiring, non-equilibrium machine that requires a constant input of nutrients that can be converted into energy to drive energetically unfavorable synthetic chemical reactions and to store energy for use in movement, locomotion, and reproduction. The unique character of organisms is their ability to carry out biosynthetic reactions of inherently high activation energy under ambient conditions. This is accomplished by the formation of biopolymers of complex structures with the ability to store information, interact, compartmentalize and regulate overall behavior. We think of higher organisms as being more complex and capable of more varied functions but simple organisms certainly have persisted successfully in forms that have not evolved very far from their original state.

The evolution of life forms took place in the presence of inorganic components available from the ambient lithosphere. Mineral ions were essential and ubiquitous factors in that environment and found use in biopolymer structure and interactions, participating in catalytic activities, metabolic reactions and also in helping in the creation of mineralized deposits and tissues used by the organisms for protection, structure, and metabolic regulatory function. The "bio" in the term biomineral implies that the high activation energy, and thus stringent or extreme temperature, pressure and concentration conditions required to produce the mineral by strictly inorganic chemical means, is bypassed by the intervention of biopolymers which alter the crystallization reaction pathways. Thus, mineral ions were undoubtedly participants in the very earliest and simplest organism development. Internalized they could be used in reactive ionic form, or sequestered into structural and storage elements, but upon the death of the organism, returned to the environment.

The cycling of mineral elements through life forms over the millennia takes place over the very long time scale, but the dynamic nature of the processes within an individual organism is on the immediate, micro time scale. This is the time domain in which most studies of biomineralization are focused, with questions of the formation and function of particular crystal forms, their location and stability. Most of the chapters in this book relate directly to those matters, but we begin with consideration of the global effects.

2. GLOBAL EFFECTS

2.1. Biogenic Minerals and Their Role in the Earth's Geology

The prebiotic earth was a slowly changing, but nevertheless seething cauldron with sharp gradients in temperature from the surface to deep interior. At the surface, lighter elements were lost to the outer space and as the surface cooled, the lithosphere was formed. The very inhomogeneous cooling process led to

differences in rock density and the lighter plutonic rocks rose to become the main constituents of the continents, punctuated by eruptions and extrusive flows of more dense basaltic rock. The less dense, largely granitic continents float on the basaltic structures of the mantle. At the earth's surface, the chemistries of weathering and erosion led inexorably to the dissolution, flow and redistribution of the mineral constituents in the surface and ocean, transport to the oceanic crust, and their reprecipitation creating sedimentary rock formations. Finally, at subduction zones the sediments are drawn back into the mantle.

There is a continuous progression of this slow circulation from magma to crust and back that recycles the mineral constituents of the surface layer. However, diagenic processes change the nature of the mineral as it recycles, and metamorphic rock structures are developed. Radioactivity in the earth's core, the input of solar energy at the surface and the leakage of lighter elements to the atmosphere all guide the earth along an irreversible path of change over the geological time scale. However, at the microscale of time in geological terms, say hundreds of years, the earth is in a near steady state condition.

The earth's age has been estimated to be about 4500 my, and the crustal layer was formed about 100 my later. The transition from an abiotic world to the presence of living organisms appears to have begun about 3800 my ago, perhaps when the reducing conditions on the weathered anerobic surface, or the oceanic crust favored the production of carboniferous molecules from the carbonate released from the rock on the earth's surface and atmosphere. Initially, all of the carbon and the oxygen had been locked within the rock. As the complexity of these molecules developed, macromolecules and macromolecular aggregates must have condensed, organized, and as chemautotrophs, ultimately became self-duplicating, hence living systems. These organisms developed over the following 500 my to form single cell and algae-like systems in the Archean era and subsequent Proterozoic (Precambrian) period during which multi-cellular soft-bodied organisms developed.

Hard-bodied organisms containing structures incorporating mineralized elements developed in endless variety in the so-called Cambrian explosion about 600 my ago. These have provided abundant evidence for their existence in the fossil record. There is evidence to suggest that throughout the period when living systems emerged they utilized inorganic ions in their biochemistry, intimately affecting their environment and, more so, the nature of the mineral distribution within the biosphere coupled to the composition of the lithosphere. A current example is the production of secondary gold grains from run-off of soluble gold(III) tetrachloride near gold mine sites in Australia by the microorganism *Ralstonia metallidurans* [1]. In culture, the resident microbiota of the soil was able to initiate the process by solubilizing 80% of the native gold in the soil within about 45 days. The *R. metallidurans* then reduced and recycled the soluble gold to form secondary gold grains covered with biofilm polymers.

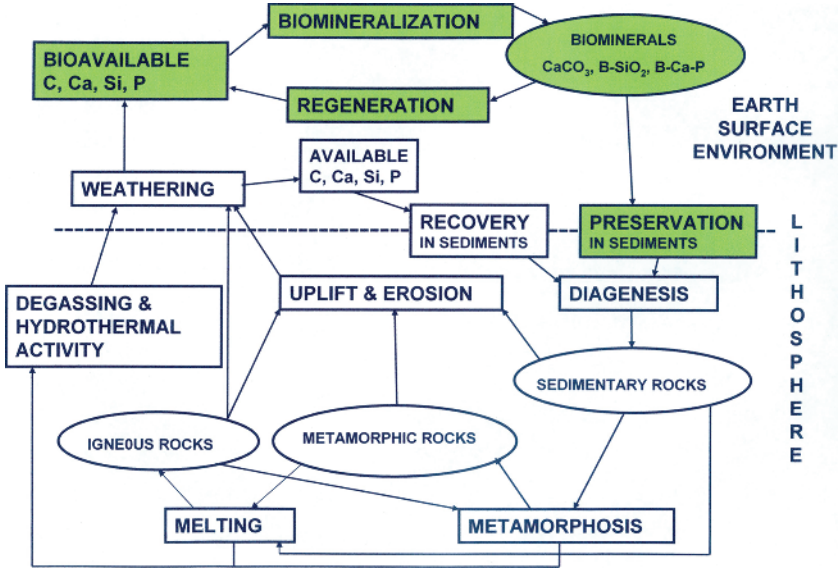

Figure 1. A composite of the abiotic and biotic effects on the rock cycle. The abiotic rock cycle makes available mineral forming elements from degassing and uplift. Erosion by weathering and surface transformation to recovered sediments is depicted in the open boxes. The presence of biota increases the rate of weathering, and, as shown in the boxes with the green background, this greatly expands the complexity of the compounds and minerals stored at the surface, adds to the diversity of the environment, and changes the nature of the materials recovered in ocean sediments. The biota-induced changes take place on a much shorter time scale than the rock cycle provides. This figure is adapted from that presented by Van Cappellen [2], with the author's permission and that of the Mineralogical Society of America.

The relationship between long time scale and short time scale processes is shown in Figure 1, modified and simplified from the elegant work of Van Cappellen [2]. Figure 1 depicts how the lithosphere interacts dynamically with the earth surface environment. In an abiotic world, the mineral components released into the surface environment by degassing and hydrothermal activity and by the uplifting of the magma through intrusive and extrusive processes were exposed to the atmospheric surface chemistry of weathering at the prevailing temperatures. New compounds of varying solubility were formed in the aqueous environment but these gradually eroded and returned to the oceanic floor as sediments where, as they accumulated at different temperatures and pressures, diagenic changes occurred, forming sedimentary rock different from the original igneous forms. Other changes took place as all was recycled at the subduction zones into the deeper mantle where additional metamorphic changes could take

place. Local conditions at all levels modulated the compositions and structures so that a world of enormous complexity and variety formed and continues to evolve without the intervention of living systems.

The appearance of simple single cell biota during the Archean era, however, added a new level of modulation of this cycle at the earth's surface (Figure 1). Operating on a very different, much shorter time scale, the biogeochemistry of the earth's surface environment now rivals in impact that of the slower geological drift. It was, in fact, the contribution of life forms in all aspects that accelerated the deposition of large amounts of sedimentary rock. The pace of such developments changed drastically about 600 my ago with the Cambrian explosion, the development of vast numbers and types of hard bodied animals whose detritus as well as their function while alive, have changed the world. The key element in Figure 1 is the superimposition of biomineralization and its role in consuming and transforming elements initially originating in emanations from the Earth's core as illustrated, but also in providing the living organisms with useful tools for metabolism, structural stability, body armor protection, and rigid elements useful in locomotion.

Obviously all of the components of the biominerals were present at the time of formation of the primordial earth, and arrived at the earth's surface over time, as described briefly above, and trace or small amounts of most elements can be found in minerals formed by biogenic systems. Although difficult to define with very much precision due to the great local variation in composition and depth, a number of studies of the average earth's crustal composition have been made [3]. The most important of these elements, relative to biology and biomineralization, are C, O, Si, S, and P, along with Ca and Mg. However, a list of the compositions of the biogenic minerals shows that three large classes are prominent and can be used to organize one's thinking [4,5]: carbonates, silicates, and phosphates.

Although one can consider the chemistry of the cycling of each of these classes between lithosphere and biosphere, it is clear that just as the rapid turnover of the earth's surface crust with weathering is coupled with the slower processes in the deep ocean and mantle, cycling of the carbonate, silicate and phosphate bearing minerals are also linked. The silicate minerals are the most abundant, but they are restricted in the sense that the majority of silica biochemistry and biogenic siliceous mineral formation takes place in the oceans. The carbonate and phosphate cycles are more prominent in the terrestrial portion of the earth's surface.

2.2. Biogenic Carbonates, Phosphates, and Silicates

Individual chapters deal with the properties of carbonate, phosphate and silicate minerals and their participation in mineralized tissues of all sorts. Here we

need only consider three factors: abundance, availability for biogenic mineral formation, and their interactions within the living organism.

2.2.1. Biosilicates

Silica-based minerals are by far the major components of terrestrial granite. On a weight basis, elemental silicon has been estimated to comprise about 22% of the mantle and crust, and as SiO_2, 45%. The silicates that participate in biogenic reactions as soluble components are primarily produced by weathering. As pointed out by Berner [6] and earlier by Garrels and Berner [7] the calcium silicates released from the surface by weathering react with atmospheric carbon dioxide to form calcium carbonate, $CaCO_3$, and silica, SiO_2, but the weathered Ca from the initial calcium silicate returns to the ocean and precipitates in the less soluble form of $CaCO_3$. The SiO_2 in the ocean is available for other biogenic interactions. From the perspective of the ocean-atmosphere weathering and recycling system the real balanced reaction is

$$2CO_2 + CaSiO_3 \rightarrow CaCO_3 + SiO_2 + CO_2 \quad (1)$$

Two moles of CO_2 are required in the reaction, with one ultimately returning from the ocean to the atmosphere, the other CO_2 is removed to the sedimentary limestone. Of course, this is a highly idealized representation, calcium silicates and carbonates are only a part of the picture. Elemental magnesium follows next after Si in abundance in the earth's surface and, as magnesium oxide (MgO), accounts for 38% of the mantle and crust. Dolomite, $MgCa(CO_3)_2$, is abundant and crustal clays and basalts containing $MgSiO_3$ are also involved in weathering and interweaving of the carbonate and silicate cycles, as depicted in the diagram modified from Garrels and Berner [7] (Figure 2). This figure emphasizes the relative sizes of the reservoirs of carbonate. The atmosphere holds about 0.055×10^{18} moles of C, the oceans 2.8×10^{18} moles, and the continental calcite and dolomite a combined 5000×10^{18} moles. The reservoir of Ca, Mg, and other silicates in the combined sedimentary, metamorphic, and igneous rock vastly outweighs these amounts. Carbon, in the form of carbonate and Ca are thus sufficiently abundant that they are not "limiting" in amount or concentration in terms of their use for synthesis in biota.

Silica dissolves in aqueous systems under mild ambient conditions (4–40 °C) at near neutral pH to form orthosilicic acid [$Si(OH_4)$]. However, the orthosilicic acid is tetrafunctional and very reactive so that at concentrations in the ppm range condensation reactions form linear and network oligomers with silane, O-Si-O-Si-O-, backbones. These network oligomers reach a limiting size and precipitate as nanoparticles, but leave some non-bonded OH groups along the

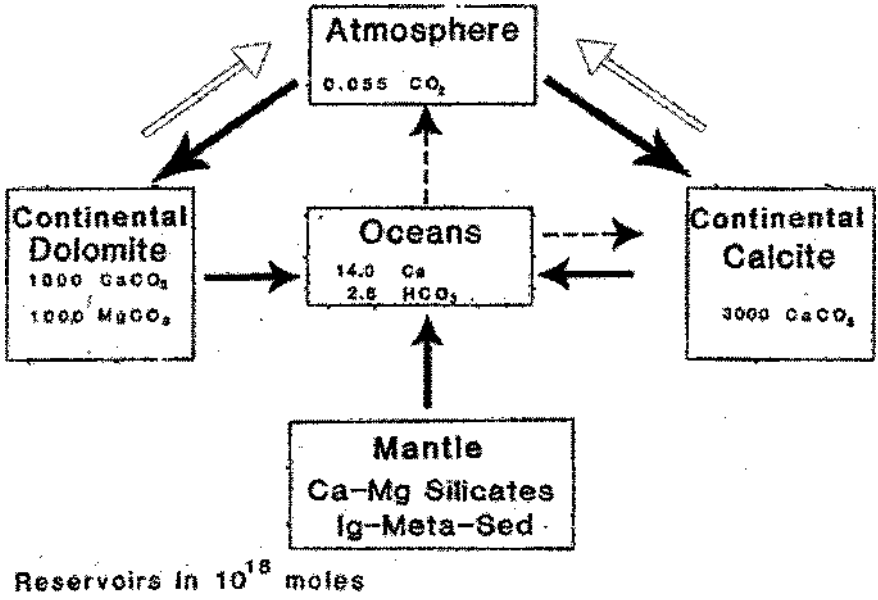

Figure 2. The relative distribution of carbonate in the major pools of the atmosphere, oceans, continental dolomite and calcite. The mantle mixture of igneous, metamorphic, and sedimentary carbonate containing rock vastly outweighs the surface reservoirs. The numbers inside the boxes represent the number of moles of CO_2 in each reservoir in units of 10^{18} moles. The arrows indicate that there are fluxes between the compartments. Note that the carbonate in the oceans is partly returned to the atmosphere, via equation (1) while some is precipitated as $CaCO_3$. Weathering, $\rightarrow$; metamorphism, $\Longrightarrow$; result of calcite precipitation, ·····►.

surface. The reaction is so rapid that, in organisms, the monomer orthosilicic acid has never been isolated, although the nanoparticles are ubiquitous [8]. This aqueous silica chemistry takes place virtually only in living organisms, which have the mechanisms available to dissolve SiO_2 and import environmental silicic acid (1 to 500 μM [9]), and to organize it into a variety of important structural forms.

Terrestrial plants, surprisingly, represent a major pool of biosilica [10] with particulate forms of biosilica in tree foliage and wood, and in grasses, which use silica for structural stiffening. Probably because of temperature–solubility relationships, arctic-alpine plants have a much lower Si content than temperate zone plants. Terrestrial plants, in general have much higher contents of Si than do aquatic plants, probably because they can locally actively extract more from the silica-rich soil than that which is available in the ocean because of the low ambient silicic acid concentration.

All of the continental weathering drainage not captured by the various plants is returned to the seas at a quite high silicate concentration; 13 ppm in runoff waters compared to the 1–2 ppm level in the oceans [11]. The soluble silica content of the oceans is actually kept well below the natural solubility of silica in water because of vast levels of its accumulation in the siliceous skeletons of the phytoplanktonic radiolarians and diatoms. The radiolarians appear to be of more ancient origin based on their fossil record of more than 600 my while diatom fossils have been placed variously at no older than 200 to 125 my [12]. However, this relative lack of diatom fossils could be attributed to the fact that the silica skeletons of diatoms are more easily solubilized than those of the radiolarians, and thus not preserved. Conclusions of this type are rather tenuous; since several studies [12] indicate that location, salinity, water temperature and water column depth all affect the diatom population such that any widely held averages allow for a large potential variation. Since the ocean is undersaturated with respect to the available soluble silica, there is a competition between species for the silica. The diatoms appear to be winning this competition as contemporary radiolaria populations have been declining.

Although radiolaria and diatoms are single cell organisms, they are highly compartmentalized and sophisticated in structure. Their appearance and structures are organized by the way in which the siliceous skeletons are deposited. The spicules are covered by a proteinaceous sheath which may regulate the diatom frustule solubility. There is no doubt that the protein components are crucial in organizing the initially amorphous silica into their exquisitely complex and species individualized forms [13] and that the entire process, including the intake of silica, is biologically mediated.

Among the metazoans the multicellular Porifera (sponges) are most primitive, and particularly interesting in terms of mineralization, since they are characterized by having skeletal spicules, which depending on the class, are siliceous, calcareous or organic. The Demospongia, representing about 90% of all sponges, synthesize siliceous spicules. As in the radiolarian and diatoms the process is controlled by the cells production of extracellular matrix macromolecules. In the Demosponge *Suberites domuncula*, collagen and galectins are the principal proteins involved with silica spicule deposition and orientation [14]. Fossil evidence for siliceous sponges goes back as far as the early Cambrian period, but the evidence is not as robust as for calcareous skeletons because of the high biosolubility of silica deposits.

The intent of this discussion is to emphasize that the evolution of complex organisms and even single cell biota made use of silica and silicates from the late Proterozoic, but more importantly, the use of silica required the pre-existence of sophisticated living systems capable of conducting the biochemistry which produced the enzymes, such as silicatein and silicase [15,16] that controlled the intake, assembly and deposition of the silica spicules in forms determined by a polymeric extracellular matrix.

2.2.2. *Biocarbonates*

The primordial biochemistry of the earliest organisms, bacteria, archaea, and eucaryota developed around the processing of carbonates and use of carbon compounds, the abundant supply of CO_2 and its dissolution in the ground water and oceans. Just as they did for the silicates, early single cell organisms capable of carrying out photosynthetic reactions, such as coccoliths, and more complex multicellular fungi, made use of the abundant metabolic carbonate to form useful structural components from highly insoluble precipitates with a variety of multivalent cations, most notably calcium ions. The calcareous coccoliths are as widespread and important for storing carbonates, and transferring them to the ocean floor as sediments, as are the diatoms for silica. Because of the stability of the carbonate precipitates, their fossil record goes back further than that of the diatoms, well into the late Proterozoic period [17]. Hard bodied animals developed with wide diversity in the oceans and on land, since the mineral deposits could be used for both structural purposes and as calcium stores for metabolic needs.

As shown by Lucas and Knapp [18] the calcareous sclerites or spicules in the coral *Leptogorgia virgulata* use the dissolved inorganic carbon in the sea water as their major source of carbon (67%) but a substantial fraction (33%) is from CO_2 generated metabolically by glycolysis. The precipitation of the calcium carbonate, and the form that the precipitate takes, amorphous calcium carbonate (ACC), vaterite, aragonite or calcite, is highly dependent on the pH, ionic strength and specific concentrations of other mineral ions such as Mg or Sr. For example, the ratio of the solubility equilibrium constants for aragonite in fresh water ($K_{s,Ar,W}$) and calcite ($K_{s,C,W}$) is $\sim$1.2 whereas in ocean water (35% salinity, pH 8.3) the ratio is $(K_{s,Ar,O})/(K_{s,C,O}) = 1.9$, and in both cases the solubility is markedly reduced: $(K_{s,Ar,W}/K_{s,Ar,O}) = 300$; $(K_{s,C,W}/K_{s,C,O}) = 470$ [19].

Water composition is also important; in the presence of Mg ions, calcite is more soluble. Since different organisms, or different compartments within the same organism, are able to selectively precipitate and retain aragonite in favor of the less soluble, more stable calcite, the species specific regulation of the crystal form can over-ride the strictly physical parameters of ambient ion concentrations, pH and so on. This is accomplished in at least two ways. In every case, the biogenic minerals are deposited or nucleated in the presence of one or more polymeric macromolecules that can influence crystal form and composition.

In most situations, the organic polymers can be synthesized directly by the organism involved, but in other cases, as in corals and algae, symbionts can contribute to either the photosynthetic accumulation of CO_2 or to the mix of macromolecules directing the mineral nucleation and form. Finally, crystal growth may not take place at the initial site of seed crystal formation, but depend on the mechanism by which the seed crystal is delivered to its growth location.

In the Scleractin coral *Astrangia danae* [20] and others, orthorhombic aragonitic crystals are embedded in trilaminar membrane-bound vesicles which are localized within the apical cytoplasm of epidermal cells. These vesicles, with crystals averaging $0.7 \times 0.1 \times 0.3\,\mu m$ in size, also contain organic matrix material, and are exocytosed through the plasmalemma to the extracellular space, where skeletogenesis takes place in the local environment of the secreted organic matrix.

Two important points emerge from this introductory discussion of carbonate-based mineralized tissues. The earliest soft-bodied organisms were based on the use of carbon compounds for the synthesis of all components, and were thus dependent on the solubility of CO_2 in water and its equilibrium transformation into HCO_3^- and CO_3^{2-}. The abundance of calcium ion and the insolubility of the $CaCO_3$ dictated the necessity of developing close control over the internal concentration and compartmentalization of the Ca^{2+} ion and equally close control of the transport of carbonates from one compartment to the next. Thus, the development of lipid membranes and, as described above, the use of lipid-bound vesicles for mineral transport, allowed the development of both intracellular and extracellular mechanisms for calcified tissue development. Along with the exocytosis of the sequestered mineral or ions was a mixture of polymeric components which provided the proper milieu, so that even outside the cell the mineral crystals could grow in a cell controlled environment.

The second point is that several forms of insoluble calcium carbonate are possible, all differing in solubility. In a strictly inorganic system, thermodynamics drives ultimately to the most insoluble, stable, and lowest energy form. In an organism with a carbonate skeleton, the end result would be calcite, which is clearly not always the case. However, the transitions between forms can be regulated by the nature of the macromolecular components and the presence of other mineral ions. Thus, organisms have developed the mechanisms to compartmentalize mineral deposition, define or specify the mineralized location both intra- and extra-cellularly, and take advantage of different mineral forms with controllable activation energies for transition from one form to another. Permutations among these factors contribute to the enormous diversity of animals with calcareous skeletal elements.

2.2.3. Biophosphates

Compared to the 45% by weight of SiO_2 if all of the phosphorus in mantle and crust were considered as P_2O_5, the comparable figure would be only a relatively miniscule 0.07% [6], but phosphate chemistry has an inordinately prominent role in living organisms. Once the evolutionary selection of hydrolysis of enol phosphate esters (such as phosphopyruvic acid, 1,3-diphosphoglyceric acid, adenosine 5'-triphosphate (ATP)) was made to use compounds with so-called high energy phosphate bonds to provide the exergonic reactions to drive the endergonic

reactions required for maintenance of life, the control of phosphate types and levels within organisms became an important problem. From the perspective of energetics, the hydrolysis of ATP yields about the same free energy as the hydrolysis of acetic anhydride, but has the advantage that the activation energy of ATP hydrolysis is high, so that ATP has a relatively long half-life in aqueous solution at physiological pH and temperature and the hydrolysis can be carried out in stepwise fashion so that the energy is delivered efficiently. The energetically equivalent acetic anhydride hydrolyzes rapidly and uncontrollably on contact with water and a large portion of the released energy would be wasted as heat.

The present day content of phosphorus in sea water is less than 0.1 ppm. A computation of the amount of P added to the oceans over time by the weathering of primary rock, based on the argument of Goldschmidt [21], would have resulted in a ~500fold increase in P content. Thus, most of the weathered phosphate not reused by animals has formed deep apatitic sediments in the oceans. In contrast to most other eukaryotic phyla which have utilized either silicate or carbonate minerals, the vertebrates have made use of the phosphates in skeletogenesis. As usual, there are some exceptions to this generalization. Several animal species use minerals in the form of calcium and magnesium phosphates in granules for purposes other than skeleton formation [4,22].

Phosphate chemistry provides two important characteristics that provide very favorable properties that justify its selection for skeletogenesis. First, as with the carbonates, the interaction of phosphoric acid, H_3PO_4, with water yields multiple pH-dependent ionic forms. These react with Ca^{2+} and other multivalent cations to form compounds of widely varying solubility. Calcium hydroxyapatite, the major mineral component of bone, has a solubility product much lower, at physiological pH, than $CaCO_3$. Second, the crystal structures of the phosphates are complex; transformations from one crystal form to less soluble forms require high activation energies, conferring the possibility of kinetic control by the stabilization of intermediate forms. Figure 3 shows the complexity of the unit cell of calcium hydroxyapatite. It corresponds to the composition $Ca_{10}(PO_4)_6(OH)_2$ and is a right, rhombic prism with $a = b = 9.432$ Å and $c = 6.881$ Å.

Figure 3A shows the projection of the unit cell on the c-axis of the basal a-b plane, while Figure 3B shows the levels of the "calcium triangles" along the c-axis at each corner of the unit cell. These drawings, from the work of Kay et al. [23] as modified by Young and Elliott [24], accentuate the bulky, diffuse charge arrangement of the PO_4 groups. In this projection two of the O atoms are at the same level along the cell z axis, depicted as if joined by heavy black lines, for example, at $z = 3/4$ while the other two O atoms are superimposed above and below P at $z = 0.93$ and 0.57. The situation is such that in the unit cell other bulky anionic groups, carbonate and citrate, can be easily substituted for the PO_4. In fact, it is the case that biological apatites are more correctly described as carbonated apatites, and bioapatites with carbonate concentrations as high as

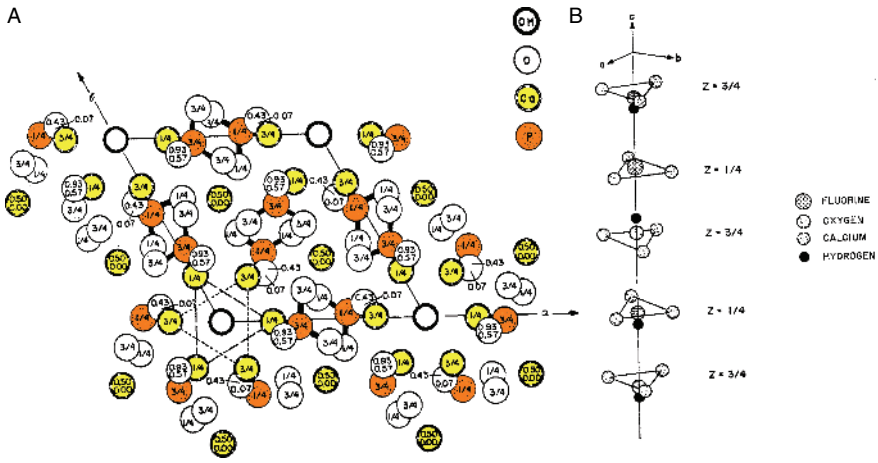

Figure 3. The unit cell structure of a pure hydroxyapatite crystal. (**A**) A projection of the unit cell contents in the a,b plane looking down the unit cell c-axis. The numbers in the atom positions represent the height above the base a,b plane in the z-axis direction. For example, the phosphorus atoms in the phosphate groups lie at $z = 1/4$ or 3/4 with two of the oxygen atoms in the same plane but with the other two above or below P at $z = 0.93$ and 0.57. Calcium ions are in yellow. (**B**). The triangular arrangements of the Ca ions in the c-axis corners of the unit cell are shown in the C-axis direction. This projection illustrates how the substitution of a fluoride or chloride ion for the hydroxyl group, alters the structure, and hence solubility, of the apatite significantly. The figure is by Young and Elliott [24], presented in a slightly modified form from that in the original paper, with permission.

12 to 15% are not uncommon. The apatite structure can be maintained up to greater than 20% carbonate [25]. Similarly, along the c-axis (Figure 3B) the calcium triangles alternate their orientation, and the position of their associated monovalent anion can vary. As depicted, the associated hydroxyl ion lies above or below the plane of the calcium triangle, and all of the O-H dipoles are aligned in the same direction along the unit cell c-axis.

If fluoride ion, with its greater electronegativity, is present, it can displace the OH and fit centrally in the plane of the calcium triangle, with the consequence of a small change in c-axis length and decreased solubility. Hydroxyapatite has a solubility product of 3.73×10^{-58} at 25°C and pH 7; fluroapatite has a solubility product of 2.51×10^{-60} [26]. On the other hand, if a chloride ion is substituting for a hydroxyl group it is displaced to a position equivalent to the H of the OH, well above the plane of the calcium triangles. This causes a profound change in crystal structure such that pure chloroapatite has a much greater solubility than hydroxyapatite, so that biochloroapatite does not form in bone in spite of the abundant chloride ions in body fluids [27].

Biological apatites rarely have the theoretical stoichiometric elemental Ca/P ratio of 10/6 (1.67), and this has been the topic of many dozens of studies. In addition to the possibilities noted above of the substitution of carbonate or citrate for PO_4, and due to the several equilibrium states of PO_4^{3-} in aqueous systems, HPO_4^{2-} is often accommodated. Tricalcium phosphate, octacalcium phosphate, brushite and whitlockite may also be found. The calcium ion can also be readily replaced with di- and tri-valent cations of approximately the same ionic radius without producing serious disruption of the characteristic apatite structure and solubility. Mg ion can replace Ca in apatite at low concentrations but above 14% Mg crystalline brushite platelets form, and are stable at pH 7.5 [28].

The rate of formation of apatite may be altered, under physiological conditions, by the presence of other cations. Lead and strontium also enter easily into the apatite structure and because of their low solubility are retained until the bone is actively resorbed. In essence, bone mineral is a very heterogeneous material, of varying composition dependent upon the presence of components in the animal's tissues at the time of deposition. Trace elements from aluminum to zirconium have been found in human tooth enamel, the tissue with the highest degree of mineralization in the body [29]. Because bone is continuously being formed and remodeled the composition of bone in an individual may vary over one's life, and this may have important interplay with the hosts physiological chemistry and structural stability.

3. MINERALS WITHIN LIVING SYSTEMS

3.1. Structural and Functional Relationships

Even in a simple inorganic solution crystallization is a complex process in which, as a first step, a cluster of ions must form and have a sufficiently high interaction energy to counter the unbalanced interactions at the surface of the cluster that would lead to easy dissolution. One can thus define a critical cluster size for nucleation of the crystal growth. The crystal then grows into some preferred ion lattice arrangement. In a complex crystal each face of the nucleated crystal may have a different affinity for individual ions so that growth of a crystal may proceed more rapidly on one set of faces than on another, producing characteristic preferred crystallographic forms. The crystal habit and rate of growth can depend upon the other components present in solution, temperature, and pressure.

Crystallization in living organisms has many layers of control added on to these basic principles. Organisms utilize their mineralized deposits for storage of mineral ions for metabolic purposes, and structural purposes such as forming skeletons for organizing shape, creating rigid elements to enable locomotion, and for creation of dermal armor as protective barriers. In each case the organism must create regulatory mechanisms by which it can control the

environment in which the mineral grows, assure that the mineral is properly placed, direct the mineral phases to their correct shape and size, and, particularly where the mineral is stored for later use, remain appropriately accessible for utilization.

In a truly classical analysis of these problems Lowenstam [30] divided the level of biological control into two broad categories: "biologically-induced" and "organic matrix-mediated" mineralization. In biologically-induced mineralization, control is principally based on location, once nucleated, the mineral adopts crystal habits similar to that which the mineral would have taken if crystallized in an inorganic medium and the crystallite aggregates would be arranged essentially randomly with respect to the nucleation site. Bacteria and Archaea, and Protoctists such as some green and red algae, have been shown to produce minerals that fit the biologically-induced model [30], based mainly on the similarity of the mineral crystal habit and that produced inorganically, but some eukaryotes also may form intracellular storage granules containing minerals with crystal habit indistinguishable from the inorganic form [31]. At the other extreme, in "matrix-mediated" mineralization, the minerals would again be nucleated specifically, but their crystal habit would be well-defined, the crystal sizes would fall in a narrow range and they would have a well-defined organization with respect to the nucleation site. Further, they could assume unique crystal forms that would not have been possible in a purely inorganic system. This is the more widespread case in most eukaryotes, although some bacteria can precipitate intracellular mineral which fits the matrix-mediated model, as described in detail in one of the later chapters. The same animal may produce both apparently biologically-induced and matrix-mediated crystalline structures in different compartments [31,32]. Thus, while it was a very important observation, and helped tremendously in organizing the thinking concerning biomineralization over the past several decades, it is probably not very useful to retain the distinction between biologically-induced and matrix-mediated mineralization. Rather, one should focus on the consideration of the regulatory processes that animals have used at each step of biomineralization: the mechanisms of nucleation, organic matrix formation, and crystal growth regulation. These subjects are all being actively explored in the new field of biomimetics, with the aim of reproducing synthetically in the laboratory what nature has developed in so many diverse ways.

3.2. Compartmentalization: The Key to Control of Crystal Form, Size, and Shape

Mineralization in eukaryotes is generally extracellular, yet is under close cellular control. As indicated above, the first task is to prepare a controlled environment for mineral formation: the animal or cell has therefore to create an extracellular compartment competent to receive the sequestered mineral ions. The term

compartment has to be considered in a broad sense as a space or locus with limited access, it may not need to have any bounding walls, but could as well be a porous polymeric gel.

Several examples will be discussed here to consider some of the various solutions that different organisms have developed to regulate mineralization and to control the environment in which mineralization can take place: (1) extracorporeal surface precipitation (tube-building polychaetes); (2) closed compartments (mollusks); (3) dynamic scaffolds acting as templates for mineral nucleation, but not retained in the final structure (tooth enamel); (4) fibrillar arrays with defined spaces or pores (vertebrate bone); (5) the assembly line or conveyor belt model (avian egg shell); and (6) the ultimate single crystal composite (sea urchin teeth).

3.2.1. Controlled Extracorporeal Biomineralization

A wonderful example of the process of extracorporeal construction of a biomineralized structure is that of mineralized tube construction by the tube-building polychaete *Phragmatopoma californica.* This sabellariid worm, anchored in place on a firm base in turbid intertidal zones where wave action provides a flow of food and mineral debris, collects particles of sand and calcareous shell bits by sweeping them into its mouth with tentacles. It selects and retains those particles of the correct size in a nearby "building organ". A thoracic gland secretes a complex mixture of at least two cystine, glycine, lysine, and 3,4-dihydroxyphenyl-L-alanine (DOPA) containing basic proteins, and a serine-rich protein, rendered acidic by phosphorylation of about 80% of its serine residues to phosphoserine. Ca and Mg ions are also added to this mixture. The gland secretion mixture is spotted at several points on each sand grain in the building organ. The building organ then deposits the sand grain and the reactive mixture of anionic and cationic proteins onto the end of the sand tube being built. The coacervate-like complex in the protein mixture is cross-linked and insolubilized in this decidedly aqueous environment by the quinone-like tanning reaction of the DOPA with the cystine residues as it is bound to the adjacent sand grains [33,34].

The tube-building polychaetes are communal animals and accumulate densely in great numbers at favorable sites. Although each individual builds only a single tube, one grain at a time, thousands of adjacent tubes bind together by the protein cement on each grain surface to form very high strength and stable reefs. Although the mineral component is not synthesized by the *P. californica* this is a clean example of biologically controlled matrix-mediated mineralization: at every step from capture and sorting of the exogenous mineral crystals, to the unique addition of an interactive, reactive organic matrix that organizes and stabilizes the mineral-organic composite. Once tube-building begins, the

P. californica remains within the tube and maintains tube integrity for the remainder of its life.

3.2.2. Controlled Mineralization in an Extracellular Compartment with a Dynamic Scaffold

Mature dental enamel in vertebrates is essentially a 98% carbonated hydroxyapatite organized in bundles (enamel prisms) of very long ribbon-like carbonated apatitic crystals with their unit cell a,b plane perpendicular to the long c-axis direction. The epithelial ameloblasts which construct the enamel are formed initially as a layer of cuboidal cells (preameloblasts) with tight junctions between neighbors. This ameloblast layer sits upon a corresponding underlying layer of mesenchymal cells (odontoblasts), which will themselves produce a second mineralized tissue, the tooth dentin which is also comprised of a carbonated apatite. The mature mineralized dentin is about 55–65% apatite by weight, and the rest of the structure is an organized network of collagen fibrils and small amounts of other proteins important for directing the mineralization process, which yields platy apatite crystals impregnating the matrix as discrete nanocrystals.

The initial odontoblasts (preodontoblasts) are also cuboidal. A basement membrane, synthesized by the preameloblasts separates these two layers. A large number of regulatory signals are passed between the two cell layers and finally, the preodontoblasts begin to mature, the basement membrane is degraded and the odontoblasts begin to secrete the collagenous matrix to form predentin, which then forms the mature mineralized dentin. We will discuss that mineralization process separately.

The preameloblasts wait until the first dentin is formed and begins mineralizing, and then they begin to elongate and mature to their secretory state. The nuclei of the ameloblasts polarize and move to the outer surface, while the elongated cells develop a secretory process, called the Tomes process, at their distal end initially in contact with the dentin surface. The elongated ameloblasts are joined by tight junctions that essentially preclude fluid transport around cells from the tooth exterior to the dentin layer. The Tomes processes actively pump mineral ions, water and an array of proteins and glycoproteins into the restricted extracellular spaces defined by the mineralized dentin layer and the space created between the neighboring Tomes processes, which are narrower than the main columnar ameloblast bodies, and not sealed by the tight junctions. The main secreted protein is amelogenin, a 25 kDa globular protein with a hydrophobic central domain, but with short N-terminal and C-terminal sequences of more hydrophilic character which emanate from the globular core. These molecules form aggregates called nanospheres, which self-assemble into dynamic linear chains of spheres, decorated periodically along the chain with C-terminal sequences projecting from the surface. These are thought to lead to nucleation

and elongation of the enamel carbonated apatite ribbons [35,36]. The matrix surrounding the growing apatite crystal prisms also contain several proteases which selectively degrade the amelogenin molecules and remove them to make space for the densely packed prisms of apatite ribbons. It is important to understand that the Tomes process spaces remain at about the same size during the period of crystal development; the epithelial layer of ameloblasts simply stays at the surface of the enamel layer that has been deposited. When the enamel reaches its genetically programmed thickness, then the ameloblasts stop secreting matrix and the cells lose their Tomes processes, a variety of enzymes clean and smooth the protein remnants on the surface, and ultimately the ameloblasts die and are removed.

Comparable to the sand tube of the *P. californica*, the mature enamel mineral is a non-living tissue, put in place for the life of the tooth. However, it is clear that every aspect of the enamel mineral structure is formed by matrix-mediated factors, under exquisite control by the ameloblasts and determined by the composition of the dynamically assembled amelogenin matrix and the competing processes of mineralization and extracellular matrix degradation [37]. The details of the enamel formation process are discussed in detail in Chapter 15.

3.2.3. Controlled Extracellular Mineralization within a Fixed Polymer Matrix

The vertebrate tooth is a particularly interesting biomineralized structure because it has two carbonated apatite structures with essentially the same crystallographic unit cell parameters but with distinctly different crystal habit immediately adjacent to each other [38]. As discussed above, the tooth enamel mineral is in the form of very long enamel prism bundles of thin ribbon-like apatite and, in its mature form has only a small fraction of organic component. The dentin mineral, on the other hand is dispersed within a collagen fiber matrix as small, thin platy crystals or aggregates of these discrete elements. The plates are not continuous but are arranged in an orderly fashion within the collagen fibrils and fibrillar matrix.

During tooth development the neural crest-derived ectomesenchymal preodontoblasts line the basement membrane that has been secreted by the juxtaposed layer of preameloblasts. The preodontoblasts also polarize and elongate as they mature to the secretory form and begin to secrete a hydrated extracellular matrix composed of collagen fibers and a variety of proteoglycans. The shape of the tooth is determined by the shape of the basement membrane laid down originally by the preameloblasts. Proteases originating in the preodontoblasts degrade the basement membrane as they begin to mature to their secretory stage and begin to produce the dentin collagenous matrix, creating the distinct dentinoenamel junction (DEJ) which remains as a fixed architectural feature of the mature tooth. Just as the mature ameloblasts retreat from the enamel mineral they have deposited,

originating at the DEJ, the odontoblasts retreat in the direction of the pulp from the matrix they have created. However, the odontoblasts remain attached to the DEJ by forming long narrow processes as they retract. The matrix of collagen fibrils surrounding these processes mineralizes.

The space occupied by the cell processes does not mineralize, creating tubules in which the cell processes may remain viable, and connect the entire matrix to the living odontoblasts. The mineralized dentin, in sharp contrast to the mature enamel, is a living tissue. The matrix immediately adjacent to the base of the odontoblastic process and the main cell body, the predentin, does not become mineralized. The collagen fibril matrix is formed and organized within the predentin before the apatite crystals form both within and between the collagen fibrils at a boundary called the mineralization front. At the region of the mineralization front proteoglycanases are activated and degrade the predentin proteoglycans that act as mineralization inhibitors. At the same time specific phosphoproteins [39] of the SIBLING family [40,41] are secreted into the extracellular matrix and interact at specific locations on the collagen fibrils and, once bound, nucleate apatite formation.

Collagen fibrils are built from rod-like collagen molecules that are packed in an oriented staggered array such that at the electron microscopic level they have a periodic cross-striated appearance, with the main periodicity, D, at about 67 nm. Since each molecule is 300 nm in length, $\sim$4.4 D, they cannot join end-to-end and thus depend on side-by-side interactions for stability. This leaves gaps of 0.6 D between molecular ends along a filament axis [42–44], and these gaps align within a microfibril creating channels with dimensions approximating the average size of the platy apatite crystals (Figure 4) [45]. The matrix phosphoproteins bind at the gap region and are presumed to nucleate the localized crystallization process [46]. Thus the mineral density shows the same average 67 nm periodicity as premineralized or demineralized collagen fibrils. Since mineral can mature and grow between fibrils the imposition of the mineral does change the collagen packing structure to differing extent at different locations in the dentin [47]. The important point to be made here is that the matrix is assembled first, and then specific nucleating matrix-interactive molecules are secreted to direct the placement of the crystals at specific locations within the matrix, while the matrix framework can limit crystal size.

In some species, particularly those where the teeth have a long life time, the dentin may contain an additional mineralized structure, the peritubular dentin. As the odontoblasts recede from the dentino-enamel junction and the process partially retracts, the empty tubular space between the mineralized collagen matrix and the process cellular membrane is sometimes filled with a dense mineral phase, not associated with the collagen and not organized in the same way, although it still contains the same kind of platy carbonated apatite crystals as the collagen matrix [48]. Recent studies in my laboratory [49], and current studies not yet published, have established that this mineral structure, called peritublar

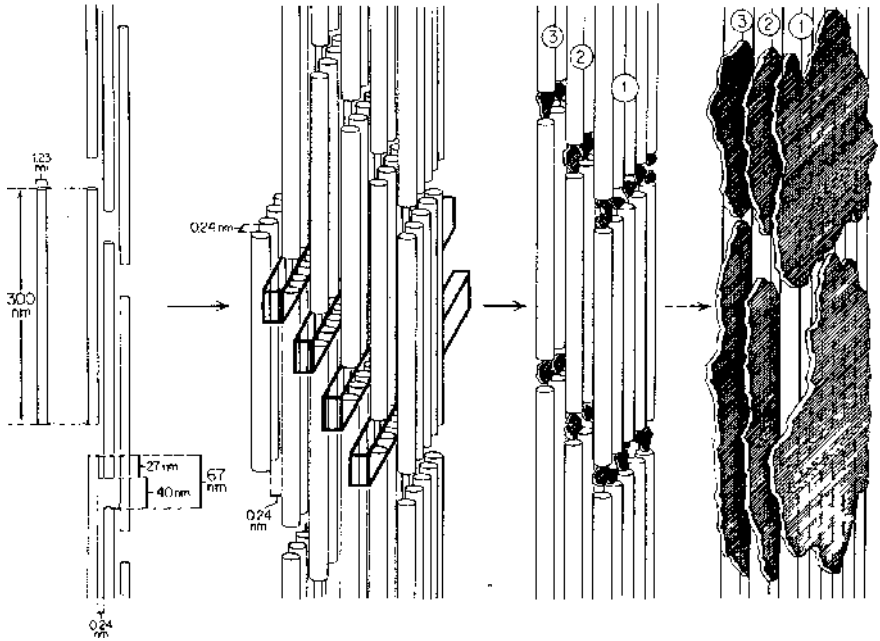

Figure 4. The progressive steps in deposition of apatite crystals within a preformed collagen matrix. From left to right: (1) The rod-like collagen molecules assemble in a regularly staggered array, which leaves gaps between the ends of the molecules in the fibrils; (2) the gaps are aligned to produce continuous channels within the fibril; (3) apatite nanocrystals are nucleated and begin to form at the entry to, or within, the gap channels; (4) as the mineralization proceeds, the mineral can outgrow the channels and fill space between fibrils. The mineral growth can occlude nucleating, and other proteins in the fibril network. From Landis [45], reproduced with permission.

dentin, is a heavily mineralized complex of proteolipids and phospholipids, also containing phosphorylated proteins not typical of those extracted from the mineralized collagen matrix. Much more work remains to be done before a more complete understanding of this mineralized phase is available.

For the purposes of this discussion, vertebrate bone could just as easily have represented the mineralization in a fixed polymer matrix of collagen fibrils. The crystals are similarly related to the direction of the collagen fibrils, filling the intrafibrillar channels and showing the collagen fibril organized periodicity, as illustrated in Figure 4. Further, mineralization is related to the presence of SIBLING proteins secreted into, and interacting with, the collagen matrix. But there are a number of differences between bone and dentin:

Foremost is the fact that the osteoblasts organize and construct their collagen matrix in a different ultrastructural form, and as they manufacture the matrix each osteoblast can become completely surrounded by the matrix except for a network

of thin processes in contact with its neighbors. At this stage, the confined, quiescent osteoblasts, now called osteocytes, remain alive and responsive to their environment.

Second, the repertoire of SIBLING proteins in the bone matrix is quantitatively and qualitatively different from that in dentin. The nucleation and crystal growth regulating proteins are related to those in dentin but are different, and this may be related to the process of bone remodeling. Skeletal bone is a tissue constantly in the process of turnover and remodeling, and very responsive to mechanical stress. Dentin, on the other hand is not removed except in pathological situations, and in the normal digestion of the root dentin in deciduous teeth in the process of being shed. Osteoclasts are specialized cells in bone which remove the bone and prepare the excavated surface for new bone addition.

Osteoclasts are not impeded in their resorptive activity by the matrix components in dentin, and in fact, polished dentin surfaces are used to test osteoclast activity; osteoclasts are seeded on the dentin surface and the number and surface area of pits created in a defined time is recorded. Odontoclasts appear on the deciduous tooth root surface only at the time of shedding and function in the same way as osteoclasts. However, differences have been observed in the signaling pathways leading to activation of the two types of cells [50]. Differences in remodeling of bone are also seen by comparing bones of different types, such as the bone of the skull and the diaphysial portion of the long bones. However, regardless of the nature of the bone brought about in response to differing mechanical and physiological requirements, the mineralization of bone, and dentin, show the same relationships between the mineral crystals and the collagen matrix. The differences in bone size, shape and permanence are determined by the cells secreting the matrix and those degrading it. Chapter 14 by Adele Boskey elaborates on these themes.

3.2.4. Mineralization in Fixed Extracellular Compartments

The hydroxyapatite-based skeletons of the vertebrates, although of particular interest to humans, represent only a small fraction of the mineralized structures produced by multicellular animals. Calcareous structures are produced in a much wider number of diverse species, and in a wide variety of forms. Chapters 2 and 5 deal with the details of the carbonate formation, but a few general comments are important to emphasize some general design concepts and problems. While the calcareous shell of every animal has an individual arrangement, in general one can say that the shell is a layered structure, and that the layers may alternate between polymeric organic sheets, and mineral crystals. These are the basis for the superb mechanical strength and fracture resistance of the biogenic carbonates.

One of the most striking, and very well studied systems is the ordered crystallization of calcium carbonate within the mollusk shell, but the systems and

mechanisms of crystallization are not so simple. In the mollusk, shell formation is initiated at the epithelial cells of the periostracum, a thin membrane of tightly connected cells that seal the shell organ from the surrounding sea water and which is tightly joined to the mantle epithelium at a structure called the periostracal groove at the growing edge of the shell. The mantle organ is a complex structure with muscle and nerve enclosed by another epithelial cell layer, the mantle epithelium. The space between the mineralizing shell and the mantle epithelium encloses the extrapallial fluid [51,52] which supplies the mineral ions as well as a number of proteins and other macromolecules that regulate and organize the crystal growth. Prismatic calcite begins to form along the inner surface of the periostracum with the a,b plane of the deposited calcite crystals parallel to the periostracum surface. Crystal growth proceeds internally most rapidly along the calcite c-axis direction, perpendicular to the periostracum surface, giving rise to elongated prisms. Growth is such that the prisms grow in diameter and become surrounded by thick interprismatic organic membranes, the orientation of the a,b plane axes of neighboring prisms is not well ordered between prisms. The interprismatic membranes form as polygonal chambers preceding the deposition of the calcite within them. Growth of the calcite prisms in the c-axial direction is not continuous and thin organic sheets, appearing as growth lines between calcite layers are deposited within each prism [53,54]. Figures 5 and 6 illustrate the arrangement of the prism walls, the prisms, and the growth lines.

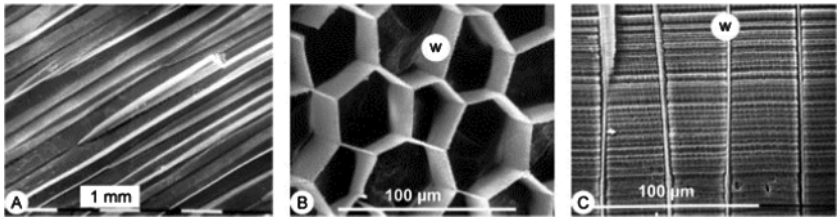

Figure 5. Structures in the prismatic calcite layer of the shell of *Pinna nobilis.* (**A**) Low magnification view of the surface of a vertical fracture through the prismatic layer, showing the axially aligned prisms. (**B**) A transverse, polished, and etched demineralized surface of the prism ends, showing the thick polygonal organic prism walls. (**C**) A tenfold higher magnification view of the vertically fractured surface, after polishing and etching as in B, showing that the prisms have regular growth lines punctuated with thin organic membranes. The prism walls (**W**) are also evident. The magnifications are indicated in each panel. Reprinted from the paper by Dauphin [53], with permission.

When the full depth of the calcite prisms is achieved, the mantle epithelium secretions change and a somewhat different organic matrix assembles

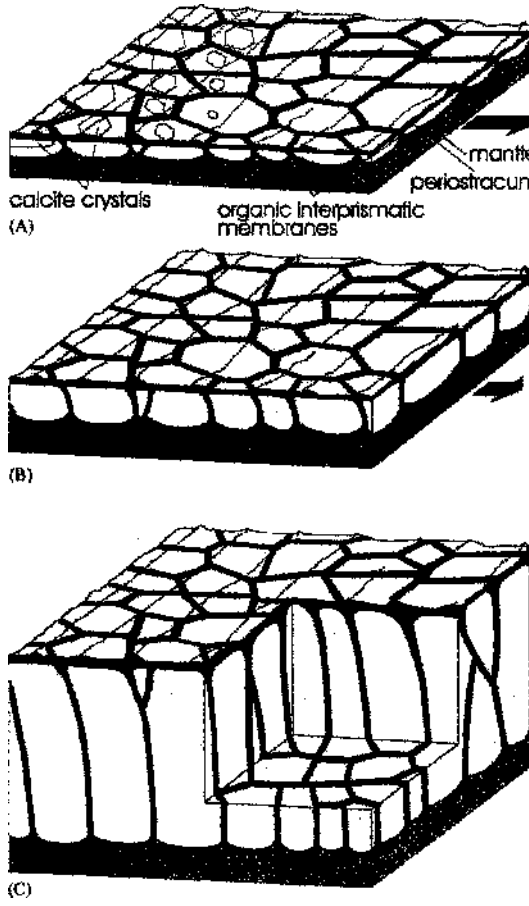

Figure 6. A schematic representation of the mode of formation of the calcite columnar prisms in mollusk shells, based on studies of bivalves of the class Pterioida. (**A)** Initiation of the prism formation. The periostracum is at the upper surface in this model, the calcite crystals begin to grow in the downward direction, utilizing the mineral and organic components within the extrapallial fluid, the solid black base. The interprismatic membranes grow along with the prisms. The solid black arrow on the right indicates the direction of the shell growth in length. (**B)** Growth of the prismatic layer continues in both thickness and length by the addition of new prism at the leading edge. (**C**) In the mature part of the shell, after extrusion of the mantle ceases, the prisms continue to elongate to their final extent. This figure is from Checa et al. [54] reproduced with permission.

[55–57] into brick-like chambers. These chambers fill with aragonite crystals to form the layer known as the nacre. The aragonite also has its unit cell c-axis perpendicular to the periostracum, but the crystals grow in the a,b plane preferentially in the direction of the b-axis and in the direction of shell

growth. They are limited in thickness by the compartment walls in the c-axial direction. Growth within a chamber seems to be initiated at more than one site, with some disorder in orientation, but with maturation the entire crystal within a chamber takes on a similar orientation, and this is related in every nacre chamber [58]. The polymeric walls between nacre plates appear to be punctuated with nanopores, and mineral bridges form between compartments. It has been suggested that these mineral bridges add considerably to the mechanical strength of the nacre [59].

Many studies have been made concerning the compositions of the proteins and polysaccharides of the nacre and prismatic calcite, and how these might relate to the shapes and mechanical properties of the shell layers [60]. One of the major problems, seemingly not yet addressed, although studied at the ultrastructural level [61], is the regulation or specification of the boundary or junction between prismatic calcite and aragonitic nacre. If one considers the properties of each layer individually, they surely differ in bending strength and other measures of mechanical strength and toughness.

This system reminds one of the enamel–dentin arrangements in vertebrate teeth where a high compressive strength, but relatively brittle enamel mineral rests upon a much more mechanically compliant base with greater resistance to crack propagation. Much more attention ought to be paid to the correlation of adjacent crystalline phases in these tissues, and, in the mollusk shell, to the problem of how the mantle epithelium cells regulate the switch from prismatic calcite assembly to nacre formation, dramatically altering the mineralized system architecture. These are very active areas, intimately connected to many aspects of the mechanical design of artificial biomimetic mineralized materials as well as those of biogenic origin.

The past few years have seen outstanding progress in studies of the macromolecular components of the shell matrices. The rapid advances in cloning of the genes has provided detailed information about the principal protein sequences and is allowing for insight into their nature and properties, but has not yet provided any direct information on the mechanisms of the mineralization processes and controls. The largest gap in our understanding of how the shell matrix is constructed is at the level of the cell biology of the mantle epithelium cells. The controls and signals regulating mineral type and specifying compartment shape and orientation are all prime topics for the next set of advances in this area. The actual structure of the aragonite tablet and its interaction with the organic phase is also in question. Studies with high-resolution transmission electron microscopy, and solid state ^{13}C and ^{1}H NMR have indicated that the aragonite platelets of the abalone *Halitosis laevigata* are covered with a continuous layer of $\sim$3 to 5 nm amorphous $CaCO_3$, not consistent with the prevailing view of some epitaxial match between the aragonite and the organic matrix surface structure [62].

3.2.5. The Avian Egg Shell – The Conveyor Belt or Assembly Line Model

A problem of substantial biological significance is the construction of the shell of the avian egg (see also Chapter 2). This is not entirely unlike the sand tube construction problem discussed above. The fertilized ovum, the "package" to be delivered, enters the tubular oviduct at the proximal infundibulum chamber where it begins its secretory passage, and the protective coats are added serially [63], by specialized cells or glandular ducts that line the tube in a spatially organized way.

In the chicken, *Gallus gallus*, the oviduct has five sections: the infundibulum, magnum, isthmus, tubular shell gland, and shell gland (uterus) [64]. The total transit time, from fertilized ovum to egg deposition is about 20 to 24 hours. Within the first hour of entry into the infundibulum, the egg passes into the region of the magnum, where the lining cells secrete the albumin coating in about 2–3 hours, the shell membranes begin to form in the isthmus where the complex inner shell membrane of types I, V, and X collagens [65] enclose the albumen surface.

Dermatan sulfate proteoglycans are added to the collagen layer as the egg moves along into the tubular shell gland, also known as the Red Isthmus, and, during the next hour, these form aggregates designated as mammillary knobs, which, with a number of other specific secreted proteins, initiate calcification and the growth of calcite. The shell then grows to its full thickness within the shell gland in asymmetric calcite crystals called palisades within the final 15 to 20 hours. A thin cuticle which terminates the growth of the calcite palisades is added. The final shell structure is illustrated in Figure 7 as a composite of a SEM and schematic, taken from the work of Dennis et al. [65].

The many vesicles indicated in the schematic drawing show the complexity of the shell, and emphasize the fact that there are many inclusion bodies whose contents and function are not known in detail. It may well be that secretions from the various glands become trapped as the egg passes from one section of the oviduct to the next, and mineralization overtakes the matrix. This may also have a function with regard to the final mechanical requirements of the shell, which may not demand more perfect structures and, in fact, add to the crack resistance of the shell. Equally interesting is the fact that the inner shell membrane of types I, V, and X collagens, added before the other shell layers, does not mineralize at all. The palisade calcite precipitation begins at the mammillary knobs, in the presence of macromolecules not present in the inner shell membrane. On the other hand, the termination of the pallisade growth appears to be linked to the presence of the inner part of the outer shell cuticle, with the formation of a thin layer of hydroxyapatite that appears to inhibit further

Figure 7. The structure of the avian egg. The left panel is a scanning electron micrograph of a fractured egg shell. Showing the collagen-based inner shell membrane (SM); the mammillary knobs (M) where mineralization begins; the calcite palisade region (Pal) that forms the major part of the shell; and the cuticle (Cu) at the external egg boundary. The right panel is a schematic drawing describing more detailed features. Of particular interest is the layer of hydroxyapatite granules at the inner surface of the cuticle. This switch to apatite deposition may have a role in limiting the calcite palisade growth. Modified from Figures 1 and 12 of Dennis et al. [65], with permission.

calcite growth [66]. Having one crystal formation as an inhibitor of another is a novel idea.

As Fernandez et al. [63] put it so eloquently in the title of an early paper, very clearly "eggshells are shaped by a precise spatio-temporal arrangement of sequentially deposited macromolecules" and the oviduct cells are programmed for that sequential delivery.

3.2.6. Echinoderm Teeth – A Cell Membrane Model

The final example of compartmental mineralization strategies is that of the echinoderm tooth. Echinoderm teeth are remarkably complex mineralized tissues with outstanding mechanical properties of strength and toughness. These derive from both the overall tooth architecture and the mixture of calcite plates, needles and prisms [67,68]. Figure 8 depicts a cross-section of a mature portion of a *Lytechinus variegatus* tooth and how it relates to the intact tooth. The cross-section in Figure 8b was obtained by scanning electron microscopy (SEM) and shows the high density calcite structures in white, the low density, essentially transparent organic matrix appear as black empty spaces [69]. In spite of having a mixture of various crystal organizations in different parts of the developing tooth, in the camarodont (T-shaped or flanged) *L. variegatus* tooth, and similar species, all of the calcite elements appear to have the properties of a single calcite crystal under X-ray scattering or polarized light analysis [70–75]. This amazing property shows that even though the tooth is a curved structure, and the individual crystal plates are separated by cell membranes, the calcite in each crystal or prism has the same crystal axial orientations. Even more amazing, the tooth composition varies with position and maturity.

The urchin tooth is a continuously growing structure, changing in concentration of organic matter from a maximum in the lightly mineralized plumula at its aboral origin, to the more heavily mineralized structure at the incisal adoral edge. The tooth captures food by a scraping process, wearing away the incisal edge by abrasion while feeding. Synthesis begins at the aboral end (Figure 8a, lower right corner) where the tooth forming odontoblasts condense in a layer on the surface of the plumula, a loose collection of collagen fibrils and other extracellular matrix components. Neighboring odontoblasts merge membranes, flatten and form plate-like multinucleated syncytia that project into the matrix with sheet-like membrane bounded structures that continue to extend in the adoral direction throughout the life of the tooth.

Using tritiated thymidine, Holland [76] showed that cell division and expansion of the number of odontoblast nuclei took place only in the plumula at the point of aggregation of the odontoblasts. The nuclei within the syncytial layers did not divide but were moved along within the syncytial layers in the adoral direction. The total transit time for a labeled nucleus to form in the plumula and then wear away at the incisal tip in *S. purpuratus* was about 120 days. Direct observation

of histological sections of the tooth shows that the syncytia are, at their widest, only a little thicker than the diameter of a nucleus.

The calcite primary plates grow in the inter membrane spaces between syncytial layers, and probably increase in size during the entire period of their existence. The compartments for mineralization in this case are comprised of the cell membranes. There are no extensive compartment walls as in the mollusk calcite or nacre mineralization. Present work suggests that transmembrane proteins with hydrophobic domains embedded in the membranes and acidic domains exposed at the membrane surface may be involved in nucleation of the calcite

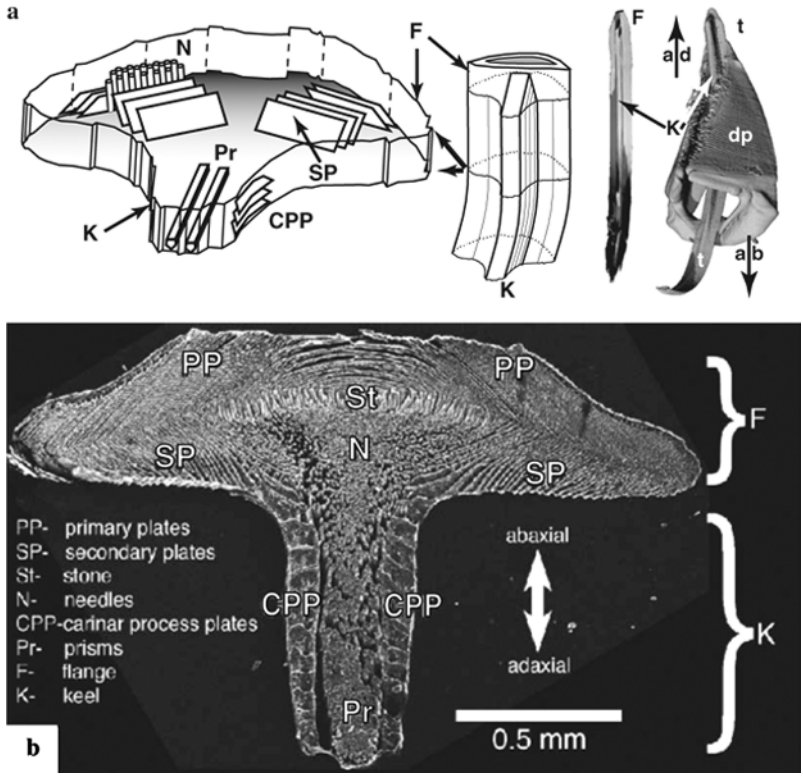

Figure 8. Structures of the tooth of the echinoderm, *Lytechinus variegatus*. (**a**) From right to left: a rendering of the mineral density from a microCT scan of a single tooth in its demipyramid (dp) housing; the flanged tooth pictured from the keel (K) to flange (F) direction; drawing of the zone at about the midpoint of the tooth length; the cross-section at the point indicated, showing the relative orientations of the primary plates (PP), secondary plates (SP), prisms (Pr); and carinar process plates (CPP). **(b)** A labeled scanning electron micrograph of a cross-section of a tooth at the position drawn in panel (a). From Robach et al. [77], reproduced with permission.

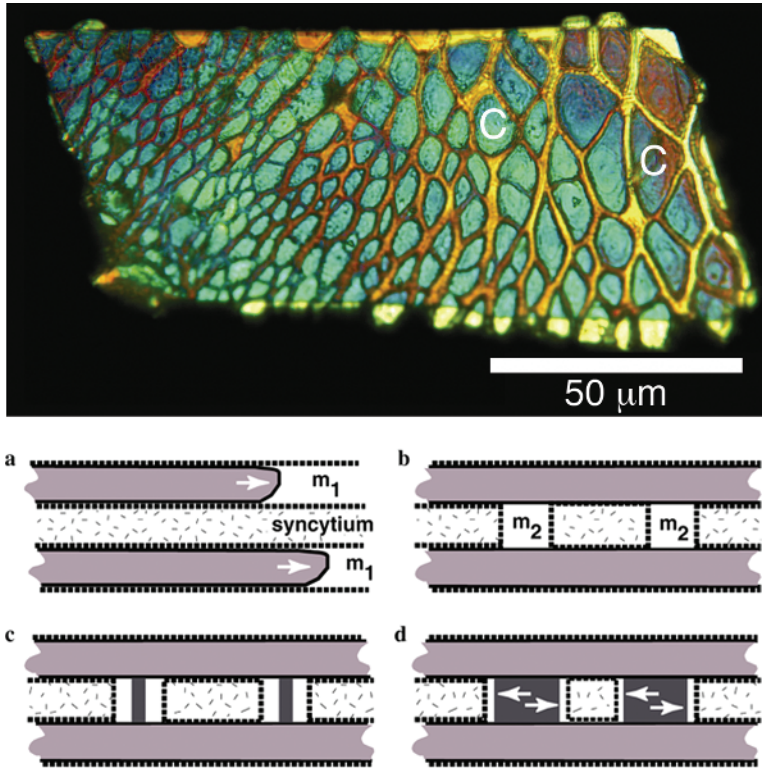

Figure 9. **Top**: A polarized light micrograph of a portion of an exposed face of a primary plate fragment from near the incisal tip of a mature tooth. The calcite surface of the tooth has many columns (C, green) of high magnesium calcite that join to the adjacent plate. The channels between the columns (yellow-orange) contain the remnants of the interplate cell syncytium layer. A comparable section at a less mature position would have a much more sparse distribution of columns and comparatively wider ribbons of cell syncytium. The orientation of the crystal axis of the plate is matched exactly by the orientations within each column, although the plates have a much lower Mg^{2+} ion concentration. **Bottom**: Schematics of transverse sections between two adjacent plates. (**a**) Early in mineralization, the calcite plates grow in size in the space (m_1) between the syncytial membranes. (**b**) The syncytial layers begin to reorganize to create new mineralization spaces, m_2. (**c**) High Mg^{2+} calcite columns begin to form within m_2 and begin to fuse the plates. (**d**) Finally, as the tooth matures, the columns expand laterally and the syncytial space shrinks. At this point the tooth is truly a single crystal because of the plate–column crystal axis match. Reproduced from Robach et al. [77], with permission.

deposition in the spaces between opposing syncytial membranes, but some proteins are occluded within the calcite plates [77]. These inclusions and resultant imperfections may have important mechanical stability consequences.

A most important feature in the strengthening of the most mature adoral segment of the tooth is the creation of calcite columns or pillars penetrating the membranes and cell syncytial cytosol and fusing adjacent plates together. The calcite pillars have the same coherent crystal structure as the main calcite plates, but they do not have the same mineral composition. It has been known for a long time that the urchin calcite has a high content of Mg^{2+} ion, and that the Mg^{2+} distribution was not uniform. Recent time-of-flight secondary ion mass spectroscopy (TOF-SIMS) examinations of urchin tooth plates show clearly that the pillars have a remarkably high Mg content, up to 43% in contrast to the 4–5% Mg of the regular calcite plates [69]. The Mg-rich pillars penetrate the syncytial cytosol, leaving channels of cytosol also containing nuclei, and other cell organelles (Figure 9). The mature incisal part of the urchin tooth is truly a complex shaped single calcite crystal.

Much remains to be learned about the matrix components of the urchin tooth, but it appears at this time that the inter-cell membrane compartment contains extracellular matrix proteins that may be involved in the crystallization process, either as trapped inclusions or as actively involved mineralization regulators. TOF-SIMS studies [69] show clearly that the Mg^{2+}-rich pillars also have a unique aspartic acid-rich protein component as compared with the main calcite plates.

4. CONCLUDING REMARKS

I have attempted to make two major points in this introduction. The first is that the Earth's biota, from the very origin of the most primitive life forms, have had and continue to have a profound and inextricable influence on the earth's structure, the lithosphere, and biosphere. The effects of the biota are emphasized and may overwhelm the natural geological drift in the physical world because of the much shorter time scale in which they operate. Since the abiotic and biotic worlds both utilize and convert minerals and mineral ions from one form to another, there is a dynamic but essentially irreversible interplay between the two systems. Nevertheless, in limited spans of a few hundreds of years, we live in a near steady state condition, and that enables us to seek generalities as to how diverse living systems utilize and control the abiotic world.

The second point, hopefully illustrated by the several examples of mineralization systems, is that there is a common scheme or grand strategy by which organisms use mineral ions to construct useful skeletal elements and mineral stores for metabolic purposes. At the time of the start of my own studies of bone and dentin about 50 years ago there was very little thought given to the questions of the relationships between mineralization in the vertebrates and invertebrates,

and a statement that there was a common thread (other than physical chemistry) between the mineralization processes would have been treated with some disdain. Even today some investigators persist in the thought that there is no connection between the two classes, and state that the formation of vertebrate bone, for example, required the development of entirely new molecular systems as compared to the formation of invertebrate shells. The examples of different systems of biomineralization presented here, and others dealt with in much greater detail in the following chapters, such as that on the magnetotactic bacteria (Chapter 11), lead me to conclude that the requirement to produce controlled deposits of mineralized structures within organisms has evolved along very similar controlled strategic pathways in every organism. One can outline a similar functional plan for each of the examples given:

1. Individual cells or aggregates of cells control their immediate environment by creating a specific chemical milieu wherein a matrix of macromolecules can be assembled (complex cell boundaries; the chondrocyte proteoglycan territorial matrix; the osteoblast osteoid; the confined compartment containing the extrapallial fluid in the space between the mantle epithelium and periostracum in the mollusk shell; and so on).
2. The structured matrix defines the space to be mineralized and the character of the space (gel matrix, pores in a fibrillar array, chitin-based compartments, amelogenin nanospheres).
3. Activation of the space by the introduction of specific matrix- and mineral-interactive molecules (phosphoproteins in the dentin, mammillary knob dermatan sulfate proteoglycan in the egg shell, Asp-rich protein in the mollusk shell) to nucleate crystal growth.
4. Introduction of mineral ions for crystal growth (diffusion, exocytosis from cell projections, lipid vesicles).
5. Limitation of crystal size by compartment walls, or introduction of inhibitors of crystal growth.

The actual tactics used in accomplishing these tasks, of course, are highly variable, from building a fixed porous collagen fibril network in bone, to an assembly line for the production of an egg shell in the avian oviduct. Most active players are the proteins, protein polysaccharides, and proteolipids that build the structural framework, the structure- and mineral-ion interactive proteins that direct the placement of the nucleation sites, and crystal growth inhibitors.

While the structural framework components are very different in each example given, the common theme in the structure-interactive, mineral-ion interactive proteins is that they appear to have domains of acidic amino acid residues that can bind mineral ions (phosphophoryn in dentin, Asp-rich proteins in mollusk and sand tube worm, C-terminal residues of amelogenin). The recent sequencing of the *Strongylocentrotus purpuratus* genome has shown many putative proteins of high homology with insect and vertebrate enzymes and regulatory proteins

(PubMed databases) and we have shown that an antibody to human and rat dentin phosphophoryn will cross-react with proteins extracted from *L. variegatus* teeth [78,79] and have cloned and sequenced a protein that is highly homologous to a human protein involved in syncytium formation [80] and shown it to also be present in the developing urchin syncytium. It is evident that some strong sequence homology is present.

The chapter on oxalate biominerals in plants (Chapter 7) and that on biomineralization in diatoms (Chapter 8) will show that acidic proteins of similar nature are also involved in those systems. There is not yet enough evidence to decide whether these homologies are the result of divergent or convergent evolution, but in the broader sense, it seems reasonable to suggest that at the very least there has been a convergent pressure for vertebrates, invertebrates and plants to select similar protein sequences as the engines to drive their biomineral formation, an indication of the importance of the process.

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ABBREVIATIONS

ACC	amorphous calcium carbonate
Asp	aspartic acid
ATP	adenosine 5′-triphosphate
DEJ	dentinoenamel junction
DOPA	3,4-dihydroxyphenyl-L-alanine
my	million years
mya	million years ago
SEM	scanning electron microscopy
TOF-SIMS	time-of-flight secondary ion mass spectrometry

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