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Non-Skeletal Biomineralization by Eukaryotes: Matters of Moment and Gravity

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Non-Skeletal Biomineralization by Eukaryotes: Matters of Moment and Gravity

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Abstract

Skeletal biomineralisation by microbial eukaryotes significantly affects the global biogeochemical cycles of carbon, silicon and calcium. Non-skeletal biomineralisation by eukaryotic cells, with precipitates retained within the cell interior, can duplicate some of the functions of skeletal minerals, e.g. increased cell density, but not the mechanical and antibiophage functions of extracellular biominerals. However, skeletal biomineralisation does not duplicate many of the functions of non-skeletal biominerals. These functions include magnetotaxis (magnetite), gravity sensing (intracellular barite, bassanite, celestite and gypsum), buffering and storage of elements in an osmotically inactive form (calcium as carbonate, oxalate

polyphosphate and sulfate; phosphate as polyphosphate) and acid-base regulation, disposing of excess hydroxyl ions via an osmotically inactive product (calcium carbonate, calcium oxalate). While polyphosphate has a wide phylogenetic distribution among microbial eukaryotes, other non-skeletal minerals have more restricted distributions, and as yet there seems to be no definitive evidence that the alkaline earth components (Ba and Sr) of barite and celestite are essential for completion of the life cycle in organisms that produce these minerals.

INTRODUCTION

Biomineralization is commonly discussed in terms of the contrast between two distinct processes, biologically-induced mineralization, associated principally with bacterial metabolism, and biologically-controlled mineralization, epitomized by skeleton formation in eukaryotic organisms. There is, however, another type of biologically-controlled mineralization that occurs across life's three domains: non-skeletal mineral precipitation within cells. These precipitates form within intracellular vesicles under tight enzymatic control, and, like skeletons, they have specific functions that contribute to overall fitness. Indeed, skeletal mineralization in protists and sponges has much in common with non-skeletal mineralization within cells, taking place inside intracellular compartments bounded by membranes and characterized by local expression of genes for the enzymes and templating molecules that guide precipitation. In a very real sense, then, the skeletal precipitation of silica by radiolaria, diatoms, and many other protists (e.g., Knoll 2003; Raven & Giordano 2009) and the formation of calcitic scales by coccolithophorid algae (e.g., Young & Henriksen 2003) may be particular examples of a broader phenomenon. Here, we examine the greater breadth of controlled biomineralization by eukaryotic microorganisms: the magnetosomes, statoliths, calcium oxalate raphides, and polyphosphate bodies found in diverse protists, with additional reference where appropriate to their occurrence in plants and animals.

MAGNETITE AND MAGNETOTAXIS

Magnetite (Fe_3O_4) has two roles in biology. One exploits its hardness: magnetite in the radulae of polyplacophoran mollusks (chitons) facilitates the scraping of food from rock surfaces. The other role makes use of its magnetic properties: magnetite plays a role in metazoan

navigation and in magnetotaxis by some motile bacteria and unicellular eukaryotes. In protists

that display magnetotaxis, small magnetite crystals occur, as in magnetotactic bacteria, within

magnetosomes. These magnetosomes appear to be intracellular vesicles with a surrounding lipid

bilayer membrane; however, detailed electron cryotomographic studies of bacterial

magnetosomes show that the magnetosomes are invaginations of the cell membrane, so that the

that magnetite crystals are topologically in the periplasm (Komeili 2007). No comparable data

are available for the magnetosomes of protists. This has been observed in a colourless

phagotrophic euglenoid from brackish sediment in a Brazilian mangel (Torres de Araujo et al.

1986) and in a colourless dinoflagellate, a ciliate and two cryptomonads, all non-photosynthetic

phagotrophs, in chemically stratified coastal saline pools (Bazylinski et al. 2000).

Magnetotaxis is distributed widely, if sporadically, in eukaryotic phylogeny, having been

documented in alveolates (ciliates and dinoflagellates), early branching chromalveolates (two

cryptomonads), and an excavate/discicristate (euglenoid), not to mention magnetite precipitation

within the Metaoza. This distribution has been interpreted as an indication that biologically-

controlled magnetite precipitation is a fundamental feature of eukaryotic biology, present in the

last common ancestor of extant Eucarya (Vali & Kirschvink 1991). Such speculation is

consistent with geological evidence that places early eukaryotic diversification within oceans

that commonly developed subsurface anoxia beneath moderately oxic surface waters (e.g., Anbar

& Knoll 2002). Confidence in broad evolutionary conclusions, however, is undermined by

uncertainties concerning the biosynthetic origin of magnetite in magnetotactic protists. Known

magnetotactic protists are phagotrophic and co-occur with magnetotactic bacteria (Torres de

Araujo et al. 1986; Bazylinski et al. 2000). This raises the possibility that the magnetosomes in

the eukaryotes are obtained from ingested bacteria, although no ingestion of the abundant

magnetotactic bacteria in the environment has been observed (Bazylinski et al. 2000, 2007), and

iron could also be obtained by phagotrophy of non-magnetotactic bacteria or colloidal iron, an

option not open to magnetotactic bacteria (Maranger et al. 1998; Nodwell & Price 2001).

Magnetosomes certainly increase the amount of iron required by cells significantly. From the

percentage of the cell volume of a colourless euglenoid occupied by magnetite (Torres de Araujo

et al. 1986), the density of magnetite, and the assumption that the dry matter is 45% carbon and

is one-third of the fresh weight, the Fe in magnetite per total cell C is 6500 µmol per mol. This is

two orders of magnitude greater than the 60 µmol Fe per mol C in 15 species of non-

magnetotactic eukaryotic marine phytoplankton from nutrient-sufficient cultures (Ho et al.

2003).

The "kleptomagnetosome" suggestion is consistent with similarities in structure of

magnetite crystals in some magnetotactic protists and in co-occurring bacteria (Bazylinski et al.

2000, 2007), although it could also be argued that there are few structure-function options for

magnetosome morphology in unicellular organisms. Magnetosome morphology in

magnetotactic euglenoids, however, is distinctly more complex than that found in bacteria or in

other unicellular eukaryotes, favoring *in situ* biosynthesis (Torres de Arranjo et al. 1986;

Bazylinski et al. 2007). Magnetosomes in both bacteria and eukaryotes occur in intracellular

vesicles; there is no indication from available electron micrographs that the eukaryote

magnetosomes occur within a bacterial endosymbiont (Bazylinski et al. 2000). Iron and oxygen

isotope signatures of biogenic magnetite in phagotrophic protists and co-occurring bacteria

(Mandernak et al. 1999) would be illuminating, although the test is one-sided. Isotopic

differences would support arguments for distinct eukaryotic and bacterial biosyntheses, whereas

similar isotopic abundances could be interpreted either in terms of ingestion or independent

precipitation from a common water body. Without question, some eukaryotes (for example, pigeons, honey bees, and chitons) precipitate magnetite, but differentiation between hypotheses that call for early origin and polyphyletic loss of magnetite biosynthesis versus polphyletic origins on various branches of the eukaryotic tree awaits careful research on the possibility of magnetosome derivation from food items.

Biogenic magnetite is characterized by crystollographically oriented chains of chemically pure crystals, commonly elongated along the [111] axis (Thomas-Keprta et al. 2000, Faivre and Schüler 2008), so in sediments it can be differentiated from magnetite of magmatic origin. (Telling biogenic from abiogenic magnetite has proven more controversial in martian meteorites, where it now appears that elongated single domain crystals may have formed during meteoritic impact; Golden et al. 2004). Because biogenic magnetite can be identified in sediments, these crystals have the potential to preserve in the geologic record; indeed, fossils of biogenic magnetite are relatively abundant and go back to the Middle Archaean (Chang et al. 1989). Geochemical data indicating that Archean oceans were neutral to reducing, with little or no free oxygen, require that anaerobic processes must have been used to oxidize 2 Fe(II) to 2 Fe(III) for each Fe as Fe(II) in magnetite. In sedimentary rocks deposited after global atmospheric oxygenation 2.4 billion years ago and after the origin of eukaryotes (in place by 1.8 billion years ago), there is the possibility of a eukaryotic origin for some magnetofossils, although allocating these later biogenic magnetites to Eukarya or Bacteria is rarely attempted. While Schumann et al. (2008) suggest a eukaryotic origin for the large (up to 4 µm long) spearhead or spindle-shaped magnetite found in marine sediments from the Paleocene-Eocene Thermal Maximum (~ 55.6 million years ago), Lippert (2008) suggests that the large magnetofossils could alternatively have

come from very large bacteria comparable to those found in some high-productivity shelf environments today.

As for the possible selective advantage of magnetotaxis in unicellular eukaryotes, all five known examples occur in two saline coastal habitats characterised by physical and chemical stratification, with oxygenated surface waters lying atop a hypoxic or anoxic water mass (Torres de Araujo et al. 1986; Bazylinski et al. 2000, 2007). Torres de Araujo et al. (1986) point out that the Brazilian mangel is within 4° of the equator, so magnetotaxis there would lead to horizontal swimming, perhaps also following a zone of specific chemical composition such as oxygen concentration at the oxic/anoxic transition zone. Such tracking of this transition zone has also been suggested for high latitude magnetotactic organisms (Frankel et al. 1997), although the obvious magnetotactic response would not yield near vertical rather than horizontal movement. Bazylinski et al. (2000) cite work showing that some microaerophilic ciliates use gravitaxis as part of a broader behavioral mechanism for aerotaxis. While magnetite particles have a higher density than the general cell contents (Table 1), a role in graviperception is unlikely in view of the unavoidable force exerted on them by the Earth's magnetic field. By increasing overall cell density magnetosomes increase the energy cost of upward movement at a given speed and decrease that of downward movement. Graviperception in the ciliates involves Müller bodies, biomineralized microconcretions that contain either strontium or barium as celestite and barite, respectively (Hemmersbach & Häder 1999; Bazylinski et al. 2000; Hemmersbach et al. 2005). These two elements also occur as the biominerals barite ($BaSO_4$) and celestite ($SrSO_4$) in charophycean algae such as desmids and Charales, with a known gravitropic role for barite particles in the rhizoids of the Charales (see below). In this case there is no known connection to aerotropism, although the Charales often grow with their rhizoids in hypoxic or anoxic

sediments. The role of barite and celestite in eukaryotic microbes is considered in more detail below.

The use of magnetotaxis or gravitaxis by protists to maintain position in relation to the oxic/anoxic transition cannot entirely substitute for a chemotactic response that detects oxygen directly (e.g., via a receptor such as haemoglobin), or through detection of an oxidation-reduction surrogate of oxygen (e.g., ferritin binding of iron). Thus far, the ecological distribution of magnetotaxis in protists is restricted to two chemically-stratified coastal marine habitats. They could perhaps also occur in inland waters, deeper marine sediments, and in such offshore pelagial marine habitats as the central Black Sea that have appropriate oxyclines.

A final evolutionary point is whether the occurrence of magnetite in magnetosomes in eukaryotes evolved in relation to magnetotaxis, or was co-opted from a function unrelated to magnetoperception.

BARITE, CELESTITE AND GRAVIPERCEPTION

Just as magnetite's magnetic properties enable magnetotaxis, the specific gravities of barite and celestite facilitate gravitaxis. Barite ($BaSO_4$) and celestite ($SrSO_4$) have densities well in excess of other cell constituents, including other common mineralized components (Table 1). Two clades of charophyte green algae are known to precipitate barite: barite (or celestite) particles occur in vesicles at the tips (poles) of the two hemicells of placoderm desmids and in some other members of the Zygnematales-Desmidiales clade, while barite, usually with some celestite, occurs in the tips of rhizoids in some Charales (Raven & Giordano 2009; see also Kreger & Boere 1969; Sievers and Schmitz 1982). The latter particles act as statocytes in graviperception, guiding positively gravitropic growth of the rhizoids.

Celestite, with a small fraction of barite (Ba/Sr ~ 0.003), is the inorganic component of skeletons and cysts made by Acantharia (Bernstein & Byrne 2004; De Decker 2004), members of the Rhizaria that are sister to the silica secreting polycystine radiolarians (Kunimoto et al. 2006). Celestite occurs as crystals in the cytoplasm of the flagellate swarmers of some colonial radiolarians/acatharians (Hughes et al. 1989; Anderson et al. 1990). Some ciliates (Alveolata) have Müller bodies containing celestite or barite that act as statocytes in graviperception of these gravitactic organisms (Hemmersbach & Häder 1999; Hemmersbach et al. 2005). Finally, barite particles occur in at least two plankonic flagellate species within the Chromista (phylum Haptophyta; class Prymnesiophyceae, order Pavlovales; Fresnel et al. 1979, Gayal and Fresnel 1979). Function is uncertain for these flagellates, but might enable graviperception. Like magnetite, then, barite and celestite occur broadly but sporadically in eukaryotic phylogeny, arguing in this case for a polyphyletic origin of $Ba/SrSO_4$ precipitation.

As far as we can determine there has been no published attempt to determine whether Ba and Sr are essential elements for barite- and celestite-producing organisms, in the sense of inability to complete their life cycle under axenic culture conditions in as near complete absence of the element under investigation as can be obtained. Of course, even if Ba and Sr turned out to be non-essential by this definition, these elements could still have significant roles in increasing fitness in the field.

$CaSO_4$ is much less common in eukaryotic microbes, though it is deposited as the main mineral in some desmids (Brook 1981) and is a minor component of the statoliths in some characeans (Schroter et al. 1975; Sievers & Schmitz 1982). It is not certain what mineral form of $CaSO_4$ is deposited in the eukaryotic microbes. In scyphozona and cubozoan medusae (Phylum Cnidaria) statoliths are formed of bassanite, ie. $(CaSO_4)_2 \cdot H_2O$ (Tiemann et al. 2002, 2006).

Bassanite is not the morph that is most readily precipitated from solution, but it has a significantly higher density than gypsum ($CaSO_4.2H_2O$), to which bassanite spontaneously transforms when placed in contact with water (Tiemann et al. 2002, 2006; Zhang & Sekine 2007).

Expanding on the occurrence of barite or celestite in graviperception, all charalean species have barite statocytes that are known to function in positive graviperception. By contrast, barite or celestite statocytes (or any other statocytes) are relatively rare in gravitropic ciliates and flagellates (Hemmersbach & Häder 1999; Hemmersbach et al. 2005). Graviperception in the absence of statocytes is apparently a result of gravity on the cytoplasmic contents acting on stretch-sensitive ion channels in what, for the moment, is the lower part of the plasmalemma. Such a mechanism does not require a particular spatial arrangement of denser components of the cytoplasm. If denser components of the cell are at one peripheral part of the cell then this part of the cell will orient at the bottom; this can cause positive gravitaxis if the placement of the ballast relative to the flagella and their direction of functioning causes downward swimming, or *vice versa* for negative gravitaxis (Hemmersbach & Häder 1999; Hemmersbach et al. 2005). This passive orientation does not involve movement of the denser components relative to the intracellular components of the cell, thereby differing from the statocyte mechanism. As does magnetite in magnetotactic protists, statocytes in swimming cells increase overall cell density, increasing the energy needed to swim upward at a given speed; conversely less energy is required for downward swimming. The function of barite crystals in the polar vacuoles of placoderm desmids is unclear.

There are well-preserved fossils both of desmids with a morphology resembling that of extant *Closterium* (Baschnagel 1966; Waggoner 1994) and of rhizoid-bearing of Charales

(Kelman et al. 2004), but neither contain barite or celestite particles, perhaps reflecting

dissolution during diagenesis.

It is hard to discuss gravitaxis and sulphate biomineralization without considering the

celestite-precipitating Acantharia. Acantharians are abundant members of the marine

microplankton. They commonly outnumber planktonic foraminifera and radiolarians, but

because they do not fossilize well, they are less well known. Acantharians leave few fossils

because at all depths the ocean is significantly undersaturated with respect to celestite, causing

their skeletons to dissolve (De Decker 2004). The cost of biomineralization for any mineral

relates to saturation levels in ambient fluids. Given the marked undersaturation of seawater with

respect to celestite, celestite precipitation within cells must require active transport of ions into

the cytoplasm. Barite forms a solid substitution series with celestite, and the presence of Ba with

a Ba/Sr of $3\pm0.8\cdot10^{-3}$ (Bernstein et al., 1998) lessens the saturation problem somewhat.

However, in past oceans, undersaturation would have been stronger, as sulfate abundance in the

oceans is higher than it has been for the great majority of Earth history (Gill et al 2007 and

references therein). Why celestite, then?

Perhaps celestite precipitation in acantharians is of Cenozoic origin, post-dating both the

increase of marine $[SO_4^{2-}]$ to near modern values and the widespread depletion of silica from

surface waters by diatoms. Functionally, the radially oriented spines of acantharian skeletons

support axopods, much as silica rods do in radiolarians, but the density of celestite presents a

potential problem – unless gravitaxis or orientation is part of skeletal function. One might

speculate that acantharians' use of celestite in skeleton formation might represent the cooptation

of a pre-existing mineralization pathway that originally evolved for gravity perception.

Certainly, this would explain the otherwise puzzling use of this mineral.   Unlike the case for

celestite, seawater is saturated with respect to barite (Rushdi et al. 2000), raising the question of why barite has not been used for skeleton formation. While this would less the physiological cost of precipitation, barite might be *too* readily precipitated, necessitating inhibitory molecules to shape skeletal elements.

As a final point about the occurrence of dense alkaline earth sulfate crystals in eukaryotic microbial cells, we underscore the obvious point that they unavoidably increase the density of the cells. This is of particular relevance for holoplanktonic cells, and meroplanktonic dispersal stages of benthic organisms (Raven & Waite 2004; Beardall et al. 2009). This ballast effect is most often considered for plankton with external silica (e.g. diatoms, parmophyceans, radiolarians, silicoflagellates, synurophytes) or calcium carbonate (e.g. coccolithophores, foraminiferans) structures. A point of relevance to this article is that the minerals are deposited internally in all of these cases (except in those foraminiferans that mineralize by agglutination of pre-existing external mineral particles: Table 2), and that the increase in density occurs as soon as the mineral is formed within the cells. Raven and Waite (2004) suggest that ontogeny may reflect evolution: the ballast effects of intracellular silica and calcium carbonate increased fitness before externalisation evolved and added skeletal and other functions that require external minerals. Such a fitness increase would be most obvious if, for example, diatoms and foraminifera evolved silicification as planktonic organisms rather than in the benthos (Raven & Waite 2004; Sims et al. 2006; Darling et al. 2009); however, in foraminifera, at least, carbonate skeletons appear to have evolved within benthic taxa, long before forams invaded the pelagic realm (Ross & Ross 1991).

PHOSPHATE MINERALS: POLYPHOSPHATE BODIES AND ACIDOCALCISOMES

Distribution in eukaryotic microbes

Apatite (actually carbonated hydroxyapatite, or dahllite; Weiner and Dove 2003) is conspicuously apparent as a biomineral in the skeletons of vertebrate animals. It also occurs in the shells of lingulid brachiopods and several other now extinct clades of Cambrian animals (summarized by Knoll 2003). Nonetheless, animal clades with skeletons of calcium carbonate greatly outnumber those that form phosphatic bones or shells, and, among algae and protozoans, phosphatic skeletons are essentially unknown. A single paper documents calcium and phosphate enrichment in surficial scales of the prasinophycean *sensu lato* (actually a basal charophycean) green alga *Mesostigma viride* (Domozych et al. 1991), but whether the observed elemental enrichment records apatite precipitation or cell wall polyphosphates is unclear (see below).

On the other hand, many eukaryotic microbes produce non-skeletal polyphosphate granules, with tens or hundreds of orthophosphate residues in a chain. Hooley et al. (2008) claim that "it (polyphosphate) has been shown to be present in all cells of all species studied", and Rao et al. (2009) state that polyphosphate is "found abundant (sic) in every cell in nature", though we adopt a more conservative stance and cite references for polyphosphate occurrence in higher taxa of eukaryotic microbes, while admitting that inability to detect polyphosphate in a given cell may be a problem of technique (below, and Table 2). The negative charge on the phosphate residues in these granules is balanced by (usually) divalent cations, such as Ca^{2+}, Mg^{2+} or Zn^{2+}. The granules are generally intracellular, often surrounded by a lipoprotein membrane.   These intracellular, membrane-bounded polyphosphate granules overlap with, but may not be entirely subsumed by, the more recently discovered acidocalcisomes, first characterised in apicomplexan (Alveolata) parasites (Docampo & Moreno 1999; Docampo et al. 2005). Polyphosphates can also

occur in the cell wall of the chlorophycean green alga *Chlamydomonas reinhardtii* (Werner et al. 2007), possibly related to the exocytosis of polyphosphate granules in this organism (Komine et al. 2000). Polyphosphate granules in the broad sense are also been reported from the Opisthokonta (many fungi), non-thecate amoebae (Amoebozoa), Plantae/Archaeoplastida (red algae, charophycean, chlorophycean, trebouxiophycean and ulvophycean green algae), Rhizaria, Alveolata (apicomplexans, dinoflagellates), Chromista: Ochrista (diatoms, chloromonads/raphidophyceans. tribophyceana), Chromista: Haptophyta (coccolithophore) and Excavata/Discicristata (euglenoids): see Table 2.

Function of polyphosphates

Polyphosphate granules do not serve a biomechanical function similar to that of dahllite skeletons in animals or the carbonate and silica skeletons found in other protists. It could be argued that phosphate is too scarce in nature to be used skeletally, at least in primary producers, as phosphorus availability should ultimately limit primary productivity. Constraints on nitrogen fixation mean that combined nitrogen (i.e. not N_2) is commonly the element that proximally limits primary production (Vitousek et al. 2002; Raven et al. 2005b; Menge et al. 2008); nonetheless, phosphate storage may enable primary producers to maximize production in water bodies characterized by patchy distribution, and therefore episodic availability, of both phosphate and fixed nitrogen.

Polyphosphate provides a biochemically accessible form of phosphorus in a form that is, in essence, an osmotically inactive form. Interestingly, Docampo and Moreno (1999) do not list phosphate storage among the possible functions of acidocalcisomes, presumably because of their perspective as parasitologists who focus on apicomplexan parasites of metazoans, where

phosphate limitation of growth is relatively unlikely. A storage role is consistent with observed changes in polyphosphate with variations in phosphorus supply, e.g., for green algae (Eixler et al. 2006; Nishikawa et al. 2006).

Raven (1987) and Raven et al. (2005a) have considered the costs and benefits of the use of polyphosphate rather than accumulation of inorganic orthophosphate in non-acidocalcisome-like vacuoles. There is at least a ten-fold saving in the volume within a cell if a certain amount of phosphate is stored as polyphosphate with chains containing hundreds of phosphate monomers rather than as dissolved monomeric orthophosphate with osmotic constraints on the possible concentration (Raven et al. 2005a). This is important for picoplankton where the cell volume is close to the minimum that is consistent with free-living existence (Raven et al. 2005a), and could also have significance for larger cells (Raven 1987). When considering polyphosphates as storage bodies, we do well to remember that while the total polyphosphate pool changes with phosphate availability in the manner expected of a storage material, phosphorus-limited algae invariably retain some polyphosphate (see also Rao et al. 2009).

At the expense of minimizing the volume associated with phosphate storage, polyphosphate depolymerisation and re-polymerisation might also help cells adjust to changes in external osmolarity. Leitao et al. (1995) showed that the marine diatom *Phaeodactylum tricornutum* responded to transfer from seawater to a medium of higher salinity by increasing the chain length of polyphosphates and a reduction in extractable polyphosphate, while transfer to a medium of low salinity increased the number of short-chain polyphosphates and total polyphosphate content. It is not clear, however, if the observed changes actually act in osmoregulation.

While the phosphate anhydride bonds in the polyphosphate have a high *in vivo* free energy of hydrolysis and so could potentially act as an energy store (Docampo and Moreno 1999), it can be readily calculated that even a polyphosphate content per cell equal to the phosphate required for a cell doubling would only last for a few minutes of providing all of the ATP needed for growth.

A further, unavoidable, outcome of storage of polyphosphate is to increase the density of the cell (Table 1), as indicated by Romans et al. (1994) for the marine diazotrophic cyanobacterium *Trichodesmium tenue*. In this species, polyphosphates provide, on average, about 20% of the ballast effect of stored polysaccharides, while nitrogen storage as cyanophycin also makes a contribution. Eukaryotic microorganisms do not synthesize cyanophycin, although storage proteins could act as an analogue, with a higher energy cost of synthesis. For phosphate, eukaryotes have the option of storage as orthophosphate in conventional vacuoles as well in the form of polyphosphate in granules.

Is the ballast effect of storage of phosphorus as orthophosphate the same as that of storage as polyphosphate? This was addressed using calculations similar to those used by Raven et al. (2005a) to determine the relative volumes of storage of a given quantity of phosphate as polyphosphate and as orthophosphate, but including values of the density of polyphosphate granules (Jacobsen et al. 1982) and of solutions of phosphate salts (Boyd and Gradmann 2002). In this case the phosphate stored as 1000 mol orthophosphate per cubic metre with a density of 1060 kg per cubic metre occupies seven times the volume of the same quantity of phosphate as polyphosphate with a density of 1950 kg per cubic metre. Ignoring the increment of cell volume in the orthophosphate case, the excess density (over water = 1000 kg per cubic metre) for orthophosphate is 420/950 or 44% of that of polyphosphate. The overall ballast effect in causing

sinking of cells or colonies is clearly greater if a given amount of phosphate is stored as polyphosphate than of orthophosphate.

These arguments are relevant to planktonic photosynthetic eukaryotes that undergo vertical migration over diel or longer time intervals in stratified waters. The rationale is that the surface waters have photosynthetically active radiation but have been stripped of nutrients (phosphorus; combined nitrogen for organisms that cannot fix nitrogen) required for the growth of primary producers, while deeper waters have limited light but higher nutrient concentrations. Acquisition of both light and nutrients can be achieved by vertical migration, employing flagellar activity (cryptomonads, dinoflagellates, raphidophytes, *Volvox*) or a balance of changes in solute composition of aqueous vacuoles and macromolecular ballast (large diatoms such as *Ethmodiscus* and *Rhizosolenia*, the latter frequently with diazotrophic *Richelia* symbionts; the large dinoflagellate *Pyrocystis*; phycoma stages of prasinophytes) rather than the balance of gas vesicles and macromolecular ballast as in cyanobacteria (Beardall et al. 2009). Under these circumstances, upward movement is impeded by the ballast effect of polyphosphate or, to a lesser extent, dissolved orthophosphate.

CALCIUM OXALATE WITHIN CELLS

Euhedral crystals of calcium oxalate have long been reported from vascular plants, where they occur within intracellular vesicles and appear to function in Ca-regulation, defense against predators, and, in some cases, a means of sequestering toxic cations, especially Al (Franceschi & Nakata 2005). A major function not mentioned by Franceschi and Nakata (2005) is that of acid-base regulation related to nitrate assimilation in shoots, synthesizing oxalic acid from the neutral gaseous CO_2. While calcium oxalate is produced in oxalate-precipitating plants grown on

ammonium, the calcium oxalate content is increased when the plants are grown on nitrate (Raven & Smith 1976). The oxalate anion produced in neutralizing excess hydroxyl ions from the assimilation of nitrate into organic matter is often in part precipitated as calcium oxalate, thus avoiding generation of excess osmolarity and turgor (Raven & Smith 1976; Raven 1977, 1985, 1986; Raven & Farquhar 1990; Andrews et al. 2009). There are, of course, consequences for acid-base regulation of oxalate precipitation in ammonium-grown land plants. In roots the excess protons can be directly secreted to the rooting medium, while in shoots excess protons from oxalic acid synthesis and precipitation as calcium oxalate in ammonium-grown plants are neutralized by organic anions other than oxalate moved up the xylem and respired in the shoots with hydroxyl ion production; the excess protons generated in organic acid synthesis in the roots are excreted to the medium (Raven & Smith 1976).

It turns out, however, that calcium oxalate crystals occur more widely within the Plantae, occurring in a number of coenocytic green ulvophycean algae (Pueschel & West 2007a, and references therein) and florideophyte red algae (Pueschel 1995, Pueschel & West 2007b 2007c), as well as in the microscopic filamentous green (charophycean) algal filament *Spirogyra hatillensis* (Pueschel 2001). Oxalic acid synthesis does not invariably involve calcium oxalate precipitation, since oxalate anions contributes about 20% to the vacuolar anionic charge in the giant-celled marine ulvophycean *Acetabularia mediterranea* (Saddler 1970) yet this organism has not been reported as precipitating calcium oxalate.. Little functional research has been done on calcium oxalates in algae, but the functions proposed for vascular plants by Franceschi and Nakati (2005) are at least plausible for other members of the Plantae. However, the absence of nitrate assimilation at a site remote from a medium that can act as a sink for excess hydroxyl ions in aquatic algae makes the stoichiometrically incontestable acid-base regulation role (Raven &

Smith 1976) less necessary, as well as being more costly of energy than hydroxyl ion excretion to the surrounding water (Raven 1985; Andrews et al. 2009). There does not even seem to be information on whether the nitrogen source has the any effect on the calcium oxalate content of algae.

Calcium oxalate has also been reported as an intracellular structure in fungi (Arnott 1995); once again its function is not well understood, but could include Ca-regulation and detoxification. Ca-regulation may also explain reports of other Ca-bearing minerals within protists, including calcium carbonate in diverse protists (Fauré-Fremiet 1957) and as aragonite in *Spirogyra* (Mann et al. 1988), gypsum in desmids (Brook 1981; Lowenstam 1986) and both calcite and Ca-phosphate (described as apatite, but possibly Ca-polyphosphate bodies) in the ciliates *Spirostomum ambiguum* (Jones 1967) and *Euplotes eurystomus* (Ruffalo 1978; Hausmann and Walz 1979). The acid-base regulation role mentioned for intracellular calcium oxalate, i.e. removal of excess hydroxyl ions in an osmotically unthreatening manner, also applies to intracellular calcium carbonate (Raven & Smith 1976; Raven 1985), provided that the inorganic carbon source for the precipitated carbonate is endogenous (respiratory) carbon dioxide, carbon dioxide entering photosynthetic cells, or bicarbonate from the medium.

DISCUSSION

The evolution of eukaryotic biomineralization

In comparisons of biologically-induced mineralization by bacteria and skeletal biomineralization by eukaryotes, bacteria invariably seem the more diverse domain. When eukaryotic biomineralization, however, is expanded to include non-skeletal minerals precipitated within cells, eukaryotes deposit nearly as many minerals as do bacteria (Lowenstam 1986). Like

most skeletal biominerals, these intracellular precipitates form in enclosed, biologically defined spaces (vesicles), guided by enzymatic activity. That is, they are at the less conspicuous end of a broad biomineralogical continuum that includes more familiar bones, shells, frustules and tests.

This being the case, it is instructive to ask why eukaryotes show only limited overlap in the minerals that they use for skeletons and other functions. The preceding discussion suggests that precipitation of specific non-skeletal minerals evolved under natural selection, exploiting the magnetic properties of magnetite, the specific gravity of barite and celestite, and the exchangable storage capacity of polyphosphates. One might equally ask why calcite, aragonite, dahllite and amorphous silica comprise the subset of eukaryotic biominerals involved in skeletal mineralization. Once again, material properties contribute to the explanation: composite materials of interlayered organic sheets and calcite or aragonite are mechanically resistant to abiological forces (especially for benthic organisms) and to forces exerted by grazers; these are functional prerequisites for many skeletons. Silica is not only rigid, resisting forces such as those that could be imposed by some grazers (Hamm et al. 2003; cf. Austin et al. 2005, who showed that diatom ingestion by foraminiferans resulted in fractured frustules, despite the absence of any obvious opposable rigid structures in these grazers), but can be fashioned into shapes of exquisite complexity within cells. Of course, function is only part of the explanation. The calcium and carbonate ions required for calcite and aragonite formation are abundant (surface seawater is strongly oversaturated with respect to these minerals at the moment, though this is subject to modification with global environmental change) and easily incorporated by organisms – all cells have fundamental mechanisms for pumping Ca^{2+} and manipulating total CO_2.

Considering in more detail intracellular mineralization in microbial eukaryotes, before the dramatic rise of diatoms to ecological prominence, surface oceans were saturated or nearly so with respect to amorphous silica, but abundances of silicified organisms was relatively low. The low concentrations of silicic acid in surface oceans and freshwaters containing diatoms required that silicic acid be concentrated to supersaturation in some compartment if silica minerals are to form (Raven 1983), an argument that also applies to formation of celestite in today's ocean. It is known that there was (polyphyletic) evolution of active transport mechanisms for silicic acid in all silicifiers (Raven & Giordano 2009): this capacity is also found in some non-silicifying prasinophycean green algae (Fuhrman et al. 1978; Nelson et al. 1984). This may help to explain the predominance of silica in intracellular skeletal biomineralization (see Table 2) but of calcium carbonate minerals in those macroscopic skeletons of invertebrate metazoans and coenocytic and multicellular algae which are precipitated extracellularly, albeit often in spaces with relatively restricted diffusive interaction with the bulk medium and so susceptible to chemical modification and enzyme activity. Of course, supersaturation of the medium with respect to the mineral form to be precipitated does not preclude intracellular mineralization, as shown by calcite formation in coccolithophores and many foraminiferans (Table 2). While bacteria can clearly form intracellular biominerals, e.g. polyphosphate, within lipoprotein membranes (e.g. Rao et al. 2009; cf. the probably periplasmic status of the apparently intracellular bacterial magnetosomes: Komeili 2007), the absence of an endomembrane system means that they do not have the possibility of exocytosis of minerals such as can occur in eukaryotes.

Perhaps only in the cases of celestite and calcium phosphate mineralization do the realms of eukaryotic non-skeletal and skeletal bimineralization overlap. It must be noted that they overlap imperfectly, at least in the case of phosphates. It is possible that biochemical pathways

originally evolved to control intracellular precipitation of polyphosphate bodies became co-opted

for skeletal phosphate precipitation (see Rao et al. 2009). As noted above, the physiological cost

of placing large amounts of phosphate in a non-exchangeable reservoir may help to explain why

primary producers store polyphosphate but do not secrete phosphatic skeletons. The celestite

found in acantharian skeletons may, however, reflect the direct evolutionary cooptation of

mineralization pathways originally used for gravitropism.

Biogeochemical consequences of eukaryotic biomineralization

Skeletons have a well understood biogeochemical importance as the major means by

which calcium carbonate and silica are deposited on the seafloor. What, if any biogeochemical

function(s) can be ascribed to nonskeletal biominerals, recognizing that any such functions are

presumably emergent properties of natural selection favouring mineral formation at the cell

level?

Diaz et al. (2008) provided evidence consistent with a significant role for polyphosphates

produced by diatoms in the formation of calcium phosphate minerals in marine sediments.

Sanigrahi and Ingall (2005) had previously emphasized the importance of polyphosphates in

increasing P fluxes in marine sediments overlain by anoxic waters, and Hopfer et al. (2007)

indicated the role of polyphosphate produced within sediments in phosphate dynamics, although

they could not quantify the involvement of microorganisms from the Archaea, Bacteria and

Eukarya. With these interactions in mind, Algeo and Ingall (2007) indicated several possible

roles for polyphosphate bodies in the deposition and retention of mineral phosphate in sediments.

Balancing this, increasing geochemical data implicate absorption onto ferric oxide particles as a

major means of getting phosphate into sediments and keeping it there, so a biogeochemical role

for polyphosphates runs parallel to other processes at work in the marine phosphorus cycle (e.g.,
März et al. 2008).

A strong case can be made for the biogeochemical importance of celestite precipitation.
In some parts of the ocean, e.g. the Indian Ocean just west of Australia, the rate of extraction of
Sr by acantharians per unit volume of seawater is so great relative to the concentration of Sr in
the top 400 m of the ocean that it very significantly exceeds the biological extraction rate of Ca
relative to the Ca concentration. This results in significant decreases in Sr/Ca in the surface
ocean; dissolution of the acantharian skeletons at greater depths restores the conservative Sr/Ca
ratio (De Decker 2004). In laboratory experiments Bernstein and Byrne (2004) showed that the
dissolution of acantharian celestite (Ba/Sr ~ 0.003) leads to the production of barite. Dissolution
of the celestite in a microenvironment leads to barite oversaturation and the production of Sr-rich
barite of the kind that is ubiquitous in the water column (Bernstein & Byrne 2004).   This shows
that Ba-containing acantharian celestite can be an important source of Sr-containing barite in the
deeper parts of the ocean, and that acantharian production in surface waters can impact
materially the distribution of both Sr and Ba in the oceans. Biologically influenced Sr
abundances in surface oceans are of interest to paleoceanographers because Sr/Ca in carbonate
skeletons has been used as a proxy for seawater temperature in ancient oceans (de Villiers 1999;
de Deckker 2004).

CONCLUSIONS

The diverse minerals considered here are united by being, for the most part, intracellular
and non-skeletal. All increase cell density, but otherwise have a diversity of functions, including
magnetoperception, graviperception, phosphate storage, calcium storage, and acid-base

regulation. In addition, intercellular polyphosphate and celestite precipitation may be evolutionarily related to extracellular skeletal dahllite and celestite, and the relatively rare intracellular deposits of calcium carbonate and of silica (Table 2) may be analogous to early stages in the polyphyletic evolution of calcium carbonate and silica skeletons that are deposited internally and then exocytosed. Research on the diversity, physiology, function, phylogenetic distribution, and geobiology on non-skeletal minerals in eukaryotic cells is still in its infancy, but continuing work has much to tell us about how eukaryotes reflect and influence their environments.

Acknowledgements

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Table 1

Density of cell components in kg m^{-3}

Magnetite	5175
Barite	4500
Celestite	3960
Dahllite	3120
Aragonite/Calcite	2710-2930
Bassanite	2750
Gypsum	2280
Opal	2170-2660
Polyphosphate	1950
Nucleic acids	1700
Polyglycan	1500
Protein	1300
Triglyceride	860
1000 mol m^{-3} solutions of intracellular solutes	985-1156
Seawater	1030
Air (as in Cyanobacterial gas vesicles)	1.2

From Weast (1979), Walsby & Reynolds (1980), Jacobsen et al. (1982), Boyd & Gradmann (2002), Zhang & Sekine (2007) and (for dahllite) http://.www.geology.neab.net/minerals/carbapat/sort.htm.

Table 2

Non-skeletal minerals in eukaryotic microbes

Based on Raven & Giordano (2009),: see also Zettler et al. (1997), Kunimoto et al. (2006).

Higher Taxon	Phylum	Class	Intracellular Minerals	References
Opisthokonta: Fungi Opisthokonta Choanoflagella-ta			Ca oxalate Polyphosphate (SiO_2 skeleton before exocytosis)	Arnott (1995) Keck & Stich (1957); Jacobsen et al. (1982); Strulu et al. 1983. Bovee (1981)
Amoebozoa			Polyphosphate (Skeletal SiO_2 particles before exocytosis in some testate species)	Keck & Stich (1957); Deslauriers et al. (1980) ; Anderson (1987, 1994); Hooley et al. (2008)
Plantae (Archaeo-plastida)	Rhodophyta	Cyanidiophyceae Bangiophyceae Floridiophyceae	Polyphosphate Polyphosphate, SiO_2 Ca oxalate Polyphosphate	Yagisawa et al. (2009); Peuschel (1995); Peuschel & West (2007a,b.c); Niemeyer (1976); Chopin et al. (1997, 2004)
Plantae (Archaeo-plastida)	Chlorophyta	Charophyceae	$BaSO_4/SrSO_4$ in intracellular vesicles in desmids and Zygnematales, statoliths in rhizoids apices of Charales. Occasional $CaSO_4$ in desmids. $CaCO_3$ (aragonite) and Ca oxalate in Zygnematales Polyphosphate	Brook (1981); Lowenstam (1986); Schroter et al. (1975); Sievers and Schmitz (1982);Mann et al. (1988); Pueschel (2001) Keck & Stich (1057)
Plantae (Archaeo-plastida)	Chlorophyta	Chlorophyceae	Polyphosphate	Keck & Stich (1957); Siderius et al. (1996); Ruiz et al. (2001)
Plantae	Chlorophyta	Prasinophyceae	(SiO_2 scales before exocytosis in	Bovee (1981)

(Archaeo-plastida)			a few) Polyphosphate	Hooley et al. (2008)
Plantae (Archaeo-plastida)	Chrorophyta	Trebouxiophyceae	Polyphosphate	Keck & Stich (1957); Bock et al. (1996); Eixler et al. (2006)
Plantae (Archaeo-plastida)	Chlorophyta	Ulvophyceae	Polyphosphate Ca oxalate	Keck & Stich (1957); Rubetsov & Kulaev (1977); Cobb (1978) Pueschel & West (2007a)
Rhizaria		Acantharia Radiolaria	$SrSO_4$ in swarmers (Skeletal $SrSO_4$ of Acantharia before exocytosis)	Hughes et al. (1989); Anderson et al. (1990)
Rhizaria		Foraminifera	(Skeletal SiO_2 in some) (Skeletal $CaCO_3$ before exocytosis in many)	Bovee (1981) Pawlowski et al. (2003); De Nooijer et al. (2009)
Rhizaria		Euglyphida Thaumato-monadina	(Skeletal SiO_2 before exocytosis in many)	Anderson (1994); Cavalier-Smith and Chao (2003)
Alveolata	Ciliata		Fe_3O_4 in a few $BaSO_4/SrSO_4$ Polyphosphate? $CaCO_3$ (calcite)	Bazylinski et al. (2000) Rieder et al. (1981) Hemmersbach and Häder (1999); Hemmersbach et al. (2005); Jones (1967), Ruffalo (1978); Hausmann & Walz (1979)
Alveolata	Apicomplexa		Polyphosphate	Scott et al (1998); Docampo & Moreno (1999); Docampo et al. (2005)
Alveolata	Dinophyta		Fe_3O_4 in a few Polyphosphate (Skeletal SiO_2 before exocytosis)	Bazylinski et al. (2000); Elgalish et al. (1980) Bovee (1981)
Chromista	Ochrista	Bacillariophyceae	(Skeletal SiO_2 before exocytosis)	Raven (1983); Raven & Waite (2004); Keck

			Polyphosphate	& Stich (1957); Leitao et al. (1995); Oku and Kamatani (1995);
		Bicoseocida	(Skeletal SiO_2 before exocytosis)	Bovee (1981)
Chromista	Ochrista	Chromomonado-phyceae	Polyphosphate	Kimura et al. (1999)
Chromista	Ochrista	Chrysophyceae	(Skeletal SiO_2 of cysts before exocytosis)	Bovee (1981)
Chromista	Ochrista	Palmophyceae	(Skeletal SiO_2 before exocytosis)	Van den Hoek et al. (1996)
Chromista	Ochrista	Phaeophyceae	Polyphosphate	Niemeyer (1976)
Chromista	Ochrista	Silicoflagellata	(Skeletal SiO_2)	Bovee (1981)
Chromista	Ochrista	Synurophyceae	(Skeletal SiO_2 before exocytosis)	Bovee (1981)
Chromista	Ochrista	Tribophyceae	Polyphosphate	Keck and Stich (1957)
Chromista	Haptophyta	Pavlovophyceae	$BaSO_4/SrSO_4$ in a few	Fresnel et al. (1979); Gayral & Fresnel (1979)
Chromista	Haptophyta	Prymnesiophyceae	(Skeletal $CaCO_3$ before exocytosis in coccolithophores) (Skeletal SiO_2 before exocytosis) Polyphosphate	Young & Henriksen (2003); Raven & Waite (2004); Yoshida et al. (2006) Dyhrman et al. (2005)
Chromista	Cryptophyta		Fe_3O_4 in a few Polyphosphate	Bazylinski et al. (2000) Heldal (1996)
Excavata/ Discicristata	Euglenophyta Bodonida Trypano-somida		Fe_3O_4 in a few Polyphosphate (SiO_2 scales before exocytosis) Polyphosphate	Torres de Araujo et al. (1986); Keck & Stich (1957) Bovee (1981) Docampo & Moreno (1999) Docampo et al. (2005)