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Sulfides in Biosystems

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INTRODUCTION

Organisms that live on and near the surface of the Earth affect the cycling of sulfur and metals and thus the formation and decomposition of sulfide minerals. Biological mediation of mineral formation can take many forms. Some organisms have evolved to synthesize minerals that are used for a particular function, such as structural support, protection against predators, hardening, or magnetic sensing. In these cases, the organism exerts strict control over the properties and the location of the mineral. The process by which such minerals form is termed biologically controlled mineralization (BCM) (Lowenstam and Weiner 1989).

Biominerals can also form as a byproduct of the metabolism of organisms, or as a consequence of their mere presence. Life can create chemical environments that result in the precipitation of minerals, and biological surfaces can serve as nucleation sites for mineral grains. In such cases, the adventitious deposition of minerals is termed biologically induced mineralization (BIM) (Lowenstam and Weiner 1989). Whereas only a few examples of the formation of sulfide minerals by BCM are known, iron sulfides form in vast quantities by BIM and affect the global cycling of iron, sulfur, oxygen, and carbon (Canfield et al. 2000; Berner 2001).

Organisms are also able to break minerals down. The dissolution of sulfides can be enhanced by biological processes, while some micro-organisms gain their energy by oxidizing the sulfur or the metal in sulfide minerals, thereby converting sulfides into dissolved species or oxides (Kappler and Straub 2005). The biological mediation of both the precipitation and the dissolution of sulfides can be used for practical purposes, such as bioremediation and bioleaching.

Over the past decade, several reviews have been published on biomineralization, many of which include details on sulfides. In the *Reviews in Mineralogy & Geochemistry* series, three volumes have been devoted to interactions between minerals and organisms (Banfield and Nealson 1997; Dove et al. 2003; Banfield et al. 2005). A further short course volume, which includes several chapters on sulfides, was published by the Mineralogical Association of Canada (McIntosh and Groat 1997). A textbook on environmental mineralogy, published by the European Mineralogical Union (Vaughan and Wogelius 2000), also contains material related to biominerals. More recently published general books on BCM include those by Mann (2001) and Baeuerlein (2000, 2004).

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The aim of the present chapter is to discuss some aspects of sulfide biomineralization and sulfide bioweathering. In order to avoid repeating the content of recent reviews, this chapter does not provide a comprehensive treatment of interactions between sulfide minerals and organisms. Instead, its primary focus is a description of the properties of biogenic sulfide minerals that distinguish them from their inorganically-formed counterparts. The relationships between mineral properties and biological functions are discussed, some aspects of sulfide formation by BIM are highlighted, and sulfide bioweathering processes are mentioned briefly. Since iron sulfides are by far the most important and abundant sulfide minerals in biosystems, most of this chapter deals with such minerals.

BIOLOGICAL FUNCTION AND MINERAL PROPERTIES: CONTROLLED MINERALIZATION OF IRON SULFIDES

Biologically controlled mineralization is a highly regulated process that results in the formation of minerals that have species-specific physical and chemical properties. These properties include size, morphology, structure, crystallographic orientation, composition, and texture. As discussed by Mann (2001), several levels of regulation combine in BCM to provide distinct mineral properties (Table 1). Chemical control through coordinated ion transport is involved in producing supersaturated solutions in spatially separated spaces such as vesicles or gaps in organic frameworks. Organic surfaces play a crucial role in providing nucleation sites and in selecting the phase and orientation of the nucleating mineral (Weiner and Dove 2003). Chemical, spatial, and morphological regulations combine to shape the growing crystals and to assemble them into complex architectures.

Minerals can serve various functions in living organisms. In association with organic materials, they can form inorganic-organic composites that have favorable mechanical properties. Well-known examples include bones that are used for structural support, teeth that are used for grinding, and shells that are used for mechanical strengthening and protection.

Table 1. Processes and mechanisms that control the properties of minerals formed by biologically controlled mineralization, based on concepts that are described by Mann (2001).

Type of Regulation	Key Factors of Mineral Formation that are Controlled	Means of Control	Result
Chemical	Ion concentration in solution	Coordinated ion transport	Supersaturation and nucleation
	Crystal growth	Promotors and inhibitors	- Controlled crystal morphology - Phase transformations
Spatial	Supersaturation and crystal growth	Vesicles or organic framework	Controlled location, size and shape of the mineral
Structural	Nucleation	Organic surfaces as templates, molecular recognition at organic/ inorganic interfaces	- Polymorph selection - Controlled crystallographic orientation
Morphological and constructional	Nucleation and growth	Organic boundaries, vectorial regulation	- Complex morphologies - Time-dependent patterning

Table 2. Sulfide minerals that are formed by biologically controlled mineralization.

Organism	Mineral	Function	References
Magnetotactic bacteria	Greigite, Fe_3S_4	Magnetic sensing	Farina et al. 1990; Mann et al. 1990; Heywood et al. 1990; 1991
	Mackinawite, FeS	Precursor to greigite	Pósfai et al. 1998a,b
	Cubic FeS (identified tentatively)	Precursor to greigite	Pósfai et al. 1998a,b
Scaly-foot gastropod	Pyrite, FeS_2	Mechanical protection	Warén et al. 2003; Suzuki et al. 2006
	Greigite, Fe_3S_4	Mechanical protection	Warén et al. 2003; Suzuki et al. 2006
	Mackinawite, FeS	Precursor to greigite	Suzuki et al. 2006

However, the biological uses of minerals are not only mechanical. Biominerals can also serve as optical, magnetic, or gravity sensing devices, and may be used for the storage of materials such as iron (Mann 2001; Baeuerlein 2004).

In contrast to some mineral groups that are common functional materials in many organisms (e.g., carbonates, phosphates, silica), only a few sulfide minerals are known to serve biological functions (Table 2). Although these sulfide minerals include common species such as pyrite (FeS_2), their formation pathways by BCM were only discovered in the last 15 years. Greigite (Fe_3S_4) is used for magnetic sensing in magnetotactic bacteria (Farina et al. 1990; Mann et al. 1990; Rodgers et al. 1990), and greigite and pyrite both serve as hardening materials on the foot of a deep-sea snail species (Warén et al. 2003; Suzuki et al. 2006). The physical and chemical properties and the apparent functions of these sulfide biominerals are reasonably well known. However, very little is understood about the specific biological control mechanisms that govern crystal nucleation and growth (as listed in Table 1).

Biologically controlled mineralization in magnetotactic bacteria

Magnetotactic bacteria contain intracellular magnetic iron oxide or sulfide minerals that are typically organized in chains. Such cells are aligned by magnetic fields, and as a result the bacteria are constrained to swim parallel to the direction of the geomagnetic field in their natural aquatic environment (Blakemore 1975). This magnetic alignment mechanism enables the bacteria to find their optimal positions in environments that are characterized by vertical chemical gradients (Frankel et al. 1997). Since geomagnetic field lines are inclined with respect to the surface of the Earth (except at the equator), the bacteria do not have to search for their optimal chemical environment in three dimensions, but are guided up and down along the field lines. Nevertheless, several questions remain about the utility of magnetotaxis; neither the benefit of magnetotaxis at the equator, nor the reason for the presence of south-seeking bacteria in the Northern Hemisphere (Simmons et al. 2005) is fully understood.

The term magnetosome refers to an intracellular magnetic mineral grain enclosed by a biological membrane. Such magnetosome membranes were shown to exist in magnetite-producing bacteria (Balkwill et al. 1980), and some of the specific membrane proteins and their encoding genes have been identified (Komeili et al. 2004; Schüler 2004; Fukumori 2000). The magnetosome membrane provides spatial, chemical, structural, and morphological regulation (Table 1) of the nucleation and growth of magnetite crystals. The membrane controls the transport of ions into the magnetosome vesicle, a delimited space in which supersaturation

can be achieved (Fig. 1). It is also likely that the membrane provides the organic template for the oriented nucleation of magnetite crystals (Bazylinski and Frankel 2004). The growth of magnetite crystals is controlled by an unknown mechanism to produce well-defined morphologies. Recently, it was found that magnetite particles are assembled into chains by an acidic membrane protein that anchors the magnetosomes to a filamentous structure (Scheffel et al. 2005) (Fig. 1).

The presence of a magnetosome membrane has never been established in sulfide-producing bacteria. Since such bacteria are not yet available in pure culture, it is difficult to determine whether the iron sulfide crystals are enclosed by membranes that are similar to those in magnetite-producing cells. Little is therefore known about the biological regulation of mineral formation in sulfide-bearing bacteria. However, the properties of the inorganic sulfide phases themselves are fairly well understood. These properties can provide indirect information about the mineral-forming process.

The biomineralization of magnetite and sulfides by magnetotactic bacteria, including their micro- and molecular biology and ecology, has been reviewed by Bazylinski and Moskowitz (1997), Baeuerlein (2003), and Bazylinski and Frankel (2003, 2004). Some of the mineralogical aspects of sulfide formation in magnetotactic bacteria are now described, and recent measurements of the magnetic microstructures of chains of greigite magnetosomes in magnetotactic cells are reviewed.

Sulfide-producing magnetotactic bacteria

Sulfide-producing magnetotactic organisms are known to exist in anaerobic marine environments, saltwater ponds, and sulfur-rich marshes (Farina et al. 1990; Mann et al. 1990; Bazylinski and Frankel 2004). The cell morphologies of sulfide-bearing magnetotactic bacteria appear to be very similar in geographically distant locations (Farina et al. 1990; Mann et al. 1990; Bazylinski et al. 1990; Pósfai et al. 1998b; Simmons et al. 2004). One organism is termed the many-celled magnetotactic prokaryote (Rodgers et al. 1990), or alternatively

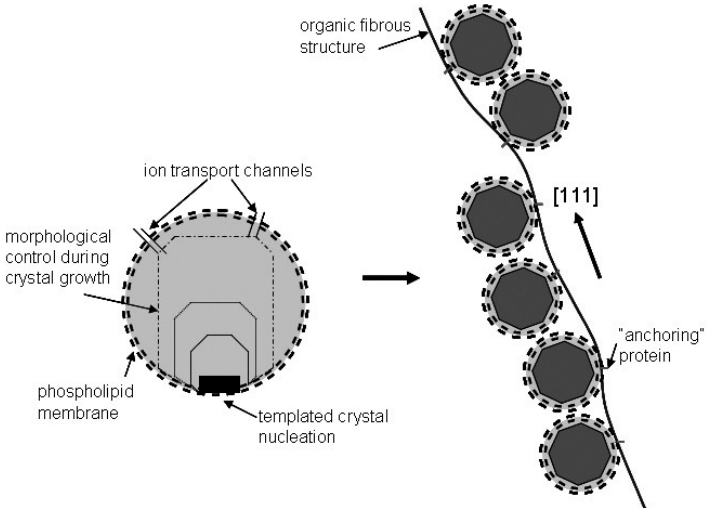

Figure 1. Stages of biologically controlled mineralization in magnetotactic bacteria, as known in the case of magnetite-producing cells. Iron sulfide-producing species may use similar strategies for mineralizaton. The inorganic crystal nucleates and grows inside a magnetosome vesicle, and then the magnetosomes are attached to a filamentous structure by an acidic protein. (Based largely on the model by Scheffel et al. 2005.)

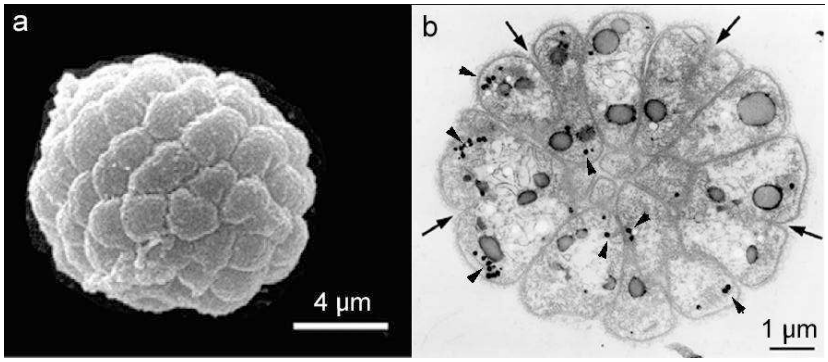

Figure 2. (a) SEM image of the magnetotactic multicellular aggregate (MMA) that consists of many cells and moves as a single unit. (b) Ultrathin section of an MMA. The arrows mark invaginations of the cell wall, indicating the sites of cell division, and the arrowheads mark iron sulfide magnetosomes. [Used with permission of Elsevier, from Keim et al. (2004) *J. Structural Biology*, Vol. 145, Figs. 3c and 5, p. 254-262.]

the magnetotactic multicellular aggregate (MMA) (Lins and Farina 1999). This organism consists of an aggregate of 10 to 30 cells that are arranged in an ordered fashion, enclosing an acellular internal compartment. Each cell contains one or more chains of greigite crystals, which are aligned approximately parallel to each other within the individual cells (Keim et al. 2004) (Fig. 2). The MMA moves as a single unit, guided by Earth's magnetic field. Other common morphological types include rod-shaped cells that may contain single or multiple chains of iron sulfide crystals (Heywood et al. 1991; Bazylinski et al. 1995) (Fig. 3). Although attempts to cultivate sulfide-producing magnetotactic bacteria in pure culture have to date been unsuccessful, fluorescent in situ hybridization studies indicated that the MMA is closely related to known sulfate reducers among the δ-proteobacteria (DeLong et al. 1993), whereas a large rod was found to be a member of the γ-proteobacteria and is likely involved in metal cycling (Simmons et al. 2004).

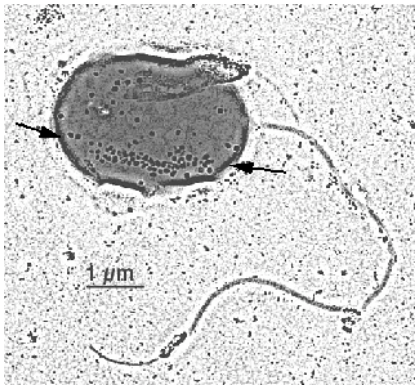

Figure 3. A single, rod-shaped magnetotactic cell that contains a double chain of iron sulfide magnetosomes between the two arrows. (Image from Kasama et al. 2006.)

Sulfide-bearing magnetotactic bacteria live below the oxic-anoxic transition zone (OATZ), where H_2S is abundant (Bazylinski and Frankel 2004). MMAs and rod-shaped cells have been observed in distinct zones below the OATZ in Salt Pond, Massachusetts, USA (Simmons et al. 2004). Whereas the concentration of MMAs was largest just below the OATZ, rod-shaped cells appeared to be broadly distributed vertically in a zone that was characterized by the absence of dissolved oxygen and by a high H_2S concentration (Fig. 4). In such an environment, the benefit of possessing an internal compass is unclear. It was speculated that intracellular iron sulfide (and oxide) crystals could serve purposes other than magnetically-assisted navigation (Simmons et al. 2004; Flies et al. 2005). In addition, populations of south-seeking magnetotactic bacteria were recently observed in the Northern Hemisphere (Simmons et al. 2006), challenging the widely-held view about the utility of magnetic navigation for these mi-

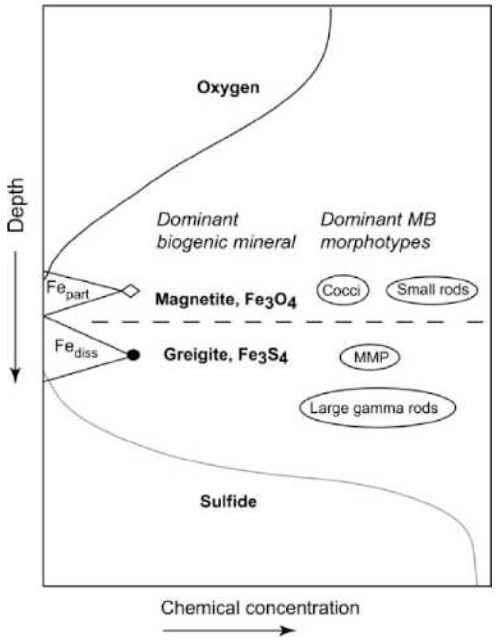

Figure 4. The positions of the types of magnetotactic bacteria (MB) in the water column of Salt Pond, Massachusetts, with respect to depth and the concentrations of oxygen and sulfide. Magnetite-bearing *cocci* and small rods predominate at the oxic-anoxic transition zone, whereas iron sulfide-bearing magnetotactic multicellular prokaryotes (MMP) and large gamma rods predominate below it. Peaks in the concentrations of particulate and dissolved iron (Fe_{part} and Fe_{diss}, respectively) are also shown. [Used with permission of American Society for Microbiology, from Simmons et al. (2004), *Applied and Environmental Microbiology*, Vol. 10, Fig. 7, p. 6230-6239.]

cro-organisms. Further studies on the physiology and ecology of magnetotactic bacteria will be required in order to establish whether the synthesis and presence of magnetosomes serve purposes other than magnetic sensing.

Structures and compositions of iron sulfide magnetosomes

The inorganic part of each iron sulfide magnetosome is typically a crystal of greigite. However, in freshly-collected cells (a few days old), mackinawite (FeS) was identified (Pósfai et al. 1998a). When the samples were stored in air, mackinawite was observed to convert into greigite. This observation suggests that non-magnetic mackinawite precipitates initially, and then converts into magnetic greigite through the loss of ¼ of its iron. Disordered crystals may represent transitional states between the mackinawite and greigite structures and suggest that the transformation takes place in the solid state. Structural similarities between the cubic close-packed sulfur substructures of mackinawite and greigite would allow such a conversion to take place by the diffusion of iron atoms, leaving the sulfur atomic arrangement intact (Fig. 5).

The transformation that was observed in the stored specimens is also thought to take place within living bacteria. The transformation is likely to be faster in living bacteria than in the stored samples, since non-magnetic mackinawite cannot be used for magnetotaxis. In addition to mackinawite, cubic FeS with a sphalerite-type structure was identified tentatively in some magnetotactic cells, based on electron diffraction patterns (Pósfai et al. 1998a). Since this initial identification of cubic FeS, several further attempts to confirm its presence have been unsuccessful. It remains to be established unequivocally that cubic FeS is also a precursor of greigite in magnetotactic bacteria.

The conversions of iron sulfides in bacteria follow similar paths as the well-known phase transformations of authigenic sulfides that form by BIM in anoxic sediments (see Luther and Rickard, 2006; this volume, and the section below on BIM sulfides). However, in marine sediments the final product of iron sulfide formation is commonly pyrite instead of greigite (Schoo-

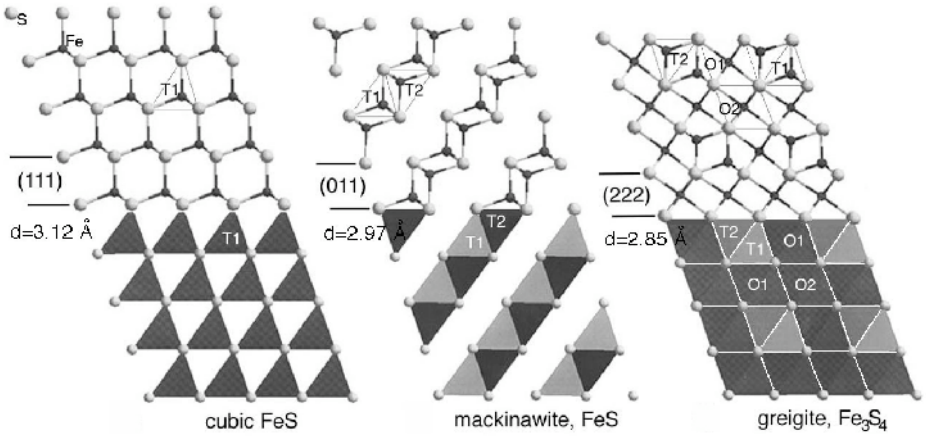

Figure 5. The structural relationships among cubic FeS, mackinawite, and greigite. Light and dark circles represent sulfur and iron atoms, respectively. The lower half of the image shows the same structures in polyhedral respresentation. T1 and T2 mark tetrahedral, and O1 and O2 mark octahedral positions. (Figure from Pósfai et al. 1998b.)

nen 2004). Rickard et al. (2001) found that mackinawite converts to either greigite or pyrite, depending on the presence or absence of carboxylic aldehydes in the solution, respectively. Even though the organic compound was present in very low concentration, it served as a switch that determined the mineral phase. Similar molecular switches have not yet been identified in magnetotactic bacteria, but the concept of a chemical control mechanism over the selection of the mineral phase is consistent with the principles of BCM that are outlined in Table 1.

Greigite magnetosomes typically exhibit patchy contrast in transmission electron microscope (TEM) images (Heywood et al. 1990; Pósfai et al. 1998b). This appearance may be related to the presence of defects that arise from the solid-state transformation of mackinawite into greigite. It may also result from thickness variations. High-resolution TEM images provide evidence that many greigite magnetosomes are aggregates of smaller, flake-like fragments that combine to form a single crystal (Kasama et al. 2006), and that such aggregates can have highly irregular shapes. Synthetic mackinawite was found to precipitate in the form of plate-like nanocrystals with an average size of a few nm (Wolthers et al. 2003; Ohfuji and Rickard 2006). The formation of primary mackinawite in magnetotactic bacteria by a similar mechanism, through the nucleation and aggregation of plate-like nanocrystals, cannot be ruled out.

Although greigite magnetosomes are typically pure iron sulfides, in some samples copper was found to substitute for iron by up to 12 at% (Bazylinski et al. 1993a; Pósfai et al. 1998b). The copper content appeared to be independent of cell type, but was related to geographical location, and therefore presumably to the copper concentration in the environment of the bacteria. When the samples of greigite-containing bacteria are stored in air, the greigite crystals oxidize partially, and an amorphous iron oxide shell forms on them (Lins and Farina 2001; Kasama et al. 2006) (Fig. 6). This phenomenon was observed to reduce the magnetic moments of the magnetosomes (Kasama et al. 2006).

Magnetic sensing with sulfide magnetosomes

Magnetotactic bacteria are the only organisms that are known to make use of the magnetic properties of iron sulfide crystals for navigation. Other organisms that navigate magnetically include algae, protists, bees, ants, fishes, turtles, and birds (Wiltschko and Wiltschko 1995;

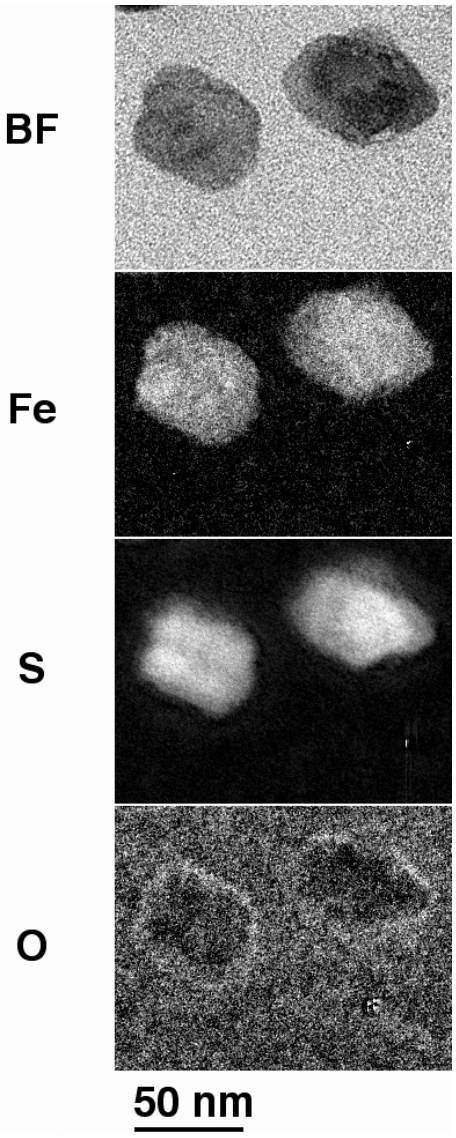

Figure 6. Three-window, background-subtracted elemental maps of two iron sulfide magnetosomes from a magnetotactic bacterium. BF: bright-field image; the images marked Fe, S, and O show the distributions of the respective elements. The magnetosomes have a crystalline iron sulfide core and an amorphous iron oxide shell.

Walker et al. 2002). In the few cases for which the mechanism of magnetic sensing is known, the mineral involved is magnetite (Kirschvink et al. 2001; Winklhofer et al. 2001; Diebel et al. 2000). Magnetite also occurs in the human brain (Kirschvink et al. 1992; Dobson 2001), but it remains to be established whether it has a biological function.

Magnetosomes in magnetotactic bacteria are typically arranged in chains, with each chain behaving as a magnetic dipole (Frankel 1984). The Earth's magnetic field exerts a torque on this dipole, and competes with the effect of Brownian motion that tends to randomize the orientation of the cell. When the magnetic moment of a cell is known, its average orientation with respect to the external magnetic field can be calculated on the basis of the Langevin function, as discussed in detail by Bazylinski and Moskowitz (1997). Both calculations and experiments show that magnetite-producing bacteria typically contain enough magnetosomes to allow their cells to migrate parallel to the small (50 μT) magnetic field of the Earth with a net velocity that is in excess of 90% of their forward velocity (Frankel 1984; Schüler et al. 1995). Since the magnetic induction of greigite (0.16 T) is only about one quarter that of magnetite (0.60 T) (Dunlop and Özdemir 1997), a cell needs a larger number of greigite than magnetite crystals (of similar size) in order to be magnetotactic (Heywood et al. 1991).

The mechanism of magnetic alignment described above requires the magnetosome crystals to be magnetized approximately parallel to each other at room temperature. The combined effects of their shape and magnetocrystalline anisotropy, as well as interparticle interactions between magnetosomes, determine the magnetic domain state, and therefore the net magnetic dipole moment, of each magnetosome. Based on theoretical considerations, Diaz-Ricci and Kirschvink (1992) calculated the size and shape-dependent magnetic properties of greigite, and determined that the sizes of bacterial magnetosomes place them at the boundary between the superparamagnetic and single magnetic domain size range for isolated crystals. They also reported that crystal shape affects the magnetic properties of greigite significantly. Whereas isolated ~70-nm crystals with prismatic habits

were calculated to be single domains, spheroidal particles of similar size were superparamagnetic at room temperature. Experimental results obtained by Chen et al. (2005) also indicate that the magnetic properties of acicular and irregularly-shaped greigite nanocrystals differ.

Measurements of the magnetic properties of greigite-producing magnetotactic bacteria are scarce. As a result of the present inability to grow sulfide-producing magnetotactic organisms in pure culture, it has not been possible to apply bulk magnetic characterization techniques to their study. Recently, Kasama et al. (2006) used off-axis electron holography in the TEM to study the magnetic properties of greigite magnetosomes in rod-shaped cells. Electron holography is a powerful and relatively specialized technique that can be applied to the study of magnetic and electrostatic fields in materials (Dunin-Borkowski et al. 2004). By using electron holography, it is possible to measure parameters such as the magnetic moments and coercivities of individual magnetosomes and their chains quantitatively, as well as to form two-dimensional images of the projected magnetic induction (Dunin-Borkowski et al. 1998, 2001).

The magnetic properties of sulfide magnetosomes were studied in a cell that was at the point of division (Fig. 7a) (Kasama et al. 2006). The structures of some of the magnetosomes in this cell were studied using selected-area electron diffraction and high-resolution TEM, their compositions were determined using energy-filtered TEM, and their three-dimensional morphologies were studied using high-angle annular dark-field electron tomography. The electron holography experiments revealed that the direction of the magnetic field is less uniform within the magnetosome chains, and undulates to a greater degree than in magnetite-containing cells. In addition, some of the greigite crystals (marked by arrows in Fig. 7b) appeared to be only weakly magnetic, with the apparent saturation magnetic induction varying between 0 and 0.16 T for individual crystals in the cell. This behavior could result either from the presence of non-magnetic sulfides other than greigite, or from the fact that some of the greigite crystals may be magnetized in a direction that is almost parallel to that of the electron beam. Since electron holograms are only sensitive to the components of the magnetic field in the plane of the specimen, i.e., perpendicular to the electron beam direction, magnetic crystals with large out-of-plane components of their magnetization would appear to be non-magnetic. Diffraction patterns obtained from several of the apparently non-magnetic crystals were found to be consistent with greigite. The diffraction patterns also showed that the greigite crystals were oriented randomly within the cell, and that their elongation directions appeared to be random. The variable degree of the apparent magnetization of the greigite magnetosomes is therefore likely to be primarily a consequence of their random orientations. Figure 7b also reveals that the magnetic contours within individual crystals are generally parallel to their axes of elongation. These observations are consistent with the calculations of Diaz-Ricci and Kirschvink (1992) that suggest that shape anisotropy has a much larger effect on the magnetization of greigite than magnetocrystalline anisotropy.

Interestingly, the multiple magnetosome chain shown in Figure 7b contains magnetite crystals in addition to the greigite magnetosomes (Kasama et al. 2006). Whereas the greigite grains are equidimensional or only slightly elongated, the iron oxide particles have distinctly elongated shapes, and their axes of elongation are aligned parallel to that of the magnetosome chain (Fig. 7c). Their elongated morphologies constrain their magnetic contours to be parallel to the chain axis (Fig. 7d). In addition, since magnetite is much more strongly magnetic than greigite, the magnetite particles contribute as much as ~30% of the total magnetic moment of the chain, which was measured by electron holography to be 1.8×10^{-15} Am^2. Whereas the randomly-oriented greigite particles produce an undulating magnetic field, the well-aligned magnetite particles provide a distinct "magnetic backbone" to the chain (Fig. 7d). The presence of both greigite and magnetite magnetosomes in the same cells was reported previously by Bazylinski et al. (1993b). The distinct shapes and orientations of these two mineral species suggest that their formation may be regulated by different biological mechanisms.

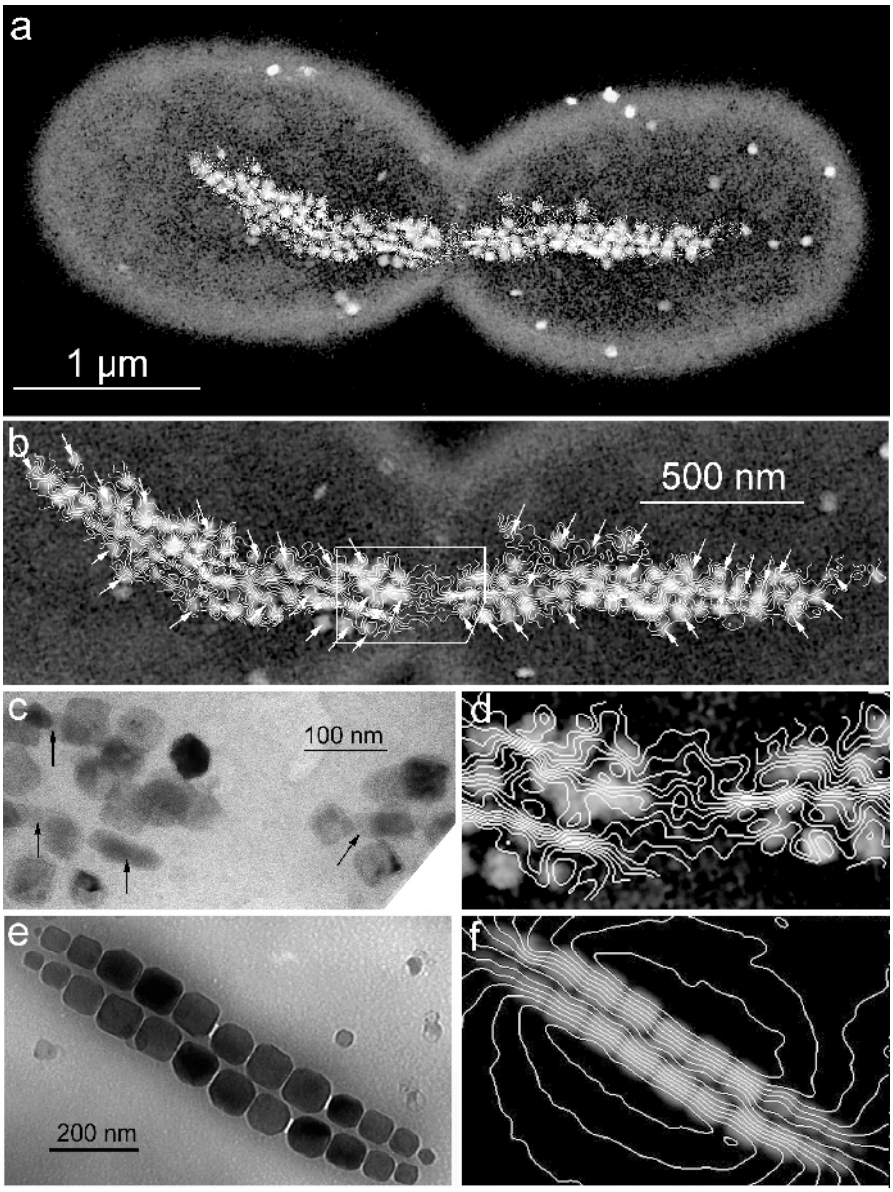

Figure 7. (a) Compositional map of a rod-shaped cell that contains iron sulfide magnetosomes. The cell was caught at the point of cell division. The image was constructed from electron energy-loss maps. (b) Magnetic induction map of the magnetosome chain in (a), obtained from electron holography. The magnitude and the direction of magnetic induction within the crystals is represented by the density and direction of the contour lines, respectively. The arrowed particles appear to be either non-magnetic or weakly magnetic. (c) Bright-field TEM image of the boxed region in (b). The arrowed particles are elongated magnetite crystals. (d) Magnetic induction map from the same area that is shown in (c). The density of the contour lines is much higher in the elongated magnetite crystals than in the equidimensional greigite crystals. (e) Bright-field image and (f) magnetic induction map obtained from a double magnetite chain from a magnetotactic coccus. In contrast to the greigite chain in (b), the magnetic contour lines are straight and their densities uniform within the particles in (f). [Based on images from Kasama et al. 2006.]

As mentioned above, the biological regulation of the nucleation and growth of magnetosomes has only been studied in magnetite-producing bacteria. The use of analogies with magnetite formation to explain control over greigite deposition in bacteria appears to be limited, because there are significant differences between the properties of sulfide and oxide magnetosomes. Some of these differences are illustrated in Figure 7f, which shows a magnetic contour map of a double magnetite chain in a cell of a magnetotactic coccus. The magnetite crystals in this cell have identical morphologies along the entire chain (Fig. 7e), and their [111] axes are aligned with the magnetosome chain within a few degrees, resulting in the same direction of magnetic induction in each crystal (Simpson et al. 2005a). In contrast, the dividing cell in Figure 7a appears to exhibit a lack of control over the shapes and orientations of the greigite crystals. As a result, the magnetic induction is highly variable along the magnetosome chain. The bacterium appears to compensate for the magnetically less efficient assembly of magnetosomes, as well as for the lower magnetization of greigite than magnetite, by forming a multiple chain that contains several times as many crystals as the magnetite chain shown in Figure 7e.

Not only the processes of crystal nuclation and growth, but also the mechanisms of chain assembly appear to be different in the magnetite and greigite producers. Whereas magnetite particles in magnetotactic spirilla were found to be aligned along a filament that runs along the long axis of the cell (Scheffel et al. 2005; Komeili et al. 2006), electron tomography experiments on the dividing cell shown in Figure 7a revealed a three-dimensional arrangement of the crystals in the multiple greigite chain (Kasama et al. 2006).

To date, the magnetic moments of magnetosome chains in three different strains of magnetite-producing bacteria (MS-1 and MV-1, Dunin-Borkowski et al. 1998, 2001; Itaipu-1, McCartney et al. 2001) and in two cells of unnamed sulfide producers (Kasama et al. 2006) have been measured experimentally using electron holography. Remarkably, in the different types of cell the magnetic moments per cell are all the same to within a factor of two. Therefore, even though the biomineralization processes and the properties of magnetosomes may vary between different groups of magnetotactic bacteria, natural selection appears to have favored structures that serve the function of magnetic sensing equally well.

Mechanical protection: iron sulfides on the foot of a deep-sea snail

Hydrothermal vents in mid-ocean ridge systems provide chemical energy for diverse populations of chemoautotrophic bacteria (as reviewed by Jannasch and Mottl 1985). The abundance of micro-organisms at deep-sea vents makes it possible for more complex organisms (such as worms, shrimp, crabs, clams, mussels, gastropods, anemones, barnacles, etc.) to thrive in an environment where no light is available. Thus, entire ecosystems depend on geochemical rather than on solar energy. Based on variations in the species composition of invertebrate communities, faunas at oceanic vents are recognized to belong to six provinces (Van Dover et al. 2001). One of these provinces is the central ridge system in the Indian Ocean, where, among many other animals, the vent fields harbor a snail that bears mineralized scales on its foot (Van Dover et al. 2001).

The sides of this gastropod's foot are covered in a tile-like fashion by black sclerites (Fig. 8). The scales consist of iron sulfide minerals (Warén et al. 2003), making this snail the first known organism that uses sulfide minerals for structural support. Initially, greigite and pyrite were described as the primary mineral phases (Warén et al. 2003; Goffredi et al. 2004), but mackinawite was also subsequently identified (Suzuki et al. 2006). The presence of greigite makes the scales magnetic. As Suzuki et al. (2006) note, "it is rare for animals to produce macroscopic materials that stick to a hand magnet." The only other known organisms that produce such structures are chiton mollusks that have magnetite-bearing radular teeth (Lowenstam 1962).

The spatial distributions, microstructures, magnetic and mechanical properties, and the isotopic compositions of the iron sulfide minerals in this organism were studied by Suzuki

et al. (2006). The sulfides were found to be present in three distinct layers, which were defined both by their positions and by their mineral species. An "iron sulfide" layer covers the outer surface of the sclerites and consists primarily of greigite. A "mixed layer" and a "conchiolin layer" occur within the organic matrix, and consist of nanocrystalline pyrite and mackinawite, respectively (Fig. 9). The greigite crystals in the iron sulfide layer are rod-shaped and highly elongated along [110], with average lengths and widths of 118 and 14 nm, respectively. The space between the greigite rods is filled by fibrous mackinawite (Fig. 10). The orientation relationship between the two phases appears to be the same as that described above for sulfides in magnetotactic bacteria, although the boundary plane is different. The pyrite in the mixed layer has an unusual appearance, since it takes the form of nanoparticles that are as small as 3 nm. Remarkably, the nanoparticles have a consistent crystallographic orientation. In the conchiolin layer, mackinawite forms ~3–10 nm particles within amorphous iron sulfide.

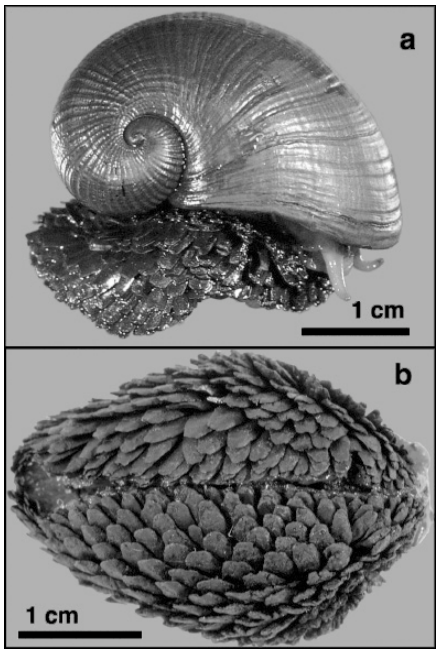

Figure 8. Two views of the "scaly-foot gastropod" that has iron sulfide sclerites on its foot. [Used by permission of Elsevier, from Suzuki et al. (2006), *Earth and Planetary Science Letters*, Vol. 242, Fig. 1, p. 40.]

The complex composite of three iron sulfide minerals and organic material results in interesting magnetic and mechanical properties. The presence of ferrimagnetic greigite raises the question of whether the snail uses this mineral for magnetic sensing. Bulk magnetic measurements reveal that most of the greigite crystals are single magnetic domains, but a significant fraction of superparamagnetic greigite is also present (Suzuki et al. 2006). Measurements of anhysteretic remanent magnetization indicate strong interparticle interactions. In addition, the ratio of natural remanent magnetization to isothermal remanent magnetization is consistent with the presence of random orientations of the greigite crystals. All of these observations suggest that the properties of the greigite crystals are not optimized for magnetic sensing, and that the snail does not use the greigite crystals as a magnetic compass (Suzuki et al. 2006).

The mechanical properties of the biomineralized layers are consistent with a hardening function. Nanoindentation studies show that the iron sulfide layer is harder and stiffer than human enamel, and stiffer than molluscan shell nacre (Suzuki et al. 2006). Whereas the minerals provide rigidity, the associated organic material provides toughness. Since the scaly-foot gastropod shares its habitat at the base of black smoker chimneys with predators such as brachyurean crabs (Suzuki et al. 2006) and other gastropods (Warén et al. 2003), it is likely that the hard and tough iron sulfide/organic composite is used for protection.

There is some ambiguity about whether the snail controls the deposition of the sequence of iron sulfide minerals. The iron sulfide layer is known to be covered by bacteria where it is overlain by adjacent sclerites (Warén et al. 2003). The phylogenetic affiliations of these episymbiotic bacteria have been studied by Goffredi et al. (2004), who found a predominance of bacteria belonging to lineages that are involved in sulfur cycling. Similar bacteria were not

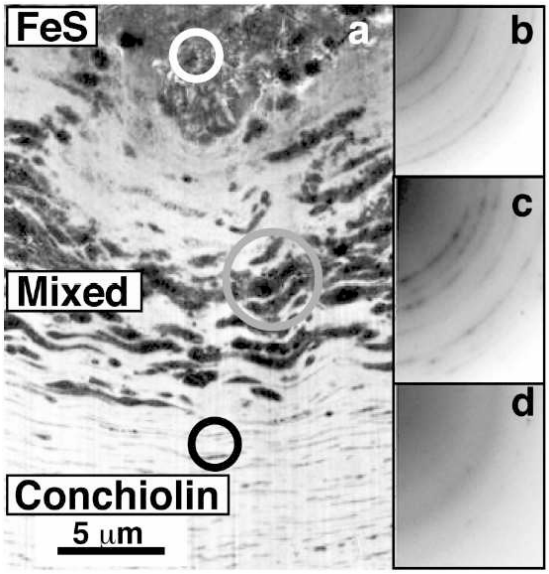

Figure 9. TEM image (a) of a cross-section of the sclerite of the scaly-foot gastropod. Selected-area electron diffraction patterns (b, c, d) obtained from the circled regions in (a), indicating (b) greigite from the FeS layer, (c) pyrite from the mixed layer, and (d) mackinawite from the conchiolin layer. [Used by permission of Elseveir, from Suzuki et al. (2006), *Earth and Planetary Science Letters,* Vol. 242, Fig. 2, p. 42.]

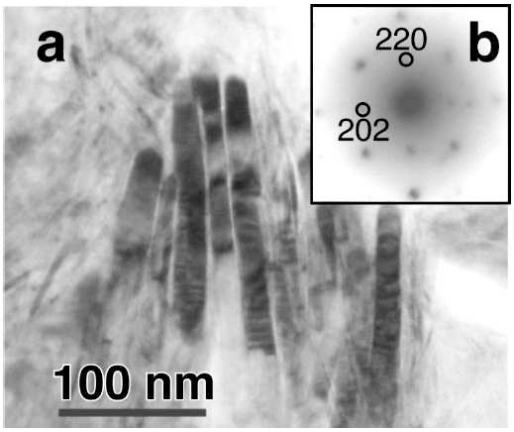

Figure 10. (a) TEM image of rod-shaped crystals from the iron sulfide layer of the sclerite of the scaly-foot gastropod, and (b) electron diffraction pattern from one of the crystals, indicating that it is greigite. The fibrous material next to the rods consists of mackinawite. [Used by permission of Elseveir, from Suzuki et al. (2006), *Earth and Planetary Science Letters*, Vol. 242, Fig. 3, p. 43.]

found on other available surfaces or among other gastropods within the same habitat. These observations prompted Goffredi et al. (2004) to speculate that iron sulfide mineralization is a consequence of the metabolism of these symbiotic bacteria. If sulfate-reducing bacteria were the source of sulfur for the sclerites, then a significant enrichment of light isotopes would be expected. However, Suzuki et al. (2006) measured the isotopic compositions of iron and sulfur and found the values to be close to those of the sulfide and iron in the hydrothermally-deposited chimneys. Thus, hydrothermal fluids appear to be a more likely source than episymbiotic bacteria of the iron and sulfur that are involved in sclerite mineralization. The presence of iron sulfides within the conchiolin tissue may also indicate the involvement of the snail in the precipitation of sulfide minerals.

Sulfide mineralization by the scaly-foot snail is the first known case of pyrite formation by BCM. The sulfide mineral assemblage in this organism is also unique in terms of its macroscopically magnetic character and its structural role. Although greigite and mackinawite

form in both magnetotactic bacteria and the scaly-foot snail, some of their physical properties and their biological functions are different in the two cases. Much research is still needed to understand the biological control of the deposition of iron sulfides in both types of organisms.

BIOLOGICALLY INDUCED FORMATION OF SULFIDE MINERALS

Biologically induced mineralization is usually considered to be an uncontrolled consequence of metabolic activity, which produces minerals that are characterized by poor crystallinity, a broad particle size distribution, and a lack of well-defined crystal morphology and chemical purity (Frankel and Bazylinski 2003). If the metabolic products diffuse away from the micro-organism, and if the mineral-forming reactions take place in solution or on sediment particles, then the precipitated products may be indistinguishable from minerals that form by purely inorganic processes. However, in many cases bacterial surfaces or extracellular polymeric materials act as passive or active nucleation sites (Fortin et al. 1997; Schultze-Lam et al. 1996; Fortin and Langley 2005). In such cases, the biological material plays a direct role in crystal nucleation, and the minerals that form may have species-specific physical or chemical properties. Thus, BIM encompasses a broad range of mineral-forming processes, many of which are unique to the particular minerals or organisms that are involved in their formation.

Many common sulfide minerals can form by BIM, but the precipitation of iron sulfides is geologically the most important and the most extensively studied problem. Recently, Rickard and Morse (2005) provided a critical review of research into iron sulfide formation, including an assessment of the "myths and facts" that have accumulated over the past 40 years. Sedimentary pyrite formation has also been reviewed by Schoonen (2004), and aspects of the formation of sulfides by BIM are discussed in this volume by Rickard and Luther (2006). Here, the key processes that are involved in BIM are described, including a brief discussion of iron sulfide formation and a review of interesting examples of zinc sulfide mineralization.

Microbial sulfate and metal reduction

The activity of dissimilatory sulfate-reducing prokaryotes (SRP), which supplies reactive sulfide ions, is key to the formation of sulfide minerals by BIM (Frankel and Bazylinski 2003). Bacteria inhabit distinct redox zones according to their physiology (as reviewed in several textbooks of mineralogy and geochemistry, e.g., Nealson and Stahl 1997; Gould et al. 1997; Aplin 2000). Micro-organisms oxidize carbon in organic matter, using a variety of terminal electron acceptors, ranging from O_2 under aerobic conditions to SO_4^{2-} in anoxic environments.

SRP represent a morphologically and phylogenetically heterogeneous group. They are generally strict anaerobes that oxidize simple organic compounds or hydrogen using sulfate ions, as shown for example by the reaction (Tuttle et al. 1969):

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S$$

In this process, the sulfur in the sulfate ion is reduced completely to sulfide, which is released into the environment. Whereas a considerable proportion of the reactive sulfide diffuses upwards and is reoxidized (Jørgensen 1977), part of it combines with metals (primarily iron) to form sulfide minerals (Berner 1970).

Since SRP can use relatively small organic molecules as electron donors, they generally depend on other microbial populations that degrade complex organic compounds. Two major groups of SRP exist, one that incompletely oxidizes organic substrates into acetate (e.g., *Desulfovibrio, Desulfotomaculum, Desulfomonas, Desulfobulbus*), and another that completely oxidizes organic matter to CO_2 (e.g., *Desulfococcus, Desulfosarcina, Desulfonema*) (Gould et al. 1997). Some hyperthermophilic archaea are also dissimilatory sulfate reducers. SRP are ubiquitous in many anaerobic environments, including lakes, swamps, soils, waste ponds,

hydrothermal systems, and even within the lithosphere (Lovley and Chapelle 1995). In terms of the amount of sulfide mineralization and its global biogeochemical effect, SRP that occur in marine sediments are most important (Schoonen 2004).

In addition to sulfate reduction, the microbial reduction of metals (such as iron and manganese) is also important in biogenic sulfide mineralization, since it may contribute to the pool of metal ions that are available for mineral formation (Rickard and Morse 2005). Dissimilatory iron-reducing prokaryotes were shown to respire using ferric iron in minerals, and to exert a strong influence on the geochemistry of many environments (Nealson and Saffarini 1994; Methe et al. 2003). Iron reducers are phylogenetically diverse, and include several genera of bacteria (such as *Geobacter* and *Shewanella*) and even archaea (Kappler and Straub 2005). Many of these organisms are phylogenetically closely related to SRP, and include species that can also reduce elemental sulfur. Iron-reducing microorganisms can even use iron from relatively poorly reactive minerals such as magnetite and sheet silicates. The potential role of such micro-organisms in dissolving iron and indirectly affecting the sulfur cycle in sediments is only now beginning to be appreciated (Rickard and Morse 2005).

The role of biological surfaces in mineral nucleation

In general, the heterogeneous nucleation of biominerals is favored kinetically over homogeneous nucleation. Biological surfaces provide excellent nucleation sites for a number of minerals, including sulfides. The properties of different types of mineral-nucleating biological surfaces were reviewed by Schultze-Lam et al. (1996), Fortin et al. (1997), Konhauser (1998), Frankel and Bazylinski (2003), and Gilbert et al. (2005).

The outer surfaces of bacterial cell walls are predominantly negatively charged at near neutral pH, irrespective of whether they belong to gram-positive or gram-negative structural types (Fortin et al. 1997). Therefore, they attract positive ions from solution and thereby initiate the nucleation of metal sulfides. In natural environments, additional biological layers exist on the cell walls. These layers include capsules that usually consist of acidic polysaccharides, S-layers that consist of regular arrays of proteins (Beveridge 1989), sheaths, stalks, and filaments (Gilbert et al. 2005). Many of these surfaces are known to induce the nucleation of metal oxides and sulfides (Fortin et al. 1997; Gilbert et al. 2005).

The ability of bacterial surfaces to bind metal ions is related to the presence of acidic functional groups. As discussed by Gilbert et al. (2005), proteins or polysaccharides that are rich in negatively charged carboxyl (COO^-) groups are the most common and effective cation-binding macromolecules in biomineral nucleation. A general sorption reaction for a metal cation M of charge z (M^{z+}) at a carboxyl binding site, as described by Ferris (1997), results in the release of a proton according to the reaction:

$$\text{B-COOH} + M^{z+} = \text{B-COOM}^{z-1} + \text{H}^+$$

Thus, the sorption of metal ions depends not only on the number of reactive chemical groups on the bacterial surface, but also on the pH and on the concentration of dissolved metal ions. The sorption of cationic species is enhanced as the pH increases and as surface groups deprotonate. As a result, the metal binding capacity of natural biofilms is enhanced significantly under circumneutral pH conditions, with respect to that in acidic metal-contaminated waters (Ferris 1997).

Iron sulfides in marine sediments

Iron sulfide minerals are ubiquitous both in modern anoxic sediments and in sedimentary rocks. The primary stages of sedimentary iron sulfide formation were identified by Berner (1970; 1984), and the topic has since been reviewed several times (Morse et al. 1987; Rickard et al. 1995; Schoonen 2004; Rickard and Morse 2005). For the past four decades, the key processes appeared to be well understood. The remaining uncertainties were related to the

importance of specific reactions, the physical and chemical properties of transient phases, and the roles of microbes. However, the most recent review by Rickard and Morse (2005) challenged many long-standing views, and identified several areas where more research is necessary. Here, primary attention is paid to the aspects of sedimentary iron sulfide formation that are related to the activities of micro-organisms.

The formation of iron sulfides in sediments is a typical example of BIM. The rate of iron sulfide formation depends primarily on the rate of microbial sulfate reduction (which also depends on the availability of organic carbon), and on the amount of competing electron acceptors including reactive Fe(III)-bearing minerals (Berner 1970) (Fig. 11). When dissolved sulfide produced by SRP reacts with Fe^{2+}, the precipitate that forms is generally termed "amorphous FeS," and appears to correspond to poorly-ordered or nanocrystalline mackinawite (Lennie and Vaughan 1996), or mixtures of mackinawite and greigite. Most earlier literature on sedimentary pyrite formation assumes that pyrite forms by the conversion of mackinawite or greigite (Schoonen 2004). However, according to Rickard and Morse (2005), these precursors are not required for pyrite formation.

Our understanding of the roles of bacteria in each pyrite-forming stage has changed considerably over the past ten years (Donald and Southam 1999; Schoonen 2004; Rickard and Morse 2005). Whereas the role of bacteria had been thought to be restricted to providing sulfide ions, it now appears that micro-organisms affect in many ways the processes that lead to the formation of iron sulfides (Fig. 11).

The mineral species. In addition to pyrite, which is the most abundant species, other iron sulfides that occur in sediments include mackinawite and greigite. The latter minerals (and pyrrhotite ($Fe_{1-x}S$)) are also termed "iron monosulfides." Significantly, mackinawite and greigite have rarely been identified in the field. In most studies, the operationally-defined category of acid volatile sulfides (AVS) is used, and is assumed to include amorphous FeS, mackinawite, and greigite. However, as pointed out by Rickard and Morse (2005), AVS is not

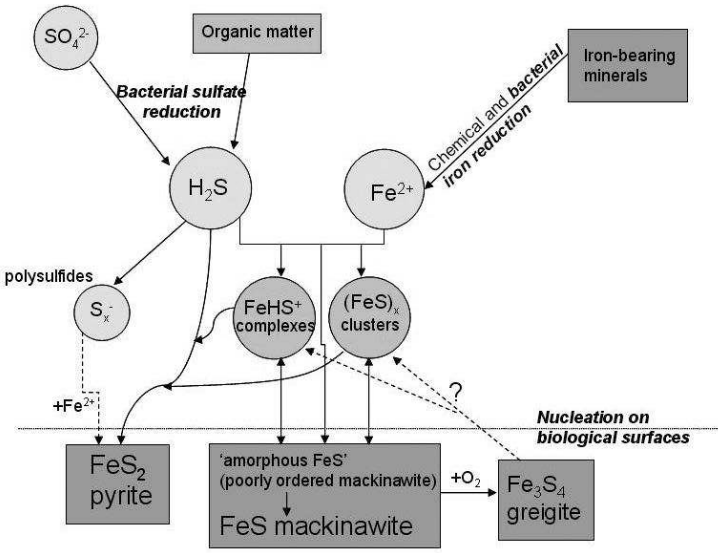

Figure 11. The primary pathways of sedimentary iron sulfide formation, based on Berner (1984) and Rickard and Morse (2005). Circles and rectangles denote dissolved and solid species, respectively. Text in italics refers to processes that involve the activity of bacteria.

equivalent to the sum of solid iron monosulfides but is a complex and variable component of the sediment. AVS likely includes dissolved iron and sulfur species and their complexes, aqueous iron sulfide clusters (FeS_{aq}) (see Rickard and Luther, 2006, in this volume), and an unidentified fraction of mackinawite and greigite. In addition, even though pyrite is insoluble in weak acids, commonly used extraction methods may partially dissolve fine-grained pyrite, which may then also contribute to AVS (Rickard and Morse 2005).

The thermodynamic constraints that determine which iron sulfide is stable in an anoxic sediment were discussed by Schoonen (2004). Iron monosulfides are predicted, by equilibrium thermodynamic calculations, to be stable over a very narrow range of p*e*-pH conditions. Marcasite is metastable with respect to pyrite and forms under acidic conditions (pH < 5) (Murowchick and Barnes 1986). Therefore, in equilibrium, only pyrite would be expected to occur in a low-temperature sedimentary environment. However, many field studies attest to the prevalence of iron monosulfides in modern marine sediments. In euxinic basins, the amount of iron monosulfides exceeds that of pyrite (Hurtgen et al. 1999). In addition, evidence has accumulated over the past 15 years that greigite is the primary carrier of magnetization in many types of sedimentary rock, some of which are as old as Cretaceous (Reynolds et al. 1994; Roberts 1995; Dekkers et al. 2000; Rowan and Roberts 2006; Pearce et al. 2006, in this volume). The presence of metastable iron monosulfides has generally been attributed to the presence of a high nucleation barrier for the formation of pyrite (Schoonen and Barnes 1991; Benning et al. 2000). If pyrite seed crystals are present, then this nucleation barrier can be overcome (Benning et al. 2000).

Availability of iron. The balance between the rate of H_2S formation and the availability of reactive iron exerts a controlling factor over FeS formation (Schoonen 2004). Raiswell and Canfield (1998) documented the importance of the mineral phase of iron oxide present in the sediment on the rate of its sulfidation. Highly reactive minerals include ferrihydrite, lepidocrocite, goethite, and hematite, with half-lives of less than a year. Magnetite and "reactive" iron silicates have half-lives on the order of ~10^2 years, whereas the half-lives of poorly reactive minerals (such as ilmenite and some silicates) are in the 10^6-year range. As discussed above, dissimilatory metal-reducing bacteria use oxidized forms of iron as terminal electron acceptors, thereby causing the dissolution of oxide minerals under anaerobic conditions (Frankel and Bazylinski 2003; Kappler and Straub 2005). The released metal ions can participate in various mineral-forming reactions, including those that produce sulfides. Although inorganic and biogenic pathways for metal reduction are not easy to distinguish in most natural systems, bacterial processes are likely to be important for supplying iron for sedimentary iron sulfide formation (Rickard and Morse 2005).

Nucleation and the physical properties of mackinawite and greigite. The role of bacteria in the nucleation of iron monosulfides is uncertain, although there is evidence that FeS nucleates preferentially on the cell envelopes of SRP. Bacterial cells and their remains were found to be prominent nucleation sites for amorphous FeS (and nanocrystalline millerite, NiS) in a contaminated lake sediment (Ferris et al. 1987). Donald and Southam (1999) found that thin layers of FeS coated both the inner and the outer surfaces of cells. Anionic cell surface polymers likely interacted with Fe^{2+}, and the immobilized cations could then react with H_2S, forming the films of FeS. Similarly, iron sulfides encrusted the surfaces of SRP in experiments by Watson et al. (2000) (Fig. 12). They formed on the surface of hematite to which SRP were attached, and initiated the precipitation of FeS (Neal et al. 2001). Thus, micro-organisms are important nucleation sites for the formation of iron sulfides.

The initial FeS precipitate is difficult to characterize because of its small grain size and poorly ordered structure. The morphologies and sizes of nanocrystals appear to be strongly affected by experimental conditions. Whereas Wolthers et al. (2003) described FeS precipitates as nanocrystals with an average size of ~4 nm, Herbert et al. (1998) found that platy macki-

nawite crystals with diameters of 100 to 300 nm precipitated in growth media of SRP, and formed 1 to 2 μm spherical aggregates. Ohfuji and Rickard (2006) showed that mackinawite precipitated as nanocrystalline particles, and presented a list of particle sizes and specific surface areas observed in various studies. Structurally, all of these studies identified the primary phase of the precipitate as "poorly ordered" or nanocrystalline mackinawite, although Wolthers et al. (2003) described two types of crystalline domains ("MkA" and "MkB"), with different *d*-values that bore little resemblance to those of mackinawite. High-resolution TEM images and electron diffraction patterns were obtained from an FeS precipitate by Ohfuji and Rickard (2006). The diffraction patters contained diffuse rings, indicating that the particles were poorly ordered (Fig. 13). The observed *d*-spacings suggested that a mackinawite-like short-range order is present, consistent with the high-resolution images.

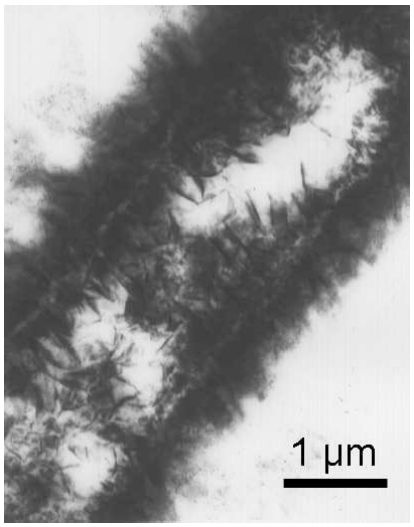

Figure 12. TEM image of a cell of a sulfate-reducing bacterium that is encrusted by iron sulfide minerals. [Used with permission from Elsevier, from Watson et al. (2000) *Journal of Magnetism and Magnetic Materials,* Vol. 214, Fig. 1, p. 13-30.]

In addition to mackinawite, greigite was also identified in several studies in the initial FeS precipitate. Herbert et al. (1998) inferred that the surfaces of the aggregated nanocrystals had a greigite composition, whereas the remaining bulk material consisted of disordered mackinawite. On the basis of magnetic measurements, Watson et al. (2000) found that greigite formed a significant fraction of SRP-precipitated iron sulfide.

Greigite also forms from mackinawite by solid-state transformation. Two basic routes have been suggested, either through iron loss (Lennie et al. 1997) or through sulfur addition

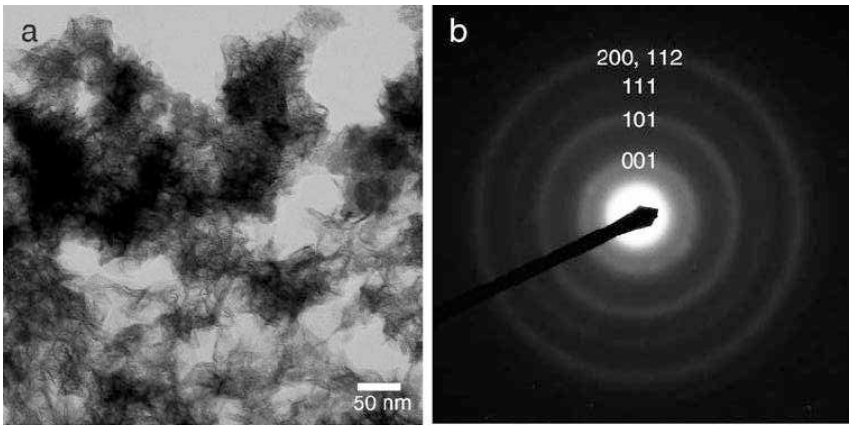

Figure 13. (a) TEM image and (b) electron diffraction pattern of precipitated mackinawite. [Used with permission of Elsevier, from Ohfuji and Rickard (2006), *Earth and Planetary Science Letters*, Vol. 241, Fig. 2a,b, p. 227-233.]

(Horiuchi 1971). It appears that the conversion of mackinawite into either greigite or pyrite can be controlled by the presence of catalytic quantities of organic compounds (Rickard et al. 2001). In the presence of aldehydic carbonyls in the solution, Fe^{2+} in the iron monosulfide is partially oxidized, whereas S^{2-} remains unchanged, forming greigite. In the absence of aldehydic carbonyls, S^{2-} is oxidized and pyrite forms (Rickard et al. 2001). It is not yet known whether similar organic switches operate in natural systems as in the laboratory experiments.

The greigite that forms from mackinawite is also nanocrystalline. This observation has important implications for the magnetic properties of sediments. The magnetic single domain range for greigite is particle-shape-dependent and extends from ~50 nm (Diaz-Ricci and Kirschvink 1992) to a poorly-constrained upper limit of 200–1000 nm (Hoffmann 1992; Diaz-Ricci and Kirschvink 1992). Crystals within this range have a high coercivity and therefore contribute significantly to the remanent magnetism of sediments. Rowan and Roberts (2006) found that single-domain and superparamagnetic greigite populations coexisted in Neogene marine sediments, providing for a complex magnetic behavior. Greigite formed with pyrite in framboids, but a later generation of very fine-grained superparamagnetic greigite appeared to grow on the pyrite crystals. Such late diagenetic changes can complicate paleomagnetic interpretations, since such crystals aquired their remanence > 1 Myr after deposition.

The formation of pyrite. Three primary pathways for pyrite formation are usually considered (Schoonen 2004), including (1) FeS oxidation by a polysulfide species (Luther 1991; Schoonen and Barnes 1991); (2) FeS oxidation by H_2S (Rickard 1997); and (3) conversion of FeS by iron loss through an intermediate greigite phase (Wilkin and Barnes 1996). The reactions are:

$$(1)\quad FeS + S_n^{2-} \rightarrow FeS_2 + S_{n-1}^{2-}$$

$$(2)\quad FeS + H_2S \rightarrow FeS_2 + H_2$$

$$(3)\quad 4\,FeS + ½\,O_2 + 2\,H^+ \rightarrow Fe_3S_4 + Fe^{2+} + H_2O$$

$$Fe_3S_4 + 2\,H^+ \rightarrow FeS_2 + Fe^{2+} + H_2$$

Experimental tests by Benning et al. (2000) showed that Reaction (2) does not produce appreciable amounts of pyrite if H_2S is the only reactant in the system with mackinawite. Pyrite formation is induced only if the aqueous sulfur species or the mackinawite is oxidized. However, the importance of Reaction (2) is supported indirectly by the persistence and large proportion of iron monosulfide in euxinic sediments. In such an environment, reactive iron is available in abundance. Consequently, dissolved sulfide is depleted by iron sulfide formation, and the lack of dissolved sulfide prevents it from reacting with FeS and converting it into pyrite (Hurtgen et al. 1999). The conversion of mackinawite into greigite via iron loss (3) was observed by Lennie et al. (1997). On the basis of an analysis of molar volume changes, Furukawa and Barnes (1995) argued that the precursor phase converts to pyrite via the iron loss pathway (Reaction 3 above).

Studies by Luther and coworkers (Theberge et al. 1997; Luther et al. 2001; Luther and Rickard 2005) demonstrated the biogeochemical importance of aqueous metal sulfide complexes (see Rickard and Luther 2006, in this volume). Highly reactive FeS_{aq} clusters appear to be key intermediaries in pyrite formation, as they react with either H_2S or polysulfide species to nucleate pyrite (Rickard and Morse 2005). In light of these results, the conversion of mackinawite or greigite into pyrite cannot be regarded as a solid-state transformation. Instead, these minerals may be partially dissolved, forming aqueous FeS clusters that react to form pyrite (Fig. 11). Since FeS clusters can form by other routes, the presence of mackinawite and greigite is not a necessary condition for pyrite formation (Rickard and Morse 2005).

Experiments by Donald and Southam (1999) indicated that the conversion of FeS to FeS_2 is promoted by the formation of a thin FeS film on the surfaces of bacterial cells. Sulfur-

disproportionating bacteria also appeared to play a role in converting organic sulfur into H_2S in experiments by Canfield et al. (1998). Since radiolabeled organic sulfur was incorporated into the final pyrite product in this study, the FeS to pyrite transformation took place via the sulfur addition pathway (reaction (1) above). Fortin and Beveridge (1997) observed the intact remains of SRP encrusted by iron sulfides, while Grimes et al. (2001) found that organic matter provided nucleation sites for the reaction of FeS to FeS_2. It appears that bacterial activity mediates both the initial precipitation of FeS and its conversion to pyrite.

Framboidal pyrite. The interesting morphologies of sedimentary pyrite have long captivated the attention of researchers. A variety of morphological types occurs, including euhedral, irregular, and ooidic pyrite (Hámor 1994). However, the most widespread and characteristic appearance of pyrite is framboidal (Schoonen 2004; Ohfuji and Rickard 2005). The term framboid refers to a spherical structure, which consists of densely-packed pyrite crystals that have similar sizes and morphologies (Fig. 14). In addition to pyrite, greigite has also frequently been found as a component of framboids (Bonev et al. 1989; Wilkin and Barnes 1997; Rowan and Roberts 2006). The diameters of framboids are in the 1–30 μm range (but most are smaller than 10 μm), while the individual constituent crystals range from ~0.1 to 2 μm (Wilkin et al. 1996). Framboids were once thought to be fossilized bacteria. They were then considered to be pyritized organic particles or colloids (Raiswell et al. 1993) or abiotic products of the conversions of magnetic precursor iron sulfides, i.e., greigite (Sweeney and Kaplan 1973; Wilkin and Barnes 1997). However, Butler and Rickard (2000) synthesized pyrite framboids in the absence of magnetic intermediates and biological intervention. They found that the framboidal texture results from rapid nucleation from a strongly supersaturated solution, through the reaction of aqueous FeS cluster complexes with H_2S (see Rickard and Luther 2006, in this volume). Thus, even though the peculiar morphologies of framboids are suggestive of biological processes, the development of framboids may be the least likely of the various stages of sedimentary pyrite formation to be affected by biogenic activity.

Since framboids form either in the water column (in euxinic environments) or during early diagenesis within the top few centimeters of the sediment, their sizes reflect the conditions of the environment of deposition. In a very thorough study of framboid size distributions, Wilkin et al. (1996) established relationships between the size distributions of pyrite framboids and the redox conditions of the depositional environment.

Framboids can have remarkably ordered architectures, forming either cubic or icosahedral close-packed structures (Ohfuji and Akai 2002). In an electron backscatter

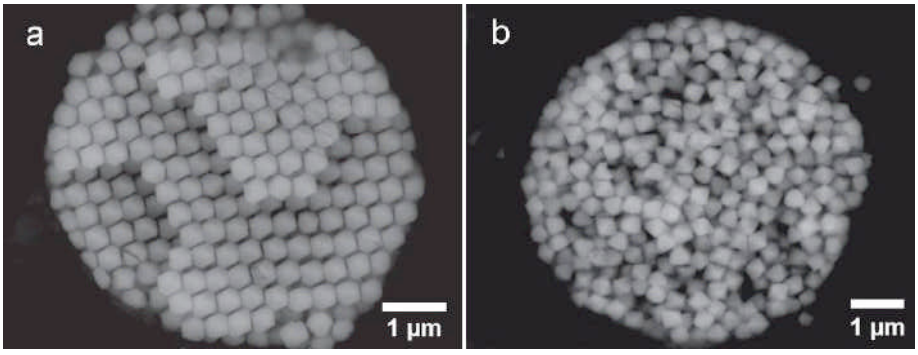

Figure 14. SEM images of synthetic pyrite framboids. (a) Morphologically ordered and (b) disordered framboid. [Used with permission of Elsevier, from Ohfuji and Rickard (2006), *Earth Science Reviews*, Vol. 71, Fig. 1a,c, p. 147-170.]

diffraction study, Ohfuji et al. (2005) distinguished morphologically ordered and disordered framboids (Fig. 14), and determined the morphological and crystallographic orientations of individual nanocrystals. Even in morphologically ordered framboids, low- and high-angle crystallographic misorientations were observed, the latter resulting from the fact that pyrite has only a two-fold axis along <100>. The results suggested that the self-organized structure results from the aggregation and subsequent reorientation of equimorphic nanocrystals.

Biogenic zinc sulfides: from mine-water to deep-sea vents

The biologically mediated precipitation of zinc sulfide has been studied recently in two widely different natural systems, in a flooded lead-zinc mine (Labrenz et al. 2000; Moreau et al. 2004) and in the tubes of a deep-sea vent worm (Zbinden et al. 2001, 2003; Maginn et al. 2002). Remarkably, the ZnS minerals that formed in these distinct environments showed similar morphological and chemical features.

Spherical aggregates of ZnS formed in a biofilm of sulfate-reducing bacteria in the flooded tunnel of a carbonate-hosted Pb-Zn deposit (Labrenz et al. 2000). The spherules were 1 to 5 μm in diameter and consisted of 1 to 5 nm, semi-randomly oriented, crystalline ZnS nanoparticles (Fig. 15). Both sphalerite and wurtzite structures occured within the nanoparticles, and stacking faults, twins, and disordered sequences of close-packed layers were observed to be present in many nanocrystals (Moreau et al. 2004). The ZnS particles were chemically pure, with no measurable iron content, and occured in layers within the biofilm, in close association with bacterial cells or extracellular polymeric material. The bacteria were shown by small-subunit ribosomal RNA gene analyses to belong to the sulfate-reducing family *Desulfobacteriaceae,* and verified to be metabolically active by fluorescence *in situ* hybridization (Labrenz et al. 2000). Some cells were encrusted and fossilized by ZnS spheroids, indicating the intimate association of bacteria and ZnS mineralization. Thus, the ZnS precipitation at this site was wholly attributable to the activity of SRP (Moreau et al. 2004).

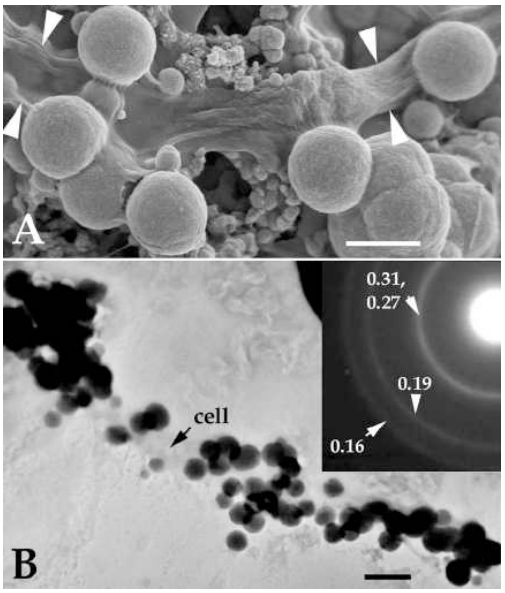

Figure 15. (a) SEM image of spherical ZnS aggregates that are associated with a biofilm (marked by arrowheads) from a flooded lead-zinc mine. (b) TEM image and selected-area electron diffraction pattern, showing that the ZnS spherules are associated with bacterial cells, and that both sphalerite and wurtzite structural elements occur in the spherules. [Reprinted with permission from Labrenz et al., *Science*, Vol. 290, Fig. 2a,b, p. 1744-1747. Copyright (2000) AAAS.]

The precipitation of pure ZnS consisting of both sphalerite and wurtzite structural elements is an interesting feature of this biomineralization. According to experimentally determined stability fields (Scott and Barnes 1972), sphalerite should form from cold (8-10 °C) groundwater. However, the presence of wurtzite is consistent with a size-dependence of ZnS phase stability, which has been predicted by molecular dynamics simulations (Zhang et al. 2003).

The extreme environment of deep-sea hydrothermal vents of the East Pacific Rise hosts the alvinellid or so-called Pompeii worms (*Alvinella*

pompejana and *Alvinella caudata*) (Zbinden et al. 2003). These animals dwell in organic tubes that contain zinc and iron sulfide minerals. The worms live on active sulfide chimney walls, where they are exposed to steep thermal and chemical gradients and intense mineral precipitation (Desbruyeres et al. 1998). Since sulfide-rich hydrothermal fluids and seawater mix within the worm tubes, the inorganic chemical deposition of sulfide minerals is possible. However, whereas hydrothermally-precipitated ZnS grains outside the worm tubes have variable iron contents and crystal sizes, the mineral grains in the tube wall have specific compositions, grain sizes, and positions within the wall structure (Zbinden et al. 2001, 2003). The specific properties of the minerals reflect the direct effects of the biological environment.

The worm tubes consist of concentric layers of fibrous organic material. The inner surface of the tube is covered by filamentous bacteria and ZnS grains. Each time the worm secretes a new layer, the bacteria and the minerals become entombed in the organic matrix of the tube (Zbinden et al. 2001, 2003) (Fig. 16). The spherical ZnS grains are aggregates of 1-5 nm crystals, and have a remarkably uniform composition of $Zn_{0.88}Fe_{0.12}S$. Powder electron diffraction patterns obtained from the round ZnS grains are consistent with the structures of both sphalerite and wurtzite (Zbinden et al. 2001). The sulfide mineral aggregates are attached to sheathed and branching bacterial filaments that occur on the inner tube surfaces (Maginn et al. 2002).

A specific feature of the mineralization associated with the Pompeii worms is that mineralogical gradients are present both from the outside to the inside and from the bottom to the top of the tubes (Zbinden et al. 2003). FeS_2 minerals predominate on the bottom outer surfaces of the tubes, with a marcasite to pyrite ratio of approximately 3:1. The relative proportion of FeS_2 minerals decreases from the bottom to the top of the tubes. Whereas Zbinden et al. (2003) found no iron sulfide in the mineralized layers within the tube wall and on the inner surfaces of the tubes, Maginn et al. (2002) observed pyrite, marcasite and other iron sulfides (presumably mackinawite and greigite) associated with ZnS inside the tubes. Although the particular features of the zinc and iron sulfide distributions may change between worm tubes observed in various sites, the mineralogical gradients indicate that the fluid compositions inside

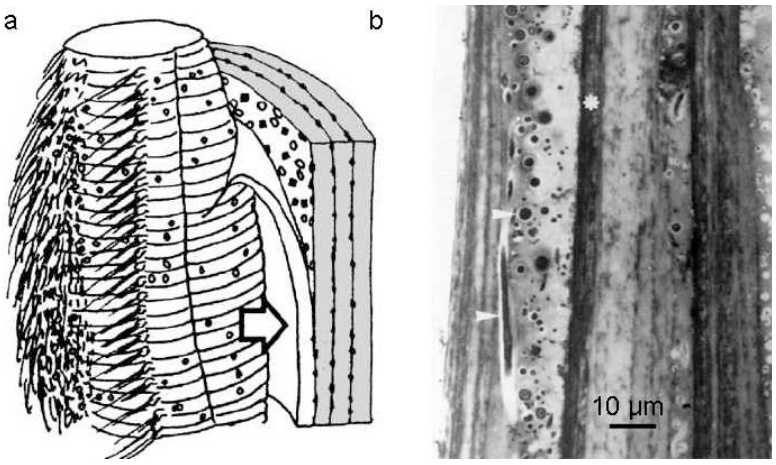

Figure 16. (a) A sketch of an Alvinellid worm and its tube. The worm secretes a new layer on the inside of the tube. The black dots represent ZnS grains and bacteria that are present on the surfaces of the tube layers. (b) Optical micrograph of a cross-section of several layers of an Alvinellid tube. The arrowheads indicate entrapped bacteria. The left side of the figure corresponds to the inside of the tube. [Used with permission of Schweizerbart (*http://www.schweizerbart.de*), from Zbinden et al. (2001), *European Journal of Mineralogy*, Vol. 13, Figs. 1, 5, p. 653-658.]

and outside the tube differ. Either the worm creates special hydrodynamic conditions within the tube, and thereby changes the ratio of vent fluid and seawater with respect to the conditions outside the tube (Desbruyeres et al. 1998), or the metabolic activity of the worm or its epibiotic bacteria causes the exclusive formation of ZnS minerals inside the tube (Zbinden et al. 2003).

There are some remarkable similarities between the ZnS minerals in the worm tubes and in the biofilm that was recovered from the groundwater in the lead-zinc mine. The sizes of the ZnS nanocrystals, and even those of the aggregate spherules, are similar in both cases. It is likely that the nanocrystals in the worm tubes have disordered layer sequences, similar to those in the sphalerite/wurtzite grains from the mine-water. Although the mineral grains from the mine are pure ZnS, while the crystals in the worm tubes contain iron, both types of biogenic ZnS are characterized by constant compositions. All of these features indicate that even minerals that are formed by BIM can have characteristics that distinguish them from inorganically-formed crystals. Although the bacteria that were found on the inner surface of the worm tube were not isolated and cultured, their close association with the ZnS grains suggests that they may be sulfate reducers, and that they could play a role in the detoxification of the fluid surrounding the worm (Zbinden et al. 2001).

BIOLOGICALLY MEDIATED DISSOLUTION OF SULFIDE MINERALS

The dissolution of sulfide minerals has important ramifications, both globally and locally, since it affects global geochemical cycles, generates acid mine drainage (AMD), and is used in industrial metal extraction. There is a long history of research on micro-organisms that oxidize sulfur or iron or both, and thereby enhance the rates of sulfide mineral weathering. Here, a few aspects of sulfide bioweathering are discussed. For comprehensive reviews on the geomicrobiology of sulfide mineral oxidation, the reader is referred to Nordstrom and Southam (1997) and McIntosh et al. (1997). The microbiological cycling of iron was recently reviewed by Kappler and Straub (2005).

Acid mine drainage

Wherever sulfide-bearing rocks or mine tailings are exposed to oxidative conditions, the sulfide minerals dissolve and produce acidic waters (Jambor et al. 2000). AMD is a major environmental concern, since the acidity of the water (generally between pH 2 and 4), as well as the presence of toxic concentrations of metals, are detrimental for many aquatic organisms. Although the oxidation of many sulfide mineral species contributes to AMD, pyrite is usually considered to be the most abundant and important mineral involved in the production of acidic waters (for a review of studies of the oxidation of various sulfides see Nordstrom and Southam 1997, and for the reaction products see Jambor et al. 2000).

Chemical and microbial processes are coupled in the generation of AMD. Under oxidative conditions, sulfide minerals react with oxygen, and the reaction is catalyzed by iron and sulfur-oxidizing bacteria. Members of the bacterial genus *Thiobacillus* are the most widely studied micro-organisms that break down sulfide minerals. Other important genera include *Leptospirillum*, *Sulfobacillus*, and some species of Archaea (Davis 1997; Nordstrom and Southam 1997; Baker and Banfield 2003). Most of these bacteria are acidophilic lithoautotrophs, i.e., they require an environment with a pH < 3 for optimal growth, and they use inorganic compounds as their source of metabolic energy (McIntosh et al. 1997). Microbial processes in the anoxic sections of mine tailings also contribute to the cycling of metals and sulfur, but these processes are still poorly understood (Fortin et al. 2002). Research into microbial communities involved in the production of AMD was recently reviewed by Baker and Banfield (2003).

The mechanism of bacterial catalysis of sulfide mineral oxidation was discussed by Nordstrom and Southam (1997). There has been some controversy over the role of a hypothesized,

direct enzymatic reaction induced by bacterial cells that attach to the mineral surface. The existence of such a reaction mechanism was supported by observations of etch pits that reflected the effects of attached cells (Bennett and Tributsch 1978). However, Nordstrom and Southam (1997) provided a comprehensive analysis of the observed rates of specific abiotic and biotic reactions that are involved in pyrite oxidation, and concluded that these data are consistent with the indirect microbial catalysis of sulfide mineral dissolution. The primary role of the bacteria is the oxidation of aqueous Fe^{2+} into Fe^{3+} under acidic conditions, according to the reaction:

$$Fe^{2+} + ¼\ O_2 + H^+ = Fe^{3+} + ½\ H_2O$$

The ferric iron then attacks the mineral surface, and pyrite is oxidized at a rate that is determined by the bacterial oxidation step:

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O = 15\ Fe^{2+} + SO_4^{2-} + 16\ H^+$$

Thus, although bacteria preferentially adhere to mineral surfaces in order to reduce the distance for the diffusion of iron between the mineral and the bacterium, there is no need to invoke an enzymatic reaction for sulfide mineral degradation (Nordstrom and Southam 1997).

The effects of cell attachment on mineral dissolution are the subject of continued interest. Edwards et al. (2001) experimented with reacting pyrite, marcasite, and arsenopyrite with the iron-oxidizing bacterium *Acidithiobacillus ferrooxidans*, the archaeon *Ferroplasma acidarmanus*, and abiotically with Fe^{3+}. Interestingly, in both the biotic and the abiotic experiments, bacillus-sized etch pits developed on pyrite, indicating that the attachment of cells is not necessary for the development of etch pits with characteristic shapes and sizes. However, attached cells of *F. acidarmanus* induced pitting on the more reactive surface of arsenopyrite (Fig. 17). Thus, the reactivity of the mineral may determine whether the surface features that develop during oxidative dissolution are related directly or indirectly to the presence of micro-organisms (see also Rosso and Vaughan 2006, in this volume).

Despite a century of research, the rate of microbially-assisted oxidation of sulfide minerals under natural conditions is still uncertain (Edwards et al. 2000). Laboratory experiments that involved the use of the same strain of *Thiobacillus ferrooxidans*, the same pyrite source, and the same experimental procedures resulted in consistent and reproducible rates, with the rate of microbial iron release being 34 times larger than the abiotic rate (Olson 1991). In contrast, field studies showed a wide variety in the degree of microbial enhancement of chemical processes. For example, in the AMD at the ore body of Iron Mountain, California, microbial

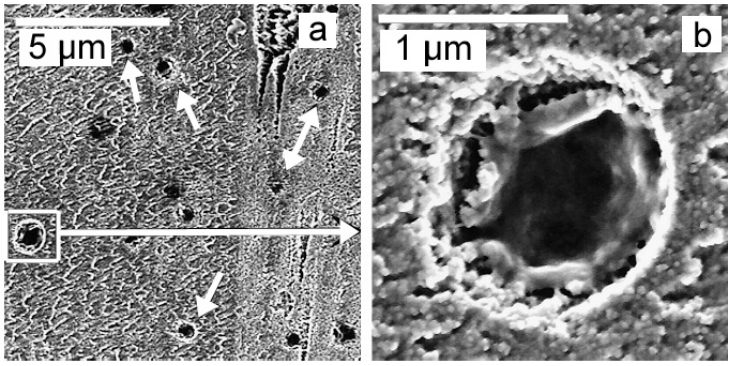

Figure 17. (a) Etch pits on the surface of arsenopyrite that was reacted with *F. acidarmanus*. (b) Magnified image of the area shown within the square in (a). The dehydrated cell in (b) is situated within a cell-sized and -shaped dissolution pit. Other cells are indicated with arrows in (a). [Used with permission of Blackwell Publishing, from Edwards et al. (2001), *FEMS Microbiology Ecology*, Vol. 34, Fig. 10, p. 203.]

enhancement of iron release was found to be much lower (~3× the rate of the abiotic reaction) than in laboratory studies (Edwards et al. 2000). Several poorly-constrained factors, including the surface area that is available for reaction, and the properties of pore fluids (that determine microbial population densities) affect the rates of microbial and abiotic contributions to AMD (Edwards et al. 2000).

In addition to the well-known case of sulfide mineral dissolution under oxidative and acidic conditions, sulfides can be dissolved at higher pH and anoxic conditions. Significant bacterially-assisted dissolution of copper from its sulfide minerals was observed in the slightly alkaline water of a tropical river, downstream of a large copper mine (Simpson et al. 2005b). Although the bacteria that were responsible for the copper release could not be isolated, indirect evidence suggested that they were lithoautotrophs. The fate of sulfide minerals and the cycling of metals in AMD are complicated further by the presence of SRP and iron-reducing bacteria in the anoxic portions of mine tailings (Fortin et al. 2002). The activity of these organisms results in the re-precipitation of sulfide minerals and a reduction in metal concentration in the solution (as discussed below in the section on bioremediation).

Microbial degradation of sulfides in marine environments

Vast quantities of sulfide minerals are present on and beneath the seafloor, in widely varying environments, including marine sediments, hydrothermal systems associated with mid-ocean ridges, and in the bare basaltic rocks on the flanks of mid-ocean ridges (Edwards et al. 2005). Wherever sulfides are in contact with the oxic seawater, the possibility of their dissolution arises. As for AMD generation, both chemical and biogenic processes are involved in the breakdown of sulfides in marine environments. However, there appear to be significant differences between the roles of bacteria in geologically distinct regions of the seafloor.

The pyrite and iron monosulfides that form by BIM within the anoxic sediments can be transported by bioturbation to the surface of the sediment, where they are oxidized chemically by O_2 (Thamdrup et al. 1994; Schippers and Jørgensen 2002). In the process, bacterial involvement may be limited to the oxidation of aqueous sulfur-bearing compounds, intermediates that result from the oxidation of pyrite, into sulfate (Kuenen et al. 1992). The bacterially-assisted oxidation of sulfide minerals under anoxic conditions was also considered by Schippers and Jørgensen (2002), who found that iron monosulfides could be oxidized by Fe^{2+}- or H_2S-oxidizing and NO_3^--reducing bacteria, but pyrite was not attacked by the same processes. Since many metals can be incorporated into iron sulfides, it is of environmental importance to trace the fate of these metals during their oxidation (Bertolin et al. 1995). Since iron monosulfides oxidize more readily than pyrite, they are prone to release incorporated metals (Holmes 1999). In general, there is no uniform behavior of pollutant metals. Whereas some metals remain in the particulate phase, others dissolve, depending on the particular environmental conditions (for a review see Schoonen 2004).

The potentially significant role of micro-organisms in the alteration of seafloor sulfide minerals at hydrothermal vents and in exposed basalt is just beginning to be addressed (as reviewed by Edwards et al. 2005). Hydrothermal metal sulfide deposits support communities of lithoautotrophic bacteria, among which sulfide- and iron- or manganese-oxidizing microbes promote the oxidative surface reaction of sulfide minerals. Verati et al. (1999) observed an external layer on black smoker chimneys that consisted of the oxidation products of sulfides and contained the imprints of bacterial cells. They concluded that bacterial sulfur and iron oxidation are responsible for the weathering of the sulfides. Schrenk et al. (2003) found diverse communities within hot, active black smoker chimney structures. Primarily archaea were found inside the chimneys, whereas in the external, cooler portions of the chimney walls bacteria dominated. In contrast, cold, inactive black smoker chimneys harbored only bacterial communities (Suzuki et al. 2004). Both iron- and sulfur-oxidizing lithoautotrophic bacteria

were cultured from extinct chimneys, indicating their likely role in the weathering of black smoker sulfides (Edwards et al. 2005).

In an *in situ* incubation study, several minerals (pyrite, marcasite, chalcopyrite, sphalerite, elemental sulfur) and a fragment of a natural black smoker chimney were left to react for two months in the vicinity of a seafloor hydrothermal system (Edwards et al. 2003). The surfaces of these minerals were colonized by bacteria, likely belonging to iron- and sulfur-oxidizing species. The colonization densities were found to correlate positively with the abiotic reactivity of the minerals, i.e., the more reactive was the mineral, the more cells colonized its surface. The only exception was elemental sulfur, which was both the most heavily colonized and the least reactive. The black smoker chimney fragment was even more heavily colonized and weathered than the individual minerals, suggesting that the weathering of natural, fine-grained sulfide structures is enhanced significantly by micro-organisms (Edwards et al. 2003).

Rock-hosted microbial communities on the seafloor are not restricted to hydrothermal chimney structures. Geographically vast regions exist on the flanks of mid-ocean ridges, which consist of unsedimented basalt and are potential targets for the colonization of lithoautotrophic micro-organisms. Iron-oxidizing bacteria were inferred to be present on the ocean basalt (Thorseth et al. 2001), and uncultured bacteria from deep-sea basalt were genetically similar to known sulfur- and iron-metabolizing bacteria (Lysnes et al. 2004).

Hydrothermal fluids mix with seawater in a shallow, sub-seafloor region, which is inferred to host a "deep biosphere" of endolithic microbial communities (Summit and Baross 2001). Such habitats are not yet accessible for direct sampling, but diffuse vents on ridge flanks are thought to offer a glimpse into the sub-seafloor biota (Edwards et al. 2005). Microbial populations in the diffuse vents were found to be distinct from those in the bottom seawater (Huber et al. 2003). The study of the interactions between microbes and minerals on and below the seafloor is a new field, which will likely bring interesting results concerning the bio-assisted precipitation and alteration of sulfide minerals.

PRACTICAL APPLICATIONS OF INTERACTIONS BETWEEN ORGANISMS AND SULFIDES

Biomimetic materials synthesis

The processes that are involved in biologically controlled mineralization provide valuable insights for materials chemists. "Bio-inspired materials chemistry" makes use of strategies learned from studies on biominerals, and has developed into a large field (Mann 2001). Various types of nanocrystals are synthesized using biomimetic approaches, including several sulfides. Here we mention a few typical examples for the concepts and strategies that are applied—for further reading we recommend the books by Mann (1997, 2001).

In BCM, crystals often precipitate in confined spaces such as phospholipid vesicles or ferritins (Table 1). Similar artificial vesicles can be used to create nanoscale reaction droplets. For example, ferritin is a spherical protein cage with an internal space about 8 nm in diameter that normally contains ferrihydrite (Mann 2001). The supramolecular structure of ferritin is remarkably stable, and the iron oxide core can be removed chemically without affecting the protein shell. Either empty ferritin cages can be used as confined reaction spaces, or the iron oxide core can be transformed chemically. The latter approach was used to produce amorphous FeS particles with controlled sizes ranging from 2 to 7 nm (Meldrum et al. 1991). Semiconducting CdS nanocrystals were synthesized both in reverse micelles and as the cores of artificial ferritin (Wong and Mann 1996). By attaching antibodies and antigens to the proteins, the ferritin cages can be linked together in solution, and a network of preformed, protein-coated inorganic nanoparticles can be engineered.

Functionalized organic structures can be used as epitaxial surfaces for templating the nucleation and growth of inorganic crystals. Oriented crystals of PbS were synthesized on self-assembled surfactant films (Langmuir monolayers) (Belman et al. 2004), while the ordered structures of bacterial S-layers proved to be an efficient template for the nucleation of ordered two-dimensional arrays of 5-nm CdS nanoparticles (Shenton et al. 1997).

Bioremediation

As iron sulfides precipitate in marine sediments, minor and trace amounts of various metals can be incorporated into the structures of iron monosulfides and pyrite. Schoonen's review (2004) contains an extensive compilation of observed metal concentrations in pyrite. Since many of the metal and metalloid impurities are toxic, it is of considerable interest to determine whether the host minerals are stable sinks for these metals. Iron sulfide formation under anoxic conditions in mine tailings and wetlands can be exploited for the immobilization of metal contaminants (Fortin and Beveridge 1997; Paktunc and Dave 2002). A wide range of metals has been co-precipitated with bacterially-produced iron sulfide (Watson and Ellwood 1994). Pesumably, greigite was one of the components of the precipitated sulfide, since the product could be divided into a weakly and a strongly magnetic fraction (Watson et al. 2000). The immobilization of heavy metals in magnetic iron sulfides offers the possibility of removing the contaminants by magnetic separation methods.

In the flooded ZnS mine that was described by Labrenz et al. (2000), coupled geochemical and microbial processes efficiently strip Zn from solutions containing <1 ppm Zn. The biofilm contains Zn in a concentration of about a million times that of the groundwater. As discussed by Moreau et al. (2004), some of the sedimentary sulfide deposits may have formed by similar BIM processes. Since metals such as As, Se, Cd, and Pb can be incorporated into or adsorbed on ZnS minerals, biomineralization may provide a suitable means to control the concentration of toxic metals in groundwater or wetlands. Such bioremediation strategies require that the minerals are relatively stable against dissolution. The solubilities of both sphalerite and wurtzite decrease with coarsening, since the growth of particles reduces the surface-to-volume ratio, decreasing reactivity with respect to oxidative dissolution (Moreau et al. 2004).

There are many possibilities for environmental applications of sulfide mineral precipitation by the mediation of bacteria. As discussed by Lovley (2003), bioremediation has been an empirical practice, but it could transform into a science thanks to new environmental genomic techniques that have become available. Experimental genomic and modelling techniques can be used to understand the physiologies of uncultured micro-organisms, and the resulting biological information, when combined with geochemical models, will be an invaluable tool for designing bioremediation strategies.

Bioleaching of metals

Whereas acidophilic, iron- and sulfur-oxidizing bacteria may be a curse when acid mine drainage is concerned, they are a blessing when used for the leaching of metals from their sulfide ores. Bacterial leaching of metals from low-grade ores is a well-established industrial technology that has been used for centuries (Rawlings and Silver 1995). Primarily mesophilic micro-organisms, such as *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, and *Leptospirillum ferrooxidans,* are applied (Hackl 1997). Historically, the targets of bacterial leaching have been sulfidic copper and refractory gold ores, but bioleaching practices are now used for the solubilization of a wide range of metals from their sulfide minerals (Nemati et al. 1998; Pina et al. 2005).

The use of bacteria in mineral processing may have additional advantageous side-effects. When *T. ferrooxidans* was added to a mixture of sulfide minerals during flotation, the cells adhered preferentially to pyrite and thus suppressed its floatability (Nagaoka et al. 1999). By

using the microbes, pyrite could be separated from chalcocite, molybdenite, millerite, and galena. A detailed discussion of bioleaching practices is beyond the scope of this chapter. The interested reader is referred to reviews by Brierley (1978) and Hackl (1997).

IRON SULFIDES AND THE ORIGIN OF LIFE

Any review of the role of sulfide minerals in biosystems would be incomplete without mentioning the hypotheses that implicate sulfides in the origins of life. However, this topic is much more complex than is possible to deal with within the scope of this chapter. Also, an entire issue of the magazine *Elements* (June 2005) was devoted to problems related to the geochemical origin of life. Therefore, here the results of research on the possible roles of sulfides in the emergence of life are discussed only in the most general terms. The reader is referred to reviews by Cody (2005) and Schoonen et al. (2004).

Several origin-of-life hypotheses assume that the first organisms were autotrophs rather than heterotrophs, i.e., that they used small inorganic molecules (such as CO_2, NH_3, H_2S, H_2O, PO_4^{3-}) for building their biomolecules. Since the conversion of the inorganic forms of biogenic elements (C, N, O, S, H, and P) requires energy or the surpassing of an activation barrier (Schoonen et al. 2004), a catalyst is necessary. Modern biocatalysts that promote the formation of organic molecules from small components include protein enzymes that contain clusters of transition metals (Fe, Ni, Co) and sulfur at their active sites (Beinert et al. 1997). The important roles of metal sulfide clusters in microbial biosynthesis inspired two distinct hypotheses by Wächtershäuser (1988, 1990) and Russell and Hall (1997), in both of which it was proposed that sulfide minerals could catalyze the production of the first biomolecules.

According to Wächtershäuser's theory (1988, 1990), the formation of pyrite could have provided the energy source for the first organism, reducing CO_2 in the process, resulting in organic molecules according to the reaction:

$$CO_2(aq) + FeS + H_2S \rightarrow HCOOH + FeS_2 + H_2O$$

The small organic molecules then presumably combined into the larger biomolecules that are necessary for life. Some of the critical points of this hypothesis have been tested both experimentally and theoretically. Iron sulfide minerals were found to promote organic reduction reactions (Blöchl et al. 1992; Kaschke et al. 1994), while Huber and Wächtershäuser (1997) reported that a (Ni,Fe)S compound enhanced reactions that were designed to emulate the carbonyl-inserting reaction in modern microbial enzymes that have key roles in inorganic carbon fixation. However, on the basis of thermodynamical considerations, Schoonen et al. (1999) showed convincingly that the FeS-H_2S/FeS_2 redox couple is unlikely to initiate the proposed prebiotic carbon fixation cycle. The key point of their study is that the reducing power of the FeS-H_2S/FeS_2 couple diminishes with increasing temperature, whereas the reduction of CO_2 and the formation of carboxylic acids require increasingly higher reducing power with temperature. Nevertheless, transition metal sulfides do act as catalysts for reactions that can form important organic molecules. Cody et al. (2001, 2004) showed that NiS and common minerals (including chalcopyrite, bornite, and chalcocite) have the capacity to convert simple organic molecules into carboxylic acids. These reactions appeared to be surface-catalyzed, since they resulted in a high degree of isomeric selectivity and the reaction yield was correlated with mineral surface area. In another interesting experiment, Bebié and Schoonen (1999) demonstrated that anionic phosphate and phosphorylated organic molecules interacted with the surface of pyrite, and suggested that phosphate could have been concentrated on metal sulfide minerals on a prebiotic Earth, promoting the selective concentration of organic molecules from aqueous solutions.

The origin-of-life hypothesis developed by Russell and Hall (1997) is based on a geochemical consideration of the conditions that may have prevailed on the young Earth.

Soon after the discovery of hot vents on the deep seafloor, it was suggested that they provided the only stable environment where life could have emerged (Corliss et al. 1981). Shock (1992) demonstrated the potential for the formation of various organic molecules when CO_2 and carbonates in seawater mix with hydrothermal solutions. Russell and Hall (1997) and Russell et al. (1998) argued that life started at a redox and pH front where the acidic and warm (~90 °C) water of the Hadean ocean merged with reduced, alkaline, bisulfide-bearing, hot (~150 °C) water emitted at diffuse submarine vents. Under these conditions, colloidal FeS precipitated spontaneously in the form of thin films. According to Russell and Hall (1997), such FeS films could have formed bubbles, creating semipermeable membranes that separated the two fluids with different chemistries. The assimilation of CO_2 was catalyzed by the nickeliferous mackinawite, and amino acids and organic sulfide polymers could be synthesized within the FeS compartments. As the concentrations of carboxylic acids and organic polymers inside the bubbles increased, these organic molecules organized themselves either as coatings on the interiors of the FeS membrane or as micelles, and gradually took over the role of separating the two contrasting fluids. Thus, the first cell-like structures emerged, in which the generation of RNA and DNA may have become possible. The hypotheses by both Wächtershäuser (1988) and Russell and Hall (1997) are great intellectual achievements, and will likely continue to motivate much interesting experimental research in the future.

CONCLUDING THOUGHTS

Sulfide minerals were likely present at the beginning of life, and may even have catalyzed the first metabolic reactions at deep-sea hydrothermal vents. Interactions between organisms and sulfide minerals were important throughout most of Earth's history. Many types of sulfur-metabolizing microbes are rooted deeply in the Tree of Life, including sulfate reducers (Canfield and Raiswell 1999). A large radiation of sulfate reducers accompanied the general radiation of bacterial life. The formation of sulfide minerals by BIM must be at least as old as the first geochemical evidence for sulfate reduction, which is found between 2.7 and 2.5 Ga. Based on the available phylogenies, sulfate reducers may have appeared even earlier, by 3.4 Ga (Canfield and Raiswell 1999). Studies of the isotopic compositions of sedimentary pyrite led to the conclusion that the bottom waters of the oceans became sulfidic around ~1.8 Ga, when increased atmospheric oxygen levels enhanced terrestrial sulfide weathering, supplying sulfate to the oceans and increasing the rate of sulfate reduction (Poulton et al. 2004). Sulfidic conditions may have persisted until between 0.8 and 0.58 Ga ago, when a second major rise in oxygen concentration took place. At this time, the widespread oxidation of marine surface sediments promoted an evolutionary radiation of sulfide oxidizing bacteria (Canfield and Raiswell 1999), setting the stage for interactions between microbes and sulfide minerals as we know them today.

The geological history of sulfide mineral production by BCM is not known with any certainty. Sulfide magnetofossils from magnetotactic bacteria were identified tentatively from Miocene rocks (Pósfai et al. 2001) and soil (Stanjek et al. 1994). Since magnetite magnetofossils were described from Archaean rocks, Kirschvink and Hagadorn (2000) hypothesized that all BCM processes originated from magnetite biomineralization by magnetotactic bacteria.

Since biologically controlled or mediated mineralization produces nanocrystalline sulfide particles, their physical, structural, and chemical characterization will likely remain an exciting and challenging field of mineralogical research. Concerning the iron sulfides that are produced by magnetotactic bacteria, the key problems to be addressed are related to biological control over crystal nucleation and growth. It has still not been established whether greigite crystals in mangnetotactic bacteria are surrounded by a magnetosome membrane, as are the crystals in magnetite-producers, or whether they are deposited in a less controlled manner,

perhaps as a consequence of the sulfate-reducing metabolism of the host cell. The mineral phases of the initially formed precipitates, as well as their conversions, also deserve further study. In addition, the possibility exists that additional sulfides with biological functions will be discovered. Given the geological and environmental importance of the bacterially-assisted formation and dissolution of sulfide minerals, interactions between microbes and sulfides will continue to be the subject of intensive research.

ACKNOWLEDGMENTS

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