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REVIEW ARTICLE

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# Synthesis of the bacterial magnetosome: the making of a magnetic personality

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**Summary** Magnetotactic bacteria synthesize intracellular, enveloped, single magnetic domain crystals of magnetite ($Fe_3O_4$, $Fe^{2+}Fe_2^{3+}O_4$) and/or greigite ($Fe_3S_4$) called magnetosomes. The magnetosomes contain well-ordered crystals that have narrow size distributions and consistent species- and/or strain-specific morphologies. These characteristics are features of a process called biologically-controlled mineralization in which an organism exerts a great degree of crystallochemical control over the nucleation and growth of the mineral particle. Because of these features, the mineral particles have been used as biomarkers although not without controversy. These unique structures impart a permanent magnetic dipole moment to the cell causing it to align and swim along geomagnetic field lines, a behavior known as magnetotaxis. The apparent biological advantage of magnetotaxis is that it aids cells in more efficiently locating and maintaining position in vertical chemical gradients common in many natural aquatic environments.

**Key words** Magnetotactic bacteria · Magnetotaxis · Magnetite · Greigite · Biomineralization

The serendipitous discovery of magnetotactic bacteria occurred 25 years ago when Richard P. Blakemore, then a graduate student at the University of Massachusetts at Amherst, microscopically observed large populations of coccoid bacteria in mud migrating unyieldingly in one direction until small magnets were brought in the vicinity of the microscope [11]. It quickly became clear to Blakemore that the swimming direction of the cocci was strongly influenced by magnetic fields and that, in general, cells swam towards the south end of a bar magnet and away from the north end. Using magnetic manipulations of collected cells and electron microscopy, this amazing phenomenon, called magnetotaxis, was found to be due to the presence of unique intracellular, electron-dense, iron-rich structures called magnetosomes [1]. Despite the fact that 25 years have elapsed since their discovery, very few species of magnetotactic bacteria have been isolated and grown in pure culture. In addition, partially because of the fastidiousness of the strains, little is known about how these microbes biomineralize their magnetic mineral inclusions at the biochemical/chemical and molecular levels.

The magnetotactic bacteria are a heterogeneous group of motile, mainly aquatic prokaryotes that exhibit a number of cellular morphologies including coccoid, rod-shaped, vibrioid and spirilloid [7, 12]. Extremely large cells and even a multicellular form are known to exist [57, 63]. Despite this morphological diversity, the magnetotactic bacteria share several important features [7], all: (i) are Gram-negative members of the Domain Bacteria (this does not preclude the possibility of magnetotactic Archaea, although none has ever been discovered); (ii) are motile by means of flagella (magnetotactic gliding bacteria or nonmotile bacteria that produce magnetosomes could also exist though they would be easily overlooked during selection); (iii) exhibit a negative tactic and/or growth response to atmospheric levels of oxygen; and (iv) possess a number of magnetosomes.

Phylogenetic analyses, based on the sequence of the 16S rRNA, show that almost all magnetite-producing magnetotactic bacteria belong to the α-subdivision of the Proteobacteria [17, 63]. Exceptions include a cultured, magnetite-producing, sulfate-reducing magnetotactic strain, RS-1 [58], which belongs to the δ-subgroup of the Proteobacteria [30], and a large, uncultured, rod-shaped, magnetite-producing bacterium, *Magnetobacterium bavaricum*, that is phylogenetically associated not with the Proteobacteria but with the Nitrospira phylum in the Domain Bacteria [63]. The 16S rRNA of only one greigite-producing magnetotactic bacterium, an unusual multicellular form, has been sequenced and the organism is associated with the sulfate-reducing bacteria in the δ-subdivision of the Proteobacteria [17]. Because the different subdivisions of the Proteobacteria are considered to be coherent, distinct evolutionary lines of descent [69], and at least one magnetite-producing species is phylogenetically linked to a major grouping in the Domain Bacteria other than the Proteobacteria, it seems likely that magnetotaxis as a trait and magnetosome synthesis have evolved several times in the past.

Nonetheless, the term "magnetotactic bacteria" lacks taxonomic significance and these microorganisms should be regarded as a diverse morphological and metabolic group of prokaryotes that share the trait of magnetotaxis [7, 12].

Magnetotactic bacteria are cosmopolitan in distribution and ubiquitous in aquatic habitats [7, 12] with few exceptions. They have not been found in significant numbers in well aerated or in acidic aquatic environments such as mine drainages. On a more local basis, the highest numbers of these organisms are found at or just below the oxic-anoxic boundary or transition zone (OATZ) also referred to as the microaerobic zone or the redoxocline. In many freshwater habitats, the OATZ is located at the sediment-water interface or just below it. However, in some brackish-to-marine systems, the OATZ is permanently or seasonally located in the water column due to the upward diffusion of hydrogen sulfide, produced by sulfate-reducing bacteria in the anaerobic zone and sediment [7]. In some cases, strong pycnoclines and other physical factors, probably including the microorganisms themselves, stabilize the vertical chemical gradients and the resulting OATZ. The OATZ occurs in the water columns of the Pettaquamscutt Estuary (Narragansett Bay, RI, USA) [3, 19] and Salt Pond (Woods Hole, MA, USA) [7] for much of the year and has been studied in some detail. Many types of magnetotactic bacteria are found at both sites. Generally, the magnetite-producing magnetotactic bacteria prefer the OATZ proper and behave as oxygen-respiring microaerophiles. Support for this also comes from the two strains of magnetite-producing magnetotactic bacteria isolated from the OATZ of the Pettaquamscutt: a vibrio, strain MV-2 [17, 48], and a coccus, strain MC-1 [17, 22, 47]. Both strains grow as microaerophiles although strain MV-2 also grows anaerobically with nitrous oxide ($N_2O$) as a terminal electron acceptor. The greigite-producers are mainly located in the anaerobic sulfidic waters below the OATZ and are likely anaerobes [3, 7], although greigite-producing magnetotactic bacteria have not yet been isolated and grown in pure culture.

## The bacterial magnetosome

Magnetosomes, defined as intracellular, single-magnetic-domain crystals of a magnetic iron mineral, either the iron oxide magnetite or the iron sulfide greigite, enveloped by a membrane or membrane-like structure [1, 26], are the signature feature of the magnetotactic bacteria and are responsible for their behavior in magnetic fields. Although most magnetotactic bacteria produce only one mineral type, a rod-shaped magnetotactic bacterium from the Pettaquamscutt Estuary contains both magnetite and greigite [3] and recently, non-magnetic iron sulfides together with greigite have been observed in some organisms [55, 56].

The iron sulfide-type magnetosomes contain particles of greigite [29, 41] or a mixture of greigite and some non-magnetic greigite precursors including mackinawite (tetragonal FeS) and possibly, sphalerite-type cubic FeS [55, 56]. Based on transmission electron microscopy (TEM), electron diffraction and known iron sulfide chemistry, the reaction scheme for greigite formation in magnetosomes appears to be:

cubic FeS $\rightarrow$ mackinawite (tetrag. FeS) $\rightarrow$ greigite ($Fe_3S_4$) [55, 56].

The de novo synthesis of non-magnetic, crystalline, iron sulfide precursors aligned along the magnetosome chain is significant because it indicates that chain formation is not necessarily a magnetism-related process [31] and that biomineralization and chain formation are separately controlled by the bacterium [3]. Reports of non-magnetic pyrite [41] and ferrimagnetic pyrrhotite ($Fe_7S_8$) [21] in magnetotactic bacteria likely represent misidentifications of greigite and other iron sulfide phases.

The morphology of the crystalline mineral phase of the magnetosome varies but is generally consistent within cells of a single bacterial species or strain [6, 7]. Even the bacterium that produces magnetite and greigite produces only one morphological form of each mineral [3]. Three general morphologies of magnetite and greigite particles have been observed in magnetotactic bacteria using TEM [6]. They include: (i) roughly cuboidal [1]; (ii) parallelepipedal (rectangular in the horizontal plane of projection) [4, 66]; and (iii) tooth-, bullet- or arrowhead-shaped (anisotropic) [38, 39]. Examples of these morphologies are shown in Fig. 1. One exception to this rule occurs in the greigite particles of an unusual many-celled "microcolony" of 20 or so similar prokaryotic cells, arranged roughly in a sphere, that is motile as an entire unit but not as separate cells [41, 57]. This organism produces mostly pleomorphic particles that lack a consistent crystalline morphology although cubo-octahedral and tooth-shaped crystals are occasionally present (Fig. 1C) [41, 56]. This particle pleomorphism is not understood but may indicate (a) less controlled biomineralization process(es) in this microorganism.

The rod-shaped bacterium that synthesizes both minerals contains different sizes of arrowhead-shaped crystals of magnetite and rectangular prismatic crystals of greigite co-organized within the same chains of magnetosomes [3]. In cells of this uncultured organism, the magnetite and greigite crystals are positioned with their long axes oriented along the chain direction. This suggests that the magnetosome membranes surrounding the magnetite and greigite particles contain different nucleation templates and that there are differences in magnetosome vesicle biosynthesis. Thus, it is possible that two separate sets of genes control the biomineralization of magnetite and greigite in this organism. In addition, environmental variables such as local molecular oxygen and/or hydrogen sulfide concentrations and/or redox conditions might regulate the type of biomineralization by cells of this organism. Cells from the more oxidized regions of the OATZ contain more or exclusively arrowhead-shaped magnetite particles while cells collected from below the OATZ in the anaerobic, sulfidic zone contain more or exclusively greigite particles [3].

Metal compositional impurities in magnetosomes are unusual. The magnetite-producers in pure culture produce stoichiometric magnetite even when grown in the presence of relatively high amounts of other transition metal ions. Several exceptions exist,

however, in magnetotactic bacteria from environmental sources. Trace amounts of titanium were found in magnetite particles of a freshwater magnetotactic coccus from a wastewater treatment pond [65]. Significant amounts of copper, ranging from about 0.1 to 10 atomic % relative to iron, were found in the iron sulfide particles of the many-celled, magnetotactic microorganism described earlier, as well as in some rod-shaped bacteria [5, 56]. The presence of copper appeared to be dependent upon where the organisms were collected and therefore, likely upon copper availability [5, 56].

High resolution TEM studies have revealed that the magnetite particles within magnetosomes are of high structural perfection and have been used to determine their idealized morphologies [36–39, 42, 47, 48]. These studies have shown the cuboidal particles to be truncated cubo-octahedra and the parallelepipedal particles to be either truncated hexahedral or octahedral prisms. The cubo-octahedral crystal morphology preserves the symmetry of the face-centered cubic spinel structure and is considered an equilibrium growth form of magnetite that is commonly found in chemically-produced magnetite particles. The hexa- and octahedral prismatic particles represent departures from this equilibrium form, presumably due to the acceleration or deceleration of mineral growth of certain crystal faces [40]. The synthesis of the tooth-, bullet- and arrowhead-shaped crystal appears to be more complex than that of the other forms [38, 39] and morphological studies suggest that their growth occurs in two stages. The first, during which the length and width develop concurrently, is the formation of a well-ordered, isotropic, cubo-octohedral single-magnetic-domain crystal while the second involves anisotropic growth of the particle along a preferred (pointed end) direction.

The magnetite and greigite crystals found in the magnetotactic bacteria fall within a narrow size range, 35 to 120 nm [6], where the particles are uniformly magnetized, permanent single-magnetic-domains. Smaller particles, termed superparamagnetic, are not permanently magnetic at ambient temperature and would not be useful to the cell. Domain walls would form in larger particles, forming a multi-domain crystal, reducing the magnetic remanence of the particle. By synthesizing single-magnetic-domains, cells maximize the magnetic remanence of the individual particles [7].

## The function of magnetotaxis

In most magnetotactic bacteria, the magnetosomes are arranged in one or more chains in which the magnetic interactions of the single particles cause their magnetic dipole moments to orient parallel to each other along the chain length. In this arrangement, the total magnetic dipole moment of the cell is the sum of the dipole moments of the individual particles and the chain behaves as a single magnetic dipole [7]. This cooperative effect between particles in the chain has now been directly shown using electron holography in the TEM, which outlines the magnetic field lines

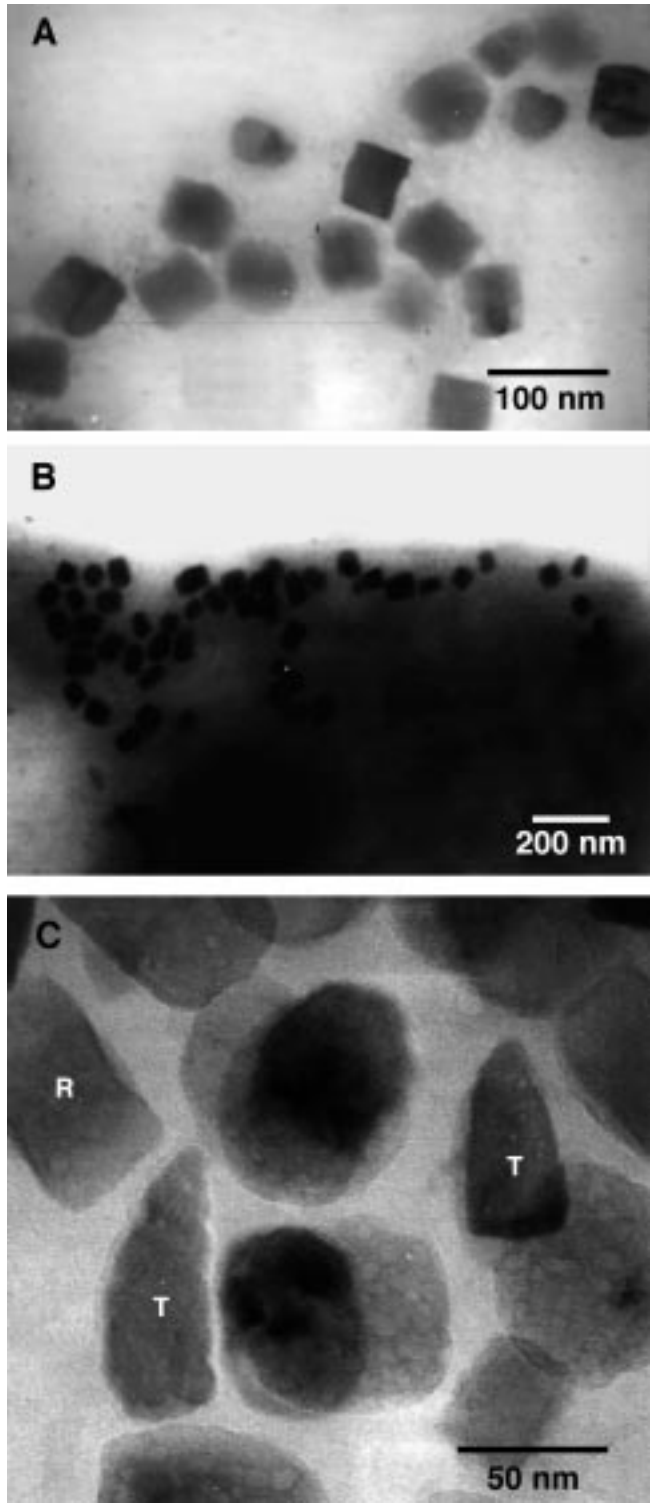


**Fig. 1** Morphologies of greigite ($Fe_3S_4$) crystals in magnetosomes of magnetotactic bacteria. (A) STEM micrograph of cubo-octahedra within an uncultured rod-shaped bacterium collected from a salt marsh pool; (B) STEM of hexagonal prisms within an uncultured rod-shaped bacterium from the same site; (C) TEM of tooth-shaped (T) and rectangular (R) greigite crystals in the many-celled magnetotactic bacterium described in the text. Magnetite ($Fe_3O_4$) crystals show the same morphologies when viewed with electron microscopy in the horizontal plane (Fig. 1C courtesy of M. Pósfai and P.R. Buseck)

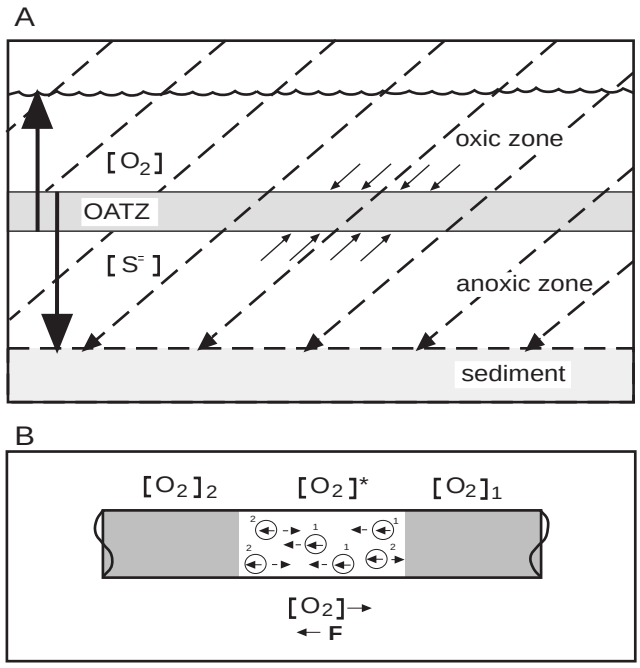


**Fig. 2** (A) Diagram of the oxic-anoxic transition zone (OATZ) in the water column of a chemically-stratified semi-anaerobic basin (e.g. Salt Pond, Woods Hole, MA, USA) containing inverse concentration gradients of oxygen ($[O_2]$), diffusing from the surface, and sulfide ($[S^{2-}]$), produced by sulfate-reducing bacteria in the anaerobic zone. Magnetite-producing magnetotactic bacteria are normally present at the OATZ while the greigite-producers are found in the anaerobic zone below the OATZ where sulfide is present. How these organisms find and maintain an optimal position in these vertical concentration gradients is not known. Axial magnetotactic species (e.g. spirilla) align along the inclined geomagnetic field lines (dashed lines) and swim up and down relying on a temporal sensory mechanism of aerotaxis to find and maintain position at the OATZ. Polarly magnetotactic species (e.g. cocci) appear to use a novel two-state aerotactic sensory mechanism to find and maintain optimal position at the OATZ. When cells of this type are in the oxic zone above the OATZ, they are in suboptimal conditions ($[O_2]$ too high) and swim downward (small arrows above OATZ). When they get below the OATZ, they are again in suboptimal conditions ($[O_2]$ too low), reverse the direction of their flagella motors and swim upward (small arrows below the OATZ) without turning around. (B) Depiction of the two-state aerotactic sensory mechanism in the magnetotactic cocci where $[O_2]$ appears to dictate the rotation of the flagellar motors. $[O_2]$ increases to the right, F is the direction of the magnetic field and $[O_2]$* represents an optimal $[O_2]$ for the cell. Under oxic conditions ($[O_2]_1$, $[O_2]$ too high), cells are or go into state 1 and swim persistently downward (northward in the northern hemisphere) parallel to F until they encounter a low oxygen threshold ($[O_2]_2$, $[O_2]$ too low) which switches the cell into state 2. In state 2, cells swim antiparallel to F until they reach a high oxygen threshold ($[O_2]_1$) which causes them to switch back to state 1 again. In both cases, the magnetic dipole of the cell is aligned along F

of force [20]. The cell has therefore maximized its magnetic dipole moment, which is generally large enough so that its interaction with the Earth's geomagnetic field overcomes the thermal forces tending to randomize the cell's orientation in its aqueous surroundings [23]. Magnetotaxis results from the passive alignment of the cell along geomagnetic field lines while it swims. Cells, live or dead, align along magnetic field lines, behave like miniature compass needles and are neither attracted nor pulled toward either geomagnetic pole. Dead cells, however, do not swim and cannot be magnetotactic.

Most non-spirillar magnetotactic bacteria have a magnetic polarity; north- or south-seeking. In wet mounts of these types of cells (e.g. magnetotactic cocci), more than 99% of the cells swim persistently in one direction and it was this observation that led to the discovery of magnetotaxis in bacteria [11]. In contrast, magnetotactic spirilla grown in liquid culture show no preference in their swimming direction and equal numbers of cells swim in either direction in wet mounts [22]. The vertical component of the inclined geomagnetic field appears to select for a polarity in the former organisms in each hemisphere by favoring those cells whose polarity leads them down towards sediments, away from toxic concentrations of oxygen in surface waters. North- (and downward-) seeking magnetotactic bacteria predominate in the northern hemisphere while south-seeking cells predominate in the southern hemisphere [13]. At the equator, where the vertical component of the geomagnetic field is zero and neither polarity is selected for, equal numbers of both polarities exist [24]. The presence of "polar" magnetotactic bacteria in "plates" at the OATZ in the water columns of chemically-stratified aquatic systems [3, 7, 22], and the fact that cultured magnetotactic cocci form microaerophilic bands of cells at some distance from the meniscus of the growth medium [22], are not consistent with this model of magnetotaxis. Otherwise north-seeking magnetotactic bacteria in the northern hemisphere would be found in the sediments or at the bottom of culture tubes. How do both cell types find and maintain position at the OATZ?

Magnetotactic bacteria propel themselves forward in their aqueous surroundings by rotating their flagella, as do most other free-swimming bacteria. Unlike cells of *Escherichia coli* and other chemotactic bacteria that exhibit a characteristic "run and tumble" motility, most magnetotactic bacteria move only bidirectionally, backwards and forwards, and do not change direction by tumbling [22]. The magnetotactic spirilla, which have a polar flagellum at each end of the cell, align along and swim up and down the inclined geomagnetic field lines while using aerotaxis to find their optimal oxygen concentration at the OATZ (Fig. 2) [22]. The microaerophilic magnetotactic cocci, which possess two bundles of flagella on one side of the cell, also swim in both directions but use magnetotaxis differently. They swim forward (north-seeking) in the northern hemisphere under oxic conditions explaining their persistent north-seeking behavior in wet mounts of oxic aqueous medium. When the oxygen concentration becomes low enough (suboptimal conditions), cells reverse direction (by reversing the direction of their flagellar rotation), without turning around, using a novel aerotactic sensory mechanism that functions as a two-way switch (Fig. 2) rather than the well-recognized aerotactic sensory mechanism used by other bacteria (e.g. *E. coli*, *Magnetospirillum magnetotacticum*). When cells go above the OATZ and the oxygen concentration becomes too high (again suboptimal conditions), they reverse direction again [22].

On the basis of the observations described above, two types of magneto-aerotaxis, called axial and polar, have been distinguished for the different mechanisms used by the magnetotactic spirilla and the magnetotactic cocci, respectively [22]. In the magnetotactic spirilla, the geomagnetic field provides an axis, not a direction, for motility along the oxygen gradient. The geomagnetic field provides both an axis and a direction of motility for the magnetotactic cocci. Both mechanisms involve the passive orientation of the cellular magnetic dipole in the geomagnetic field. Both cell types use magnetotaxis in conjunction with aerotaxis to find and maintain optimal position in a vertical oxygen gradients. Magnetotaxis is particularly advantageous to microorganisms in vertical concentration gradients because it increases the efficiency of finding and maintaining an optimal position relative to the gradient by reducing a three-dimensional search problem to a one-dimensional search problem [22]. Magnetotaxis may also interact with other forms of chemotaxis involving molecules or ions other than oxygen, such as sulfide, or with redox- or phototaxis in bacteria that inhabit the anaerobic zone (e.g. greigite-producers) in chemically-stratified waters and sediments.

Cellular magnetotaxis is a direct consequence of the cell possessing magnetosomes. Bacteria cannot think and can only react to a stimulus and therefore did not originally synthesize magnetosomes for magnetotaxis (a teleological argument). In addition, many obligately microaerophilic bacteria find and maintain position at the OATZ without magnetosomes, cultured magnetite-producing magnetotactic bacteria form microaerophilic bands in the absence of a magnetic field and some greigite-producing magnetotactic bacteria produce gas vacuoles presumably for buoyancy (Fig. 3).

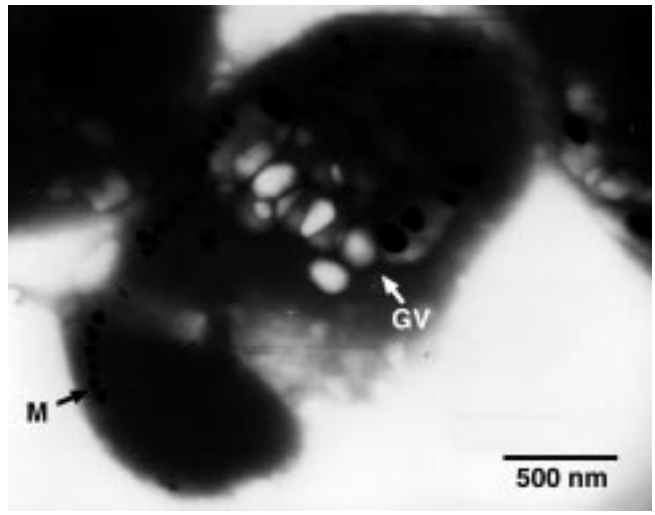


**Fig. 3** Scanning-transmission electron micrograph of a rod-shaped bacterium that presents greigite-containing magnetosomes (M) and gas vacuoles (GV). This organism was collected from a salt marsh pool

## Microbial biomineralization processes in magnetite and greigite formation

Magnetosomes contain well-ordered crystals with narrow size distributions and specific particle morphologies. These characteristics are indicative of a direct mechanism of mineralization termed "biologically-controlled mineralization" (BCM) [7], in which the organisms appear to regulate the biomineralization process to a high degree, exerting a great degree of crystallochemical control over the nucleation and growth of the mineral particles. This, in turn, suggests that BCM processes are under specific metabolic and genetic control.

Magnetite and greigite can also be formed by a second, indirect means of mineral formation called "biologically-induced mineralization" (BIM) [34]. In BIM, biomineralization is not controlled by the organism and occurs indirectly as a result of metabolic activities of the organism and subsequent chemical reactions. In most cases, the organisms secrete and/or produce (a) metabolic product(s) that react(s) with a specific ion or compound in the environment resulting in the production of extracellular mineral particles that are an unintended byproduct of metabolic activities. BIM-type particles are formed extracellularly, are poorly crystallized, have a broad size distribution and no defined morphology. In addition, the lack of control over biomineralization in BIM can result in decreased mineral specificity and/or the inclusion of impurities in the particles. Mineral particles produced by BIM share the same crystallochemical features as those produced chemically in the absence of bacteria [35]. Therefore, particles formed by BIM are generally indistinguishable from those particles produced non-biogenically in inorganic chemical reactions under similar conditions. The implication in BIM is that minerals nucleate in solution or form from poorly crystallized mineral species already present [7].

Magnetite is formed through BIM by some dissimilatory-iron reducing bacteria that respire with Fe(III) as amorphous Fe(III) oxyhydroxide [33] under anaerobic conditions. Fe(II), produced by the cells, subsequently reacts with excess Fe(III) oxyhydroxide in the environment to form magnetite. Magnetite particles formed by these microorganisms are (i) extracellular, (ii) irregular in shape with a relatively broad size distribution and (iii) poorly crystallized [62]. Although magnetite formation by BIM has only been shown to occur in cultures of *Shewanella putrefaciens* and *Geobacter metallireducens* ($\gamma$-, and $\delta$-subgroups, respectively, Proteobacteria) [32, 33], it is likely that magnetite can be produced by any Fe(III)-reducing bacterium under suitable environmental conditions.

Some sulfate-reducing bacteria produce particles of greigite using BIM processes. In this case, sulfate-reducing bacteria respire with sulfate anaerobically, releasing hydrogen sulfide. Sulfide ions react with excess iron present in the growth medium forming magnetic particles of greigite and pyrrhotite as well as of a number of other non-magnetic iron sulfides including mackinawite, pyrite (cubic $FeS_2$) and marcasite (orthorhombic $FeS_2$) [7]. The mineral species formed in these bacterially-

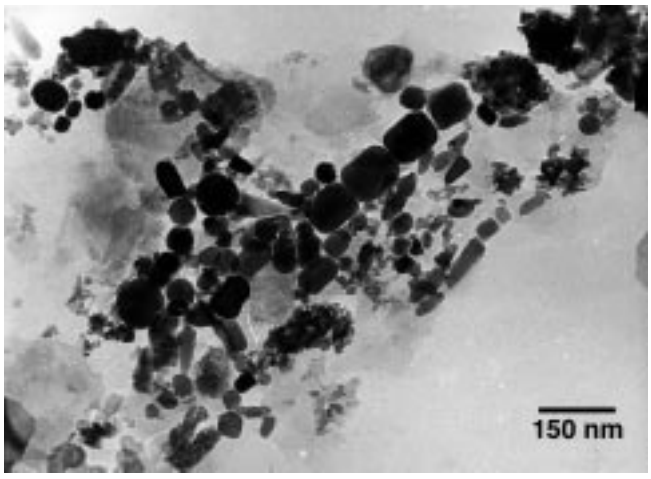


**Fig. 4** Transmission electron micrograph of a magnetic separate from surface sediments collected from the Irish Sea. Note the presence of parallelepipedal, cubo-octahedral and tooth-shaped forms of magnetite that are presumably "magnetofossils" left from magnetotactic bacteria (Courtesy of Z. Gibbs)

catalyzed reactions are dependent upon the pH of the growth medium, the incubation time and temperature, the $E_h$, the presence of specific oxidizing and reducing agents and the type of iron source in the growth medium. Microorganisms modify or contribute to many of these factors such as pH, $E_h$, etc. Most studies of iron sulfides produced through BIM by sulfate-reducing bacteria involve the motile Gram-negative bacterium *Desulfovibrio desulfuricans* (*d*-subgroup of the Proteobacteria, Domain Bacteria), but because all sulfate-reducing bacteria release sulfide ions when respiring with sulfate, it is likely that all are capable of producing iron sulfide minerals through BIM under appropriate environmental conditions. Although none of the iron sulfides produced by the sulfate-reducing bacteria have been examined in any detail using modern electron microscopy techniques, it is likely that they have similar characteristics to those of BIM magnetite and cannot be distinguished from inorganically-produced forms.

The ability to distinguish between the various types of bacterially-produced (BCM vs BIM) as well as inorganically-produced magnetite and greigite has great environmental and paleobiogical significance. When magnetotactic bacterial cells die and lyse, their magnetosomes can be deposited as "magnetofossils" into the sediments, where they contribute to the sedimentary paleomagnetic and mineral magnetic records [7, 16]. The narrow single magnetic domain size range and apparently unique crystal morphologies provide a means of identifying magnetosome mineral particles using electron microscopy of magnetic extracts from sediments.

Magnetite particles presumably derived from magnetotactic bacteria have been separated from many recent freshwater and marine sediments (Fig. 4) [7, 16] as well as from ancient sediments approximately two billion years old [16]. Moreover, magnetite particles resembling those from magnetotactic bacteria have been found in the Martian meteorite ALH84001 collected in Antarctica and interpreted as partial evidence for ancient life on Mars [46]. These crystals range from about 10 to 100 nm, are in the superparamagnetic and single-magnetic-domain size range, and are cuboid, teardrop and irregular in shape. Pyrrhotite and possibly greigite particles, about 100 nm, were also identified. The iron sulfide particles vary in size and shape. Both the magnetite and the iron sulfide particles are embedded in a fine-grained carbonate matrix on the rim of carbonate inclusions within the meteorite. These findings have raised serious debate on the interpretation and use of such particles as biomarkers both on Earth and in extraterrestrial materials. In a recent study [18], inorganically-produced and biogenic magnetite crystals from several strains of magnetotactic bacteria were examined by electron microscopy. Crystal defects such as twinning are relatively common in both types of magnetite and not useful as a distinguishing character. Statistical analysis of the sizes and shapes might provide robust criteria for discerning biogenic and nonbiogenic magnetite crystals although more studies involving crystals formed by cells in nature (versus those synthesized by cultured cells) must be performed [18].

## Physiology of magnetotactic bacteria and links to magnetosome synthesis

Only a handful of magnetotactic bacterial species have been grown in pure culture. Most of these are magnetotactic freshwater spirilla that synthesize cubo-octahedral particles of magnetite. Although magnetite synthesis has not yet been linked to the physiology of a magnetotactic bacterium, it is important to understand the physiology of these bacteria and the conditions under which they synthesize magnetosomes in order to find this link. One point is clear, however; magnetite is formed by physiologically diverse magnetotactic bacteria under aerobic and anaerobic conditions.

The first magnetotactic bacterium to be isolated and grown in pure culture was *Magnetospirillum* (formerly *Aquaspirillum*; [59]) *magnetotacticum* strain MS-1 [14]. This species, isolated from a freshwater swamp, is the most studied magnetotactic bacterium. Cells are helical, possess an unsheathed polar flagellum at each end of the cell and synthesize cubo-octahedral crystals of magnetite. This organism is obligately respiratory and grows chemoorganoheterotrophically using organic acids as a source of energy and carbon. Although this strain uses nitrate as a terminal electron acceptor during denitrification, cells still require a small amount of molecular dioxygen for growth and are therefore obligate microaerophiles [2]. Cells produce more magnetite when grown with nitrate than with oxygen as a terminal electron acceptor, yet molecular oxygen must still be present for magnetite synthesis, with the optimal concentration for maximum magnetite yields being 1% (v/v) oxygen in the headspace of cultures and concentrations greater than 5% being inhibitory [15]. In an effort to understand the relationship between nitrate and oxygen utilization and magnetite synthesis, Fukumori and co-workers examined electron transport and cytochromes in *M. magne-*

*totacticum*. Tamegai et al. [65] reported a novel "cytochrome $a_1$-like" hemoprotein present in greater amounts in magnetic cells than nonmagnetic cells. They did not find a true cytochrome $a_1$ that was once considered to be one of the terminal oxidases, along with an *o*-type cytochrome, in *M. magnetotacticum* [52]. A new *ccb*-type cytochrome *c* oxidase [64] and a cytochrome $cd_1$-type nitrite reductase [68] were isolated and purified from *M. magnetotacticum*. The latter protein is of particular interest because it shows Fe(II):nitrite oxidoreductase activity that may be linked to the oxidation of Fe(II) in the cell and thus to magnetite synthesis [68].

Cells of *M. magnetotacticum* reduce Fe(III) and translocate protons when Fe(III) is provided anaerobically [61] suggesting that cells conserve energy during the reduction of Fe(III). This reduction may be linked to growth [27] but has never been shown to directly support an energy-consuming process (e.g. amino acid uptake).

Matsunaga et al. [44] isolated a magnetotactic spirillum physically similar to *M. magnetotacticum*, designated *Magnetospirillum* strain AMB-1, which is much more oxygen tolerant than other magnetotactic species and forms colonies on the surface of agar plates. This species, like *M. magnetotacticum*, synthesizes cubo-octahedral crystals of magnetite, is obligately respiratory, has a chemoorganoheterotrophic mode of nutrition and uses organic acids as sources of energy and carbon. Cells, like those of *M. magnetotacticum*, form more magnetosomes when grown with nitrate. However, unlike *M. magnetotacticum*, this strain grows under anaerobic conditions with nitrate and synthesizes magnetite without molecular oxygen [45]. Growth and inhibitor studies [44, 45] show that *Magnetospirillum* AMB-1 uses nitrate as a terminal electron acceptor although the products of nitrate reduction were not reported. Another similar microaerophilic, freshwater magnetotactic spirillum, *M. gryphiswaldense* [59], also produces cubo-octahedral crystals of magnetite. How nitrate affects magnetite synthesis and growth of *M. gryphiswaldense* is not known.

A marine magnetotactic vibrio, strain MV-1, was isolated from a sulfide-rich salt marsh pool [4]. Cells possess a single polar unsheathed flagellum and synthesize hexahedral prismatic crystals of magnetite when growing either microaerobically or anaerobically with nitrous oxide ($N_2O$) as the terminal electron acceptor. Cells appear to produce more magnetite under anaerobic conditions than under microaerobic conditions [4]. Cells of this nutritionally versatile species grow chemoorganoheterotrophically with organic or amino acids as carbon and energy sources, chemolithoautotrophically with thiosulfate and sulfide as energy sources, and carbon dioxide as the sole carbon source. Cells use the Calvin-Benson cycle for carbon dioxide fixation as cell-free extracts from thiosulfate-grown cells show ribulose bisphosphate carboxylase/oxygenase activity. A virtually identical strain, designated MV-2, was isolated from the Pettaquamscutt Estuary [17, 48].

Strain RS-1 is a Gram-negative, sulfate-reducing bacterium that grows and produces bullet-shaped particles of magnetite only under anaerobic conditions [58]. Cells are helicoid-to-rod-shaped and possess a single polar flagellum. Little is known about the physiology of this strain. Cells grow chemoorganoheterotrophically using certain organic acids and alcohols as carbon and energy sources and do not use nitrate as a terminal electron acceptor.

Several other pure cultures of magnetotactic bacteria exist (DA Bazylinski, unpublished data), but they grow poorly and little is known about them. Strain MC-1 produces hexahedral prisms of magnetite and grows chemolithoautotrophically with thiosulfate or sulfide as an electron and energy source [47]. Strain MV-4, a new marine spirillum, produces elongated octahedrons of magnetite and grows chemolithoautotrophically with thiosulfate or chemoorganoheterotrophically [48].

## Magnetosome formation

**Chemistry/biochemistry** The initial step in magnetosome synthesis in magnetotactic bacteria is iron uptake. Free reduced Fe(II) is very soluble [51] and is easily taken up by cells by non-specific means. However, because free Fe(III) is so insoluble, most microbes rely on iron chelators that bind and solubilize Fe(III). These chelators, called siderophores, are defined as low molecular weight (<1 kDa), specific ligands that facilitate the solubilization and transport of Fe(III) [28]. They are generally produced under iron-limited conditions, and high iron concentrations repress their synthesis.

A hydroxamate siderophore was reported to be produced by cells of *M. magnetotacticum* grown under high but not low iron conditions [54], the reverse of what is normally observed. However, this unusual finding was never confirmed. Frankel et al. [25] assumed that iron uptake by this organism probably occurred via a non-specific transport system. Although iron is supplied as Fe(III) chelated to quinic acid, the growth medium also contains reducing agents (e.g. ascorbic acid) potent enough to reduce Fe(III) to Fe(II). Thus, both forms of iron are present and it is not known which form is taken up by cells.

Nakamura et al. [50] did not detect siderophore production by *Magnetospirillum* AMB-1 and concluded that Fe(III) uptake by cells was mediated by a periplasmic binding protein-dependent iron transport system. Spent medium stimulated iron uptake by cells of *M. gryphiswaldense* but there was no evidence for the presence of a siderophore [60]. Iron for magnetite synthesis in this species is taken up as Fe(III) and the process appears to be energy-dependent [60].

Only one study has addressed the chemistry of magnetite synthesis after iron uptake. Frankel et al. [25] examined the nature and distribution of major iron compounds in *M. magnetotacticum* using $^{57}$Fe Mössbauer spectroscopy. They proposed a model in which Fe(III) is taken up by the cell by non-specific means and reduced to Fe(II) as it enters the cell. It is then reoxidized to form a low-density hydrous Fe(III) oxide that is then dehydrated to form a high-density Fe(III) oxide (ferrihydrite) that was detected in cells. In the last step,

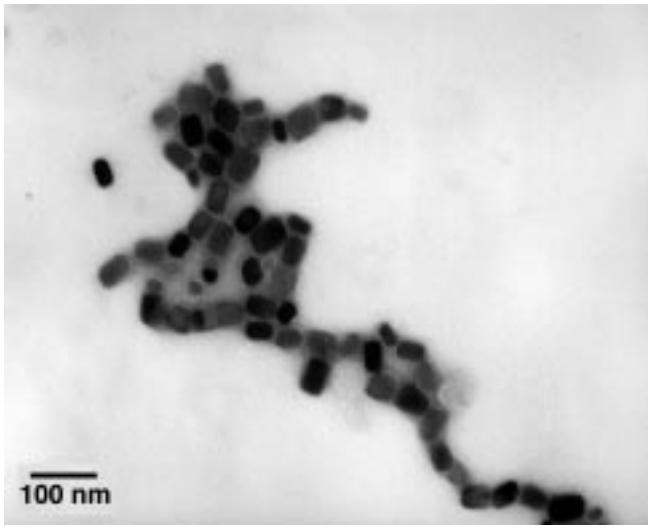


**Fig. 5** Transmission electron micrograph of a negatively-stained preparation (2% sodium phosphotungstate, pH 7.0) of purified magnetite-containing magnetosomes from strain MV-1. The "magnetosome membrane" is visualized as an electron-lucent area surrounding each magnetite crystal. This membrane is easily removed with detergents like sodium deodecyl sulfate

one-third of the Fe(III) ions in ferrihydrite are reduced and, with further dehydration, magnetite is produced.

The size and shape of the mineral phase of the magnetosome reflect the strict control the magnetotactic bacteria exert over the biomineralization processes involved in magnetosome synthesis. In all cultured and some non-cultured strains of magnetite-producing magnetotactic bacteria, the magnetosome mineral phase appears to be enveloped by a coating, the "magnetosome membrane" (Fig. 5) [26]. Although it is not known whether all greigite-producing magnetotactic bacteria produce a magnetosome membrane, a similar structure has been occasionally observed. In *M. magnetotacticum*, the magnetosome coating is not contiguous with the cell membrane and appears to consist of a lipid bilayer containing phospholipids and numerous proteins, several of which are unique to this membrane and not the outer or cell membrane [26]. It is the magnetosome membrane that is presumably the locus of control over the size and morphology of the inorganic particle as well as the structural entity that anchors the magnetosome at a particular location within the cell. However, it is not known if the magnetosome membrane is premade as an "empty" membrane vesicle prior to the biomineralization of the mineral phase. Empty and partially filled vesicles have been observed in iron-starved cells of *M. magnetotacticum* [26] but have not been commonly observed in other magnetotactic strains. The unlikely alternative is that nucleation of the mineral phase occurs prior to membrane formation. In any case, most biochemical and molecular biological studies directed at understanding the biomineralization processes involved in magnetosome formation are focused on the magnetosome membrane.

**Molecular biology** Little is known about the molecular biology of magnetosome formation. A genetic system in the magnetotactic bacteria is obviously required for molecular studies of magnetosome synthesis. Technical problems have hampered research in this area over the years, including for example, the lack of a significant number of magnetotactic bacterial strains for comparison, the fastidiousness of the organisms in culture and the elaborate techniques required for the growth of these organisms and the inability of most strains to grow on the surface of agar plates. Two general approaches to this problem have been taken by investigators: (i) the generation and biochemical and genetic comparisons of non-magnetotactic mutants; and (ii) biochemical investigation of the magnetosome membrane.

Waleh and co-workers [9, 67] showed that some genes of *M. magnetotacticum* can be functionally expressed in *E. coli* and that the transcriptional and translational elements of the two microorganisms are compatible. They cloned, characterized and sequenced the *recA* gene from *M. magnetotacticum* [9, 10]. They also examined iron uptake by *M. magnetotacticum* and cloned and characterized a 2 kb DNA fragment from *M. magnetotacticum* that complemented the *aroD* (biosynthetic dehydroquinase) gene function in *E. coli* and *Salmonella typhimurium*. The *aroD* mutants of these strains cannot take up iron from the growth medium and when the 2 kb DNA fragment from *M. magnetotacticum* was introduced into these mutants, their ability to remove iron from the growth medium was restored [8] suggesting that the 2 kb DNA fragment is important in iron uptake in *M. magnetotacticum*. However, although the cloned fragment restored iron-uptake deficiencies in siderophore-lacking, iron-uptake deficient mutants of *E. coli*, it did not mediate siderophore biosynthesis [8].

Okuda et al. [53], also working with *M. magnetotacticum*, found three proteins, with apparent molecular weights of 12, 22 and 28 kDa, unique to the magnetosome membrane and absent from the cellular membrane fraction. N-terminal amino acid sequence of the 22 kDa protein led to a 17 bp oligonucleotide probe for the genomic cloning of the gene encoding for that protein. The protein exhibits significant homology with several proteins of the tetratricopeptide repeat protein family that includes mitochondrial protein import receptors and peroxisomal protein import receptors. Thus, although the role of the 22 kDa magnetosome membrane protein in magnetosome synthesis has not been elucidated, it may function as a receptor interacting with associated cytoplasmic proteins [53].

*Magnetospirillum* AMB-1, the oxygen-tolerant species [46], forms colonies on the surface of agar plates. In air, colonies are white and typically contain non-magnetic cells but under an incubation atmosphere of 2% oxygen, cells form black-brown colonies consisting of magnetic cells. This feature facilitated the selection of non-magnetic mutants (which form white colonies) obtained by the introduction of transposon Tn*5* into the genome of *Magnetospirillum* AMB-1 by the conjugal transfer of plasmid pSUP1021 that contains the transposon [43]. Introduction of this plasmid and transposon into *M. magnetotacticum* was also successful but colony formation

by this strain was not. Three regions of the *Magnetospirillum* AMB-1 chromosome appear to be required for magnetosome synthesis [43, 49]. One region includes a gene, designated *magA*, that encodes for a protein homologous with cation efflux proteins, the *E. coli* potassium ion-translocating protein, KefC, and the putative sodium ion/proton antiporter, NapA, from *Enterococcus hirae*. The *magA* gene was expressed in *E. coli* and membrane vesicles prepared from cells that contained the *magA* gene product took up iron when ATP was supplied indicating that energy is required for iron uptake. The *magA* gene was expressed to a much greater degree when wild-type cells were grown under iron-limited conditions rather than iron-sufficient conditions under which they produced more magnetosomes [49]. The nonmagnetotactic Tn*5* mutant over-expressed the *magA* gene under iron-limited conditions although it did not synthesize magnetosomes. The role of the *magA* gene in magnetosome synthesis is thus unclear.

## Final comments

This paper shows that much more work remains to be done in elucidating the molecular basis for magnetosome synthesis. However, the knowledge gained will have an impact that goes beyond microbiology. Structures virtually indistinguishable from magnetosomes have been found in protozoa and a number of animal species including the brain of humans [31]. In some cases, there is reasonable evidence that the presence of the structures is related to some type of magnetoreception in the Earth's geomagnetic field. The fact that many organisms biomineralize single magnetic domain crystals of similar morphologies suggests the intriguing idea that they share the same or a similar set of genes responsible for biomineralization. Studying the magnetic personality in humans may have a scientific basis after all!

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