Journal of Molecular Microbiology and Biotechnology

J Mol Microbiol Biotechnol 2013;23:63–80
DOI: 10.1159/000346543

Published online: April 18, 2013

The Bacterial Magnetosome: A Unique Prokaryotic Organelle

Brian H. Lower[a] Dennis A. Bazylinski[b]

[a]School of Environment and Natural Resources, The Ohio State University, Columbus, Ohio, and [b]School of Life Sciences, University of Nevada, Las Vegas, Nev., USA

Key Words
Biomineralization · Greigite · Magnetite · Magnetosome · Magnetotactic bacteria

Abstract
The bacterial magnetosome is a unique prokaryotic organelle comprising magnetic mineral crystals surrounded by a phospholipid bilayer. These inclusions are biomineralized by the magnetotactic bacteria which are ubiquitous, aquatic, motile microorganisms. Magnetosomes cause cells of magnetotactic bacteria to passively align and swim along the Earth's magnetic field lines, as miniature motile compass needles. These specialized compartments consist of a phospholipid bilayer membrane surrounding magnetic crystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4). The morphology of these membrane-bound crystals varies by species with a nominal magnetic domain size between 35 and 120 nm. Almost all magnetotactic bacteria arrange their magnetosomes in a chain within the cell there by maximizing the magnetic dipole moment of the cell. It is presumed that magnetotactic bacteria use magnetotaxis in conjunction with chemotaxis to locate and maintain an optimum position for growth and survival based on chemistry, redox and physiology in aquatic habitats with vertical chemical concentration and redox gradients. The biosynthesis of magnetosomes is a complex process that involves several distinct steps including cytoplasmic membrane modifications, iron uptake and transport, initiation of crystallization, crystal maturation and magnetosome chain formation. While many mechanistic details remain unresolved, magnetotactic bacteria appear to contain the genetic determinants for magnetosome biomineralization within their genomes in clusters of genes that make up what is referred to as the magnetosome gene island in some species. In addition, magnetosomes contain a unique set of proteins, not present in other cellular fractions, which control the biomineralization process. Through the development of genetic systems, proteomic and genomic work, and the use of molecular and biochemical tools, the functions of a number of magnetosome membrane proteins have been demonstrated and the molecular mechanism for the biomineralization of magnetosomes in these organisms is beginning to be revealed.

Introduction

The bacterial magnetosome [Balkwill et al., 1980] is a unique prokaryotic organelle that comprises a magnetic mineral crystal, either magnetite (Fe_3O_4) [Frankel et al., 1979] or greigite (Fe_3S_4) [Mann et al., 1990], and its enveloping membrane. Unlike most other intracellular inclusions in prokaryotes which are compartmentalized in a relatively thin monolayer of protein only (e.g. sulfur globules) [Shively, 1974], the magnetosome membrane is

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Dennis A. Bazylinski
School of Life Sciences
University of Nevada at Las Vegas
Las Vegas, NV 89154-4004 (USA)
E-Mail dennis.bazylinski@unlv.edu

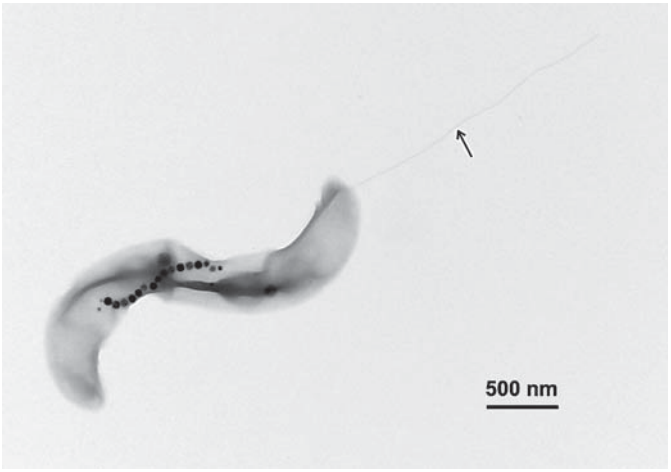

Fig. 1. Transmission electron microscopy image of a cell of a magnetotactic bacterium collected from the Olentangy River, Columbus, Ohio, USA. Note the chain of electron-dense magnetosomes containing cuboctahedral crystals of magnetite running along the long axis of the cell and the single polar long flagellum (at arrow).

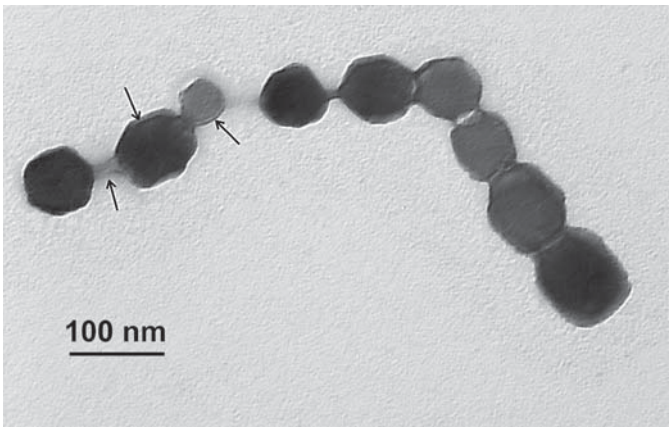

Fig. 2. Transmission electron microscopy image of a chain of purified magnetosomes from *M. marinus*. Arrows denote magnetosome membrane.

a true phospholipid bilayer [Gorby et al., 1988]. These structures are biomineralized by a diverse group of aquatic, motile prokaryotes called the magnetotactic bacteria [Bazylinski and Frankel, 2004]. Each bacterium typically contains 10–20 magnetosomes, each containing a single inorganic nanocrystal that is typically between 35 and 120 nm in diameter (fig. 1) [Bazylinski and Frankel, 2003, 2004; Frankel et al., 1998]. Magnetosomes also contain a unique set of proteins that appear to control the biomineralization of the nanocrystals [Bazylinski and Frankel, 2004; Komeili, 2012; Schüler, 2008]. The composition, size and morphology of the magnetic crystals vary from species to species, but are highly conserved within bacteria of the same species or genus [Bazylinski and Frankel, 2004].

Although originally described by Salvatore Bellini in 1963 [Bellini, 1963, 2009], interest in magnetotactic bacteria did not burgeon until 1975 when Richard Blakemore independently described the magnetotactic behavior of these microorganisms and observed the 'chains of iron-rich particles' (i.e. magnetosomes) within their cells responsible for their magnetotactic behavior [Blakemore, 1975]. Magnetotactic bacteria are microaerophiles or anaerobes that are predominantly found at the oxic-anoxic interface (OAI) or transition zone where oxygenated water (or sediment) meets oxygen-deficient water (or sediment) [Bazylinski and Frankel, 2004]. These microbes exhibit a great deal of diversity in terms of their morphology (both cellular and mineral), physiology, phylogeny and even mode of magnetotaxis. Despite their diversity and relatively high abundance in freshwater and marine habitats, the isolation and cultivation of these organisms has proven to be difficult due to their fastidious nature and lack of suitable enrichment media. Recently, the isolation of new strains of magnetotactic bacteria, the development of new techniques for genetically manipulating these strains and the sequencing and annotation of several magnetotactic bacterial genomes has led to great strides in our understanding of the genetics, biochemistry and molecular biology of these microorganisms and their biomineralization processes. Despite this, details regarding the chemical/biochemical pathways of magnetite and greigite synthesis and the precise roles of most magnetosome-associated proteins remains unresolved. The major purpose of this paper is to present some general background on magnetotactic bacteria and what is currently known regarding magnetosome biomineralization.

The Bacterial Magnetosome

The bacterial magnetosome consists of an organic and inorganic phase: an organic phospholipid bilayer surrounding an inorganic magnetic iron mineral crystal of magnetite or greigite (fig. 2). The composition, size and morphology of the magnetic crystals are under strict con-

trol within the magnetosomes as well as the arrangement of the magnetosomes within the cell [Bazylinski and Frankel, 2004]. This is characteristic of a biologically controlled mineralization, consisting of complex biogeochemical processes that involve several key steps and is under genetic control [Bazylinski and Frankel, 2004]. Based on the oldest so-called magnetofossils (magnetosome crystal remains of magnetotactic bacteria) [Chang et al., 1989], magnetosome synthesis probably represent the first example of biologically controlled mineralization of this planet.

Almost all known freshwater magnetotactic bacteria synthesize magnetite within their magnetosomes. Magnetotactic bacteria that biomineralize iron sulfide nanoparticles are most common in marine, estuarine and salt marsh environments but have now been found in nonmarine saline and some freshwater habitats [Lefevre et al., 2011]. Some magnetotactic bacteria biomineralize both minerals [Bazylinski et al., 1993, 1995; Lefèvre et al., 2011c]. Iron oxide magnetosomes consists entirely of the mineral magnetite (Fe_3O_4) and there is no evidence for the synthesis of precursors to magnetite. Iron sulfide magnetosomes contain nanocrystals of greigite (Fe_3S_4) or a mixture of greigite and nonmagnetite iron sulfide mineral phases including mackinawite (tetragonal FeS) or sphalerite-like, cubic FeS, which appear to be precursor phases for greigite [Heywood et al., 1990; Pósfai et al., 1998a, b]. The synthesis of greigite is believed to follow the transformation pathway of cubic FeS to tetragonal FeS (mackinawite) to Fe_3S_4 [Pósfai et al., 1998a, b].

In general, the morphology of magnetosome crystals is consistent within a single species of magnetotactic bacterium [Bazylinski and Frankel, 2004]. This is more true of those that biomineralize magnetite than those that synthesize greigite as several different morphologies of greigite appear to be present in some uncultured cells. Three main magnetosome crystal morphologies, regardless of whether they consist of magnetite or greigite, have been identified. These morphologies include: (1) cuboctahedral [Balkwill et al., 1980; Heywood et al., 1990; Mann et al., 1984a, b]; (2) parallelepipedal or elongated prismatic [Bazylinski et al., 1988; Heywood et al., 1990; Moench, 1988] and (3) tooth- or bullet-shaped [Lefevre et al., 2011c; Mann et al., 1987a, b; Pósfai et al., 1998a, 1998b].

Almost all individual magnetosomes crystals, regardless of composition, are between 35 and 120 nm in diameter [Bazylinski and Frankel, 2004; Frankel et al., 1998] which places them in the stable single magnetic domain size range [Butler and Banerjee, 1975; Diaz-Ricci and Kirschvink, 1992; Frankel and Moskowitz, 2003]. Single magnetic domain crystals of magnetite and greigite are the smallest particles that can be made of these minerals and still be permanently magnetic at ambient temperature. Smaller superparamagnetic crystals do not have stable, remanent magnetization at ambient temperature and would be useless to the bacteria for magnetotaxis. Larger particles tend to form multiple domains, reducing the remanent magnetization of the crystal. By forming single magnetic domain crystals, magnetotactic bacteria have maximized the magnetic remanence per unit volume of material [Frankel, 1984; Frankel and Blakmore, 1980; Frankel and Moskowitz, 2003].

Magnetosomes are usually positioned as one or more chains that traverse the long axis of the cell if the cell is not coccoid (fig. 1) [Bazylinski, 1995; Bazylinski and Moskowitz, 1997; Frankel and Moskowitz, 2003]. While it is thought that magnetic interactions between individual magnetic magnetosomes within the chain cause each magnetosome moment to orient parallel to one another, thus minimizing the magnetostatic energy of the chain and maximizing the magnetic dipole moment of the bacterium [Frankel, 1984; Frankel and Moskowitz, 2003], cytoskeletal elements appear to play a major role in magnetosome chain formation and anchoring the chain within the cell [Katzmann et al., 2010; Komeili et al., 2006]. In the chain motif, the total dipole moment of the bacterium is the sum of the moments of the individual magnetic magnetosomes which provides a means for the bacteria to passively align with Earth's geomagnetic field lines as they swim [Frankel, 1984; Frankel and Blakemore, 1980; Frankel and Moskowitz, 2003].

The organic phase of the magnetosome, the magnetosome membrane (fig. 2), is the structure that controls the biomineralization of magnetite or greigite in magnetotactic bacteria [Schuler, 2008]. The magnetosome membrane consists of fatty acids, glycolipids, sulfolipids, phospholipids and proteins [Gorby et al., 1988; Grunberg et al., 2004]. The composition of fatty acids contained within the magnetosome membrane is similar to those found in the cytoplasmic membrane but distinct from the outer membrane originally suggesting that the magnetosome membrane is derived from the cytoplasmic membrane [Tanaka et al., 2006]. This was later confirmed in *Magnetospirillum* using electron cryo-tomography [Komeili et al., 2006].

Ecology of Magnetotactic Bacteria

Magnetotactic bacteria are ubiquitous in chemically-stratified sediments and water columns of almost all aquatic habitats and are worldwide in distribution [Bazy-

linski and Frankel, 2004]. These organisms are excellent examples of gradient-loving organisms, which likely explains the known difficulties in their isolation in axenic culture. Their presence is dependent on the presence of an OAI (also known as the oxic-anoxic transition zone, OATZ) that generally represents opposing gradients of oxygen from the surface and reducing compounds from the anoxic zone (e.g. sulfide) with a concomitant redox gradient in sediments or water columns. While the highest numbers of magnetotactic bacteria have been observed at the OAI of sediments or chemically stratified water columns [Moskowitz et al., 2008], different species of magnetotactic bacteria occupy different positions within the gradients that are dependent on specific chemical/redox conditions. Typically magnetite-producing magnetotactic bacteria are found quite close to the OAI while greigite-producing species are found below the OAI when the anoxic zone is sulfidic [Moskowitz et al., 2008].

Magnetotactic bacteria are thought by many to be mesophiles restricted to habitats with pH values near neutrality. Recently, however, several extremophilic species have been described. Uncultured, moderately thermophilic species have been found in several hot springs in northern Nevada with a probable upper growth limit of about 63°C [Lefèvre et al., 2010] and in California [Nash, 2008]. Moreover, several cultured strains of obligately alkaliphilic magnetotactic bacteria have been isolated from different aquatic habitats in California including the hypersaline, extremely alkaline Mono Lake [Lefèvre et al., 2011b]. These organisms appear to be strains of the known, nonmagnetotactic *Desulfonatronum thiodismutans* [Pikuta et al., 2003], and have an optimal growth pH of about 9.0. None yet have been found in strongly acidic habitats (e.g. acid mine drainage).

Biological Advantage to Magnetotaxis and Magnetosomes

In the Earth's geomagnetic and other magnetic fields, cells of magnetotactic bacteria experience a torque causing the cells to passively align along magnetic field lines as they swim, the definition of magnetotaxis [Blakemore, 1975; Frankel and Blakemore, 1980]. While a number of theories or models have been proposed on how this benefits magnetotactic bacteria in nature, it seems that the most accepted model involves magnetotaxis helping cells to be more efficient at chemotaxis, i.e. at finding an optimal position in vertical chemical and redox gradients [Frankel et al., 1997, 2007].

In Blakemore's [1975] original model, it was assumed that all magnetotactic bacteria had a polar preference in their swimming direction and were microaerophiles. He proposed that magnetotaxis helped to guide cells to swim downward to less oxygenated regions of aquatic habitats. Once cells reached their preferred microhabitat (e.g. surface sediments) they would cease swimming and perhaps attach to sediment particles until environmental conditions (e.g. oxygen concentration) changed. This idea was supported by the fact that, in general, magnetotactic bacteria are primarily north-seeking in the northern hemisphere and south-seeking in the southern hemisphere, both of which would swim downward along the Earth's inclined geomagnetic field lines [Blakemore et al., 1980]. However, this model did not explain a number of later observations, for example, the presence of large, stable populations of magnetotactic bacteria at the OAI in the water columns of certain habitats [Simmons et al., 2004; Moskowitz et al., 2008] and how magnetotactic cocci form microaerophilic bands of cells in semi-solid oxygen gradient medium [Frankel et al., 1997]. If this model was complete, cells of magnetotactic bacteria in these situations should continue to swim downward and be in the sediment or at the bottom of the culture tube.

Frankel et al. [1997] later described two forms of magnetotaxis, more appropriately called magneto-aerotaxis since the magnetotactic bacteria studied were not only magnetotactic, but also strongly aerotactic as microaerophiles. In axial magneto-aerotaxis, cells do not have a polar preference in their swimming direction and use the magnetic field as an axis while swimming in both directions under oxic conditions. In polar magneto-aerotaxis (fig. 3), cells have a polar preference in their swimming direction under oxic conditions, i.e. cells are north-seeking in the northern hemisphere. Both types of cells form microaerophilic bands of cells in semi-solid oxygen gradient media and can swim in the opposite direction if need be based on chemical/redox conditions, although they appear to use a different mechanism [Frankel et al., 1997, 2007]. For either type of magnetotaxis, once cells are aligned along the inclined magnetic field lines, their search for an optimal position in a vertical chemical/redox gradient has been reduced from a 3-dimensional search (for nonmagnetotactic bacteria such as *Escherichia coli*, for example) to one dimension, thereby increasing the efficiency of chemotaxis (e.g. aerotaxis) [Frankel et al., 1997, 2007].

Despite the fact that this model of magneto-aerotaxis seems to fit well for many magnetotactic bacteria, particularly those microaerophiles that require sulfide as an

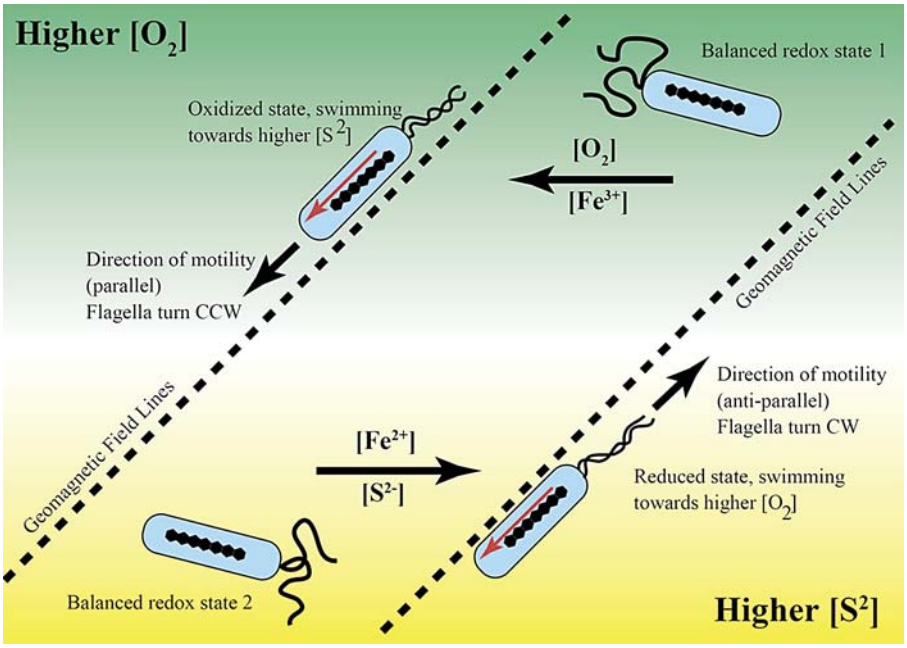

Fig. 3. Model of how polar magnetotaxis might help to guide bacteria in the northern hemisphere, depending on their internal redox state, either downward to accumulate reduced sulfur species or upward to oxidize stored sulfur with oxygen. It has been proposed that magnetotactic bacteria displaying polar magnetotaxis alternate between two internal redox states. In the oxic zone, cells enter an oxidized state resulting from the consumption of most of the stored sulfur, the electron donor. In this state, cells swim to deeper anoxic layers (swim northward; flagellum rotates in one direction) where they could again access the electron donor (sulfide). Eventually, they would enter a reduced state in which they would have accumulated a large amount of electron donor which cannot be efficiently oxidized without oxygen, leading to a surplus of reduction equivalents. Cells must therefore return to the microoxic zone (swim upward; flagellum rotates in opposite direction) where oxygen is available to them as an electron acceptor. In either case, cells remain aligned in the magnetic field and the direction of swimming is dictated by the direction of flagella rotation which in turn is affected by the redox state. Cells of axial magnetotactic bacteria simply swim up and down the magnetic field lines using chemotaxis to find their optimal location in the double inverse gradient. In either case, magnetotaxis makes chemotaxis more efficient by reducing a 3-dimensional search problem to one of a single dimension. Diagram modified from Spring and Bazylinski [2006].

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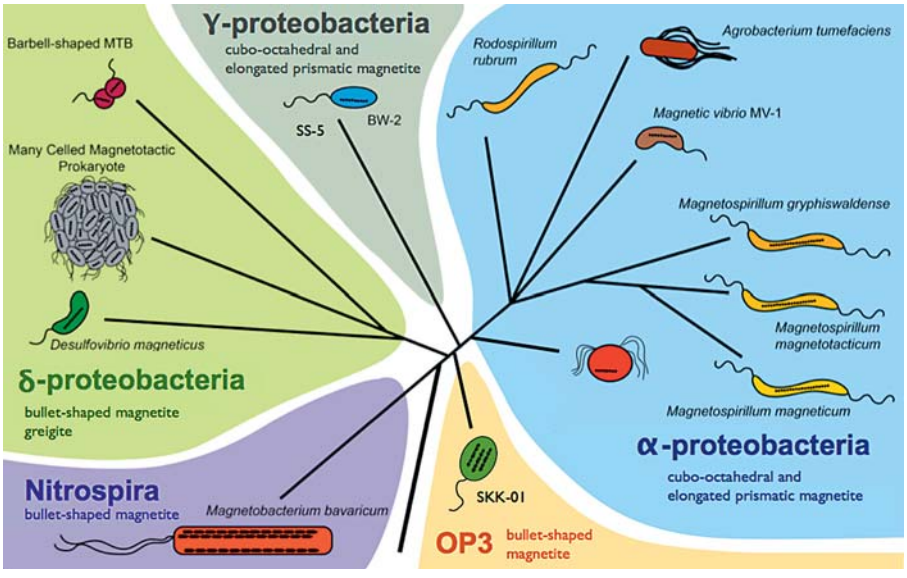

Fig. 4. Phylogenetic distribution of known cultured and uncultured magnetotactic bacteria in five major evolutionary lineages. These include the Alpha-, Gamma- and Deltaproteobacteria classes of the Proteobacteria phylum, the Nitrospirae phylum and the candidate division OP3, part of the Planctomycetes-Verrucomicrobia-Chlamydiae bacterial superphylum. Magnetotactic species in this grouping are shown as cells with dark inclusions. Species from different groups produce specific types of magnetosome crystals as shown.

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electron donor as well as oxygen as a terminal electron acceptor (the OAI is the only location for a bacterium to access both compounds in many environments), it does not explain the behavior of other magnetotactic bacteria or answer some very important questions regarding biomineralization of magnetosomes. For example, Simmons et al. [2006] and Shapiro et al. [2011] described populations of polar magnetotactic bacteria in natural habitats of the northern hemisphere whose cells were mostly south-seeking. Based on the model of polar mag-

netotaxis described above, these organisms would be selected against because they would presumably continue to swim southward/upward towards surface waters in the northern hemisphere and would die from high, toxic levels of oxygen. Other important questions include: if magnetotaxis really made aerotaxis more efficient, why are there many obligately microaerophilic, nonmagnetotactic bacteria at the OAI; why do some cultured species biomineralize far more magnetite under anaerobic conditions when no gradient is present in the medium (e.g. *Magnetovibrio blakemorei*), and why do cells of nonmagnetotactic mutants of microaerophilic magnetotactic bacteria that do not biomineralize magnetosomes behave the same way as cells of the magnetotactic wild-type in semi-solid oxygen gradient cultures? These models really present a consequence of possessing magnetosomes and do not explain the reason for taking up so much iron in the first place. It seems logical that there are physiological reasons for magnetosome biomineralization (e.g. detoxification of free iron ions in the cell; decomposition of toxic oxygen radicals produced during respiration such as hydrogen peroxide [Blakemore, 1982; Guo et al., 2012]) but, to date, any convincing physiological link remains elusive.

A recent proposal that will surely raise significant discussion is the possibility that magnetosomes provide little to no selective advantage for the organism and that as long as the trait is not particularly disadvantageous, there is no selective pressure to lose the ability to biomineralize magnetosomes [Komeili, 2012]. Magnetosomes may thus simply be a vestigial remnant of a trait possibly useful in the past or the byproduct of the activity of a set of selfish genes [Komeili, 2012]. This is a particularly intriguing notion because cells of several strains of magnetotactic bacteria in culture seem to easily lose the ability to make magnetosomes [Dubbels et al., 2004; Fukuda et al., 2006; Komeili et al., 2006] although this observation is difficult if not impossible to extrapolate to natural environments.

Phylogeny of Magnetotactic Bacteria

The phylogenetic diversity of magnetotactic bacteria based on their 16S rRNA gene sequences is relatively extensive although appears to be restricted to phyla within the domain Bacteria that are considered recent groups that are not deeply branching. None have been found to be phylogenetically associated with the Archaea. At present, known representatives of the magnetotactic prokaryotes are phylogenetically affiliated with five major lineages within the domain Bacteria, three within the Proteobacteria (fig. 4). Most known cultured and uncultured magnetotactic bacteria belong to the Alpha-, Gamma- and Deltaproteobacteria classes of the Proteobacteria phylum, while several uncultured species belong to the Nitrospirae phylum and one strain to the candidate division OP3, part of the Planctomycetes-Verrucomicrobia-Chlamydiae bacterial superphylum (fig. 4) [Kolinko et al., 2011].

There is an interesting correlation between the phylogenetic groups of magnetotactic bacteria and the mineral phase of their magnetosomes (fig. 4). The organisms within the most deeply branched groups, which includes the candidate division OP3, the Nitrospirae and the Deltaproteobacteria, all biomineralize bullet-shaped crystals of magnetite in their magnetosomes [Lefèvre et al., 2011d]. Some Deltaproteobacteria also produce greigite magnetosomes [Lefèvre et al., 2011c]. Organisms in the later diverging groups, the Alpha- and Gammaproteobacteria, biomineralize only cuboctahedral and elongated prismatic crystals of magnetite [Lefèvre et al., 2012]. Because of this correlation and the great variation and large number of crystal flaws in the bullet-shaped magnetite crystals, these crystals might represent the first magnetosome mineral phase, the most primitive magnetosomes [Abreu et al., 2011].

Physiology of and Biogeochemical Cycling by Magnetotactic Bacteria

The physiology of the magnetotactic bacteria is quite diverse and, like magnetosome crystal composition, correlates with their phylogenetic group. Many cultured species have been shown to mediate important reactions in the biogeochemical cycling of elements including iron, nitrogen, sulfur and carbon while some uncultured forms appear to be implicated in these same reactions.

Little is known regarding the physiology of the magnetotactic bacteria of the most deeply branching groups, the candidate division OP3 and the Nitrospirae, as none have been isolated in axenic culture. It has been assumed however, that some are microaerophilic sulfide-oxidizing chemolithoautotrophs including *Candidatus* Magnetobacterium bavaricum and related organisms of the Nitrospirae that exist mainly in the microaerobic zone of sediments and contain internal sulfur globules [Jogler et al. 2010; Lefèvre et al., 2011a; Spring et al., 1993] while others, moderate thermophilic strains whose closest phylogenetic relatives are *Thermodesulfovibrio* species, may be sulfate-reducing bacteria [Lefèvre et al., 2010].

Table 1. Genomes of magnetite-producing magnetotactic bacteria

Bacterium	Class of Proteobacteria	Status	Size, Mb	Plasmids	Reference
D. magneticus	Deltaproteobacteria	complete	5.25	2	Nakazawa et al., 2009
M. marinus	Alphaproteobacteria	complete	4.5	0	Schübbe et al., 2009
M. gryphiswaldense	Alphaproteobacteria	incomplete	4.3	1	Jogler and Schüler, 2007; Richter et al., 2007
M. magneticum	Alphaproteobacteria	complete	4.97	1	Matsunaga et al., 2005
M. magnetotacticum	Alphaproteobacteria	incomplete	4.3	possible	Bertani et al., 2001
M. blakemorei	Alphaproteobacteria	incomplete	3.7	0	Not published

In general, almost all cultured magnetotactic bacteria grow chemoorganoheterotrophically utilizing organic acids as a carbon and electron source with a few exceptions of those that appear to be obligate chemolithoautotrophs. Many of those in the Alphaproteobacteria are also capable of chemolithoautotrophic growth using sulfide or thiosulfate or both as electron sources [Bazylinski et al., 2004; Geelhoed et al., 2010]. All strains in this group use the Calvin-Benson-Bassham cycle for autotrophy [Bazylinski et al., 2004; Geelhoed et al., 2010] with the exception of *Magnetococcus marinus* which uses the reverse or reductive tricarboxylic acid cycle [Williams et al., 2006]. All magnetotactic Alphaproteobacteria are mesophilic and grow under microaerobic or anaerobic conditions or both. Many species in the Alphaproteobacteria catalyze important reactions in the nitrogen cycle. For example, all species tested fix atmospheric dinitrogen as evidenced by their ability to reduce acetylene to ethylene under nitrogen-limiting conditions [Bazylinski et al., 2000, 2012a, 2012b; Bazylinski and Blakemore, 1983a; Williams et al., 2012] and most of the described species of *Magnetospirillum* are capable of denitrification [Bazylinski and Blakemore, 1983b; Li et al., 2012]. Blakemore et al. [1985] first noted that cells of *Magnetospirillum magnetotacticum* produced more magnetosomes under microaerobic conditions when grown with nitrate as an additional terminal electron acceptor. Recently Li et al. [2012] showed that the periplasmic nitrate reductase is required for anaerobic growth in *M. gryphiswaldense* and that the deletion of the *nap gene* impaired microaerobic respiration and magnetite biomineralization, resulting in fewer, smaller and irregular crystals during denitrification probably by disturbing the proper redox balance required for magnetite synthesis.

The two known magnetotactic bacteria strains affiliated with the Gammaproteobacteria also grow chemolithoautotrophically with reduced sulfur compounds as electron sources but show little potential for chemoorganoheterotrophic growth [Lefèvre et al., 2012]. Autotrophy is through the Calvin-Benson-Bassham pathway. Both appear to be obligate mesophilic microaerophiles and one species is capable of nitrogen fixation while the other is not [Lefèvre et al., 2012].

The known cultured magnetotactic Deltaproteobacteria are all anaerobic, chemoorganoheterotrophic, dissimilatory sulfate-reducing bacteria [Lefèvre et al., 2011b, 2011c; Sakaguchi et al., 1993]. One strain, *Desulfovibrio magneticus*, is also capable of growth through the fermentation of pyruvate to acetate and hydrogen [Sakaguchi et al., 2002].

Genomics of Magnetotactic Bacteria

Genomic analyses has provided valuable insight into how magnetosome genes are organized in different magnetotactic bacteria as well as to the magnetosome genes common to groups of magnetotactic bacteria. Magnetotactic bacteria whose genomes are complete or mostly complete and from which data can be obtained are shown in table 1. The sequencing of genomes of a number of others is in progress.

As a genomic approach to identifying specific magnetosome-related genes, cross comparisons of genomes of the magnetotactic Alphaproteobacteria including *M. magneticum*, *M. magnetotacticum*, *M. gryphiswaldense*, and *M. marinus* showed a core genome of approximately 890 genes shared by all four strains [Richter et al., 2007]. In addition, 152 genus-specific genes were shared by the three *Magnetospirillum* strains, while 28 genes were identified as group specific, i.e. they occur in all four magnetotactic Alphaproteobacteria, but exhibit no or weak simi-

larity to genes from nonmagnetotactic bacteria [Richter et al., 2007]. This later group of genes included nearly all the *mam* and *mms* genes previously shown to control magnetosome formation. If the genome sequence of the sulfate-reducing, deltaproteobacterium *D. magneticus* is included in this analysis, the number of signature genes conserved in these five species decreases to only nine.

The magnetosome-related *mam* and *mms* genes are organized as clusters that are in relatively close proximity to each another within the genomes of almost all magnetotactic bacteria studied. These clusters, in turn, appear to be organized as a larger unit, a genomic magnetosome island (MAI), in some species. Mobile elements, tRNA genes that can act as insertion sites for integrases [Blum et al., 1994; Reiter and Palm, 1990; Reiter et al., 1989] and a different guanine + cytosine content compared to the rest of the genome [Dobrindt et al., 2004] are typical, common, important features of genomic islands [Mahillon and Chandler, 1998; Mahillon et al., 1999]. In *M. gryphiswaldense*, the putative MAI is about 130 kb in size, contains three tRNA genes upstream of the *mms* operon, has a slightly different guanine + cytosine content versus the rest of the genome, and contains 42 mobile elements as transposases of the insertion sequence type and integrases [Ullrich et al., 2005]. In addition, many hypothetical genes and pseudogenes are present [Schübbe et al., 2003; Ullrich et al., 2005] which apparently have no function as their deletion had no effect on either growth or magnetosome formation [Lohsse et al., 2011]. While this is excellent evidence that this genomic region represents a bona fide MAI in *M. gryphiswaldense*, and probably in other magnetotactic bacteria with some variations [Abreu et al., 2011; Fukuda et al., 2006; Jogler et al., 2011; Nakazawa et al. 2009; Richter et al., 2007; Schübbe et al., 2009], the situation is not so clear in some species such as *M. marinus* whose magnetosome gene cluster lacks many of the features of typical genomic islands [Schübbe et al., 2009].

Gene or genomic islands are thought to be distributed to different bacteria through horizontal gene transfer and to undergo frequent gene rearrangements, and thus may be a major pathway for the evolution of bacterial genomes [Juhas et al., 2009]. Distribution of the MAI through horizontal gene transfer could explain the phylogenetic diversity of the magnetotactic bacteria while variations of the MAI in different magnetotactic bacteria may be the result of rearrangements within the MAI occurring over time.

There are some examples of magnetosome or magnetosome-like genes outside of the putative MAI in some magnetotactic bacteria. Rioux et al. [2010] identified a cluster of *mam*-like genes (referred to as a genomic islet), including *mamKDLJEFQ*-like genes, in the genome of *Magentospirillum magneticum*, distant from the known MAI. There is also some evidence for magnetosome membrane proteins genes present on a cryptic plasmid rather than the genome in *D. magneticus* [Matsunaga et al., 2009].

The organization of *mam* and *mms* genes in described *Magnetospirillum* species is relatively well conserved. The organization of these genes is less conserved in other unrelated magnetotactic strains [Jogler et al., 2011; Schübbe et al., 2003, 2009; Ullrich et al., 2005]. In *M. gryphiswaldense*, all *mam* and *mms* genes are located on a segment of DNA about 45 kb in length and most magnetosome gene clusters are organized as three operons. Experimental evidence shows that these operons, the *mamAB*, *mamGFDC* and *mms6* operons, are each transcribed as single long mRNAs. The transcription starting points of each mapped closely upstream of the first genes in each operon [Schübbe et al., 2006].

The *mamAB* cluster represents a segment of DNA about 16.4 kb in length and contains not only the *mamA* and *mamB* genes but 15 other genes as well [Grünberg et al., 2001]. It is the only known operon in *Magnetospirillum* that contains genes that are absolutely essential for magnetite biomineralization and thus magnetosome formation. Other operons in *Magnetospirillum* appear to have important accessory functions in controlling the size and shape of the magnetite magnetosome crystals [Lohsse et al., 2011; Murat et al., 2010; Ullrich and Schüler 2010].

Of the accessory operons, the *mamGFDC* cluster is about 2.1 kb in length and located about 15 kb upstream of the *mamAB* operons. It comprises four genes encoding for a group of abundant magnetosome membrane proteins involved in controlling the size of magnetite crystals [Scheffel et al., 2008]. The *mms6* cluster is about 3.6 kb in length and located 368 bp upstream of the *mamGFDC* operon and comprises five genes [Schübbe et al., 2003]. One magnetosome gene encoding for a magnetosome membrane protein, *mamW*, lies not within these operons, but is located about 10 kb upstream of the *mms6* operon [Ullrich et al., 2005].

Recently, a magnetotactic bacterium, called strain BW-1, was isolated that biomineralizes both magnetite and greigite magnetosomes [Lefèvre et al., 2011c]. The arrangement of magnetosomes genes in this organism is more complex in that the genome contains at least two known sets of magnetosomes genes: one containing magnetosome genes similar to those of the magnetite-produc-

ing *D. magneticus* and *M. marinus* and the other with genes similar to the greigite-producing *Candidatus* Magnetoglobus multicellularis. This suggests that the former set is responsible for the biomineralization of magnetite and the other for the biomineralization of greigite although this has not yet been proven experimentally.

Genetics of Magnetotactic Bacteria

Ideally, the best and most common, method of assigning definitive functions to specific genes in prokaryotes is through single gene knockouts with subsequent analysis of the mutant phenotype. However, it has proven difficult to genetically manipulate strains of magnetotactic bacteria to determine the function of specific magnetosome genes. This is mainly due to the difficulty in getting cells to form colonies on agar plates, which is, in turn, due to their preference for microaerobic conditions and oxygen toxicity. Several approaches to grow these organisms on plates included the addition of compounds to scavenge toxic radicals to the growth medium (e.g. catalase, activated charcoal) and/or to incubate plates under low concentrations of oxygen. However, these approaches have led only to working genetic systems for *M. gryphiswaldense* [Schultheiss and Schüler, 2003] and *M. magneticum* [Matsunaga et al., 1992]. Nonmagnetotactic mutants of these and other strains unable to synthesize magnetosomes are easily detected because magnetite-forming colonies are dark-brown to black in color compared to the lighter-colored colonies of nonmagnetic mutants [Dubbels et al., 2004; Schultheiss and Schüler, 2003]. Moreover, the magnetic response of a culture, as long as the cells are not spherical, which is related to the number of magnetosomes per cell, is easily tested by light scattering measurements of cell suspensions in variable magnetic fields by 'C_{mag}' values using a spectrophotometer [Schüler et al., 1995; Zhao et al., 2007].

The establishment of genetic systems in *M. gryphiswaldense* and *M. magneticum* have now allowed for general transposon mutagenesis [Komeili et al., 2004; Matsunaga et al., 1992; Schultheiss and Schüler, 2003; Schultheiss et al., 2004] and for the extrachromosomal expression of genes and the integration of reporter genes like luciferase or green fluorescent protein genes *(gfp)* and their derivatives which further facilitated studies involving subcellular localization of proteins possibly involved in magnetosome biomineralization [Komeili et al., 2004; Matsunaga et al., 2000a, 2000b; Nakamura et al., 1995; Schultheiss et al., 2004] in these species. The addition of genomic information together with the introduction of suicide vectors into these species allow for the construction of site-directed gene knockouts for the determination of precise roles of specific genes in magnetite magnetosome biomineralization [Komeili et al., 2004, 2006; Murat et al., 2010; Pradel et al., 2006; Scheffel et al., 2006]. Use of the Cre-lox system has allowed the targeted excision of large DNA regions (60 kb and more) from the genome of *M. gryphiswaldense*, thereby facilitating functional analysis and genomic engineering [Lohsse et al., 2011; Ullrich and Schüler, 2010].

Biochemistry and Molecular Biology of Magnetosome Biomineralization

A general understanding of the molecules responsible for magnetite or greigite biomineralization in magnetosomes of magnetotactic bacteria was lacking until the early 1990s when two species of magnetotactic bacteria, *M. magneticum* strain AMB-1 and *M. grysphiswaldense* strain MSR-1, were isolated in pure culture [Matsunaga et al., 1991; Schleifer et al., 1991]. The relative ease with which these microorganisms can be grown, the development of genetic systems in these species [Komeili et al., 2004; Matsunaga et al., 1992; Murat et al., 2010; Schultheiss and Schüler, 2003; Schultheiss et al., 2004], and the advent of systematic proteomic and genomic technologies has allowed scientists to use both strains as model organisms for understanding the molecular mechanism of magnetite and greigite biomineralization in magnetotactic bacteria. Other cultured species such as *M. marinus* strain MC-1 and *M. blakemorei* strain MV-1, have offered additional important insight into the biochemistry and genes involved in the biomineralization of magnetosomes although reliable genetic systems have not yet been established for these organisms [Richter et al., 2007].

Purifying magnetosomes from cells is a relatively tedious process and is facilitated by the use of magnetic separation techniques [Bazylinski et al., 1994; Gorby et al., 1988]. Isolating magnetosome-associated proteins, however, is a fairly easy process that involves the use of detergents (e.g. sodium deodecyl sulfate) to remove the magnetosome membrane from purified magnetosomes followed by one- or two-dimensional gel electrophoresis [Dubbels et al. 2004; Gorby et al., 1988; Grünberg et al., 2004]. Through these methods, some proteins isolated and identified in this manner have been found to be unique to the magnetosome membrane and absent from other cellular protein fractions [Gorby et al., 1988; Grün-

Table 2. Magnetosome membrane proteins and their putative functions

Protein	Putative function	Reference
MamA, Mam22, Mam24	Serve as scaffold proteins to coordinate assembly of functional protein complexes within the magnetosome	Abreu et al, 2011; Grünberg et al., 2011; Komeili et al., 2004; Matsunaga et al., 2005; Nakazawa et al., 2009; Okuda et al., 1996; Schübbe et al., 2009; Taoka et al., 2006; Yamamoto et al., 2010
MamB, MamM, MamN, MamV	Transport iron into the magnetosome, initiate crystal formation, form protein-protein complexes	Grünberg et al., 2001; Murat et al., 2010; Uebe et al., 2011b
MamC/Mms12/Mms13, MamD/Mms7, MamG/Mms5, Mms6	Control nanocrystal size and morphology during biomineralization process	Arakaki et al., 2003; Fukuda et al., 2006; Taoka et al., 2006
MamE, MamO, MamP	Homologous to HtrA-like serine proteases, function in proper localization and arrangement of proteins within the magnetosome membrane	Grünberg et al., 2001; Murat et al., 2010; Quinlan et al., 2011; Yang et al., 2010
MamI, MamL, MamQ	Catalyze initial step in magnetosome membrane formation and invagination	Murat et al., 2010
MamJ, MamK	Assembly of the magnetosome chain	Katzmann et al., 2010, 2011; Komeili et al., 2006; Scheffel and Schüler, 2007; Scheffel et al., 2006; Schübbe et al., 2003
MamR, MamS, MamT	Control crystal size and morphology	Grünberg et al., 2004; Murat et al., 2010

berg et al., 2004; Matsunaga et al., 2009; Okamura et al., 2000]. To date, 30–40 proteins have been isolated from the magnetosome membrane fraction of these organisms [Grünberg et al., 2004]. These proteins are known as the Mam (magnetosome membrane) or Mms (magnetic particle membrane specific) proteins and their respective *mam* and *mms* genes. Putative functions for these proteins have been determined using site-directed mutagenesis, in vitro experiments that examine mineral precipitation in the presence of recombinant proteins, and comparisons of the DNA-derived amino acid sequences with known proteins.

Almost all that is known regarding the biomineralization of magnetosomes involves studies of magnetotactic bacteria that synthesize magnetite. The magnetosome biomineralization process itself is a complex orchestration of several different steps, all of which must be occurring in the cell simultaneously. In this section we discuss these steps in conjunction with putative roles of specific magnetosome membrane proteins in the process (listed in table 2).

The first step in magnetosome synthesis involves the invagination of the cytoplasmic membrane, which may or may not pinch off to form a magnetosome membrane vesicle [Komeili et al., 2006; Katzmann et al., 2010]. It is still unclear if the membranes of mature magnetosomes remain attached to the cell's cytoplasmic membrane or if they detach during the synthesis of magnetite to become true intracellular vesicles [Faivre et al., 2007; Katzmann et al., 2010]. Several magnetosome Mam proteins are thought to play a role in magnetosome invagination/vesicle formation. Based on gene deletion studies, *mamB*, *mamI*, *mamL* and *mamQ* are essential for the formation of the magnetosome membrane in *M. magneticum* [Murat et al., 2010]. When any of these genes were deleted from *M. magneticum*, neither magnetosomes nor magnetosome membranes were formed [Murat et al., 2010]. MamI and MamL each encode for small 70-amino acid polypeptides with putative transmembrane domains [Murat et al., 2010]. Both proteins are unique to magnetotactic bacteria and lack homology to any other known proteins. MamL has a C-terminus consisting of 15 positively charged amino acids that is believed to form an alpha helix [Murat et al., 2010]. These positively charged amino acid motifs are known to interact with and even cross membranes in a number of different systems [Schmidt et al., 2010] and thus may interact with the inner portion of the cytoplasmic membrane in a manner

that helps to shape the magnetosome membrane, perhaps catalyzing the initial step in the invagination process [Komeili, 2012]. While the specific function of MamQ is unknown, its primary sequence displays similarity to the family of conserved proteins which include the LemA protein in *Listeria monocytogenes* [D'Orazio et al., 2003], a protein that contains coil-coil repeat domains, which may facilitate the formation, bending and shaping of the magnetosome membrane [Komieili, 2012].

The *mamA* gene (which corresponds to *mam22* and *mms24* in some magnetotactic bacteria) is present in the genomes of all magnetotactic bacteria examined [Abreu et al., 2011; Grünberg et al., 2001; Komeili et al., 2004; Matsunaga et al., 2005; Nakazawa et al., 2009; Okuda et al., 1996; Schübbe et al., 2009]. Amino acid sequences of MamA proteins show high similarity to proteins of the tetratricopeptide repeat (TPR) protein family [Okuda et al., 1996]. MamA is thought to be important in protein-protein interactions that presumably occur in magnetosomes synthesis and construction of the magnetosome chain [Okuda et al., 1996; Okuda and Fukumori, 2001] since multiple copies of TPRs are known to form scaffolds within proteins to mediate protein-protein interactions and to coordinate the assembly of proteins into multisubunit complexes [Ponting and Phillips, 1996]. In fact, X-ray crystal structure analysis of MamA showed that it contains multiple potential binding sites. This finding, taken into consideration with the functions of known TRP proteins, suggests that MamA serves as a scaffolding protein to coordinate the assembly of oligomeric protein complexes [Zeytuni et al., 2011]. A deletion of *mamA* in *M. magneticum* resulted in the production of shorter magnetosome chains leading to the suggestion that MamA activates magnetosome vesicles and may be involved in magnetite crystal maturation [Komeili et al., 2004; Murat et al., 2010].

Iron uptake by cells is obviously necessary for magnetosome synthesis and probably occurs continuously as long as iron is available to the bacterium. Although iron can account for greater than 3% of the dry weight of a magnetotactic bacterial cell, an amount that is several orders of magnitude higher than of nonmagnetotactic bacteria [Blakemore, 1982; Heyen and Schüler, 2003; Schüler and Baeuerlein, 1998], magnetotactic bacteria have not yet been shown to possess novel iron uptake systems. *Magnetospirillum* species take up both Fe(II) and Fe(III) for magnetosome synthesis [Matsunaga and Arakaki, 2007; Schüler and Baeuerlein, 1996; Suzuki et al., 2006] and the process appears to occur relatively quickly [Heyen and Schüler, 2003; Schüler and Baeuerlein, 1997, 1998]. Several studies implicate siderophores in iron uptake by some magnetotactic bacteria [Calugay et al., 2003; Dubbels et al., 2004; Paoletti and Blakemore, 1986]. However, a specific role in magnetosome synthesis for these low molecular weight iron chelators [Neilands, 1984, 1995] has not been established, and, in one case, because siderophores are only formed when iron is depleted in the growth medium [Calugay et al., 2003], they do not appear to be involved in magnetite synthesis. Other research suggests that copper-dependent iron uptake proteins might be important for transporting iron into the cell [Dubbles et al., 2004] and that the ferrous iron transport protein B gene *(feoB1)* plays an accessory role in magnetosome formation in *M. gryphiswaldense* [Rong et al., 2008].

Once inside the cell, iron must then be transported into the magnetosome invagination or vesicle, an important question that remains unresolved. If the magnetosome membrane is truly an invagination of the cytoplasmic membrane then iron would only have to be transported across the outer membrane to the periplasmic space where it would be accessible to the magnetosome membrane invagination. If, on the other hand, magnetosomes were true vesicles that were physically separate from the cytoplasmic membrane, then iron would also have to be transported across the cytoplasmic membrane and then the magnetosome membrane to enter the vesicle where the mineral is synthesized. Results from Mössbauer spectroscopic analysis of cells of *M. gryphiswaldense* suggest a mechanism by which iron required for magnetite biomineralization is processed throughout the cytoplasmic membrane directly to the magnetosome membrane without iron transport through the cytoplasm, suggesting that pathways for magnetite formation and biochemical iron uptake are distinct [Faivre et al., 2007]. Magnetite formation then presumably occurs via membrane-associated crystallites, whereas the final step of magnetite crystal growth is possibly spatially separated from the cytoplasmic membrane [Faivre et al., 2007]. There is also evidence that the ferric uptake regulator (Fur) transcription factor in this species plays a key role in the biomineralization process [Uebe et al., 2010; Yijun et al., 2007]. Yijun et al. [2007] showed that disruption of a *fur*-like gene in *M. gryphiswaldense* resulted in defects in iron accumulation and magnetosome formation. Uebe et al. [2010] later showed that deletion of the *fur* homolog in *M. gryphiswaldense* resulted in synthesis of fewer magnetite crystals. There was also a change in the overall distribution of cellular iron with a higher portion bound to a ferritin-like molecule [Uebe et al., 2010]. These results

suggest magnetosome magnetite iron in this organism iron might be recruited from the cytoplasmic pool. Qi et al. [2012] provide evidence that Fur in *M. gryphiswaldense* directly regulates genes involved in oxygen as well as iron metabolism, thereby influencing magnetosome biomineralization.

Specific magnetosome membrane proteins are also probably involved in transporting and confining iron in the magnetosome invagination/vesicle. The MagA protein was the first protein discovered thought to be important in iron transport to the magnetosome in *M. magneticum* [Nakamura et al., 1995]. Recent evidence, however, suggests that this protein is not involved in magnetosome synthesis [Uebe et al., 2011a]. The genes *mamB* and *mamM* are present in the genomes of all magnetotactic bacteria examined thus far, while the gene for a third homologous protein, MamV, is only present in *M. magnetotacticum* and *M. magneticum* [Abreu et al., 2011; Grünberg et al., 2001, Matsunaga et al., 2005; Nakazawa et al., 2009; Schübbe et al., 2009]. The amino acid sequences of these proteins exhibit high homology to metal transporter proteins known to facilitate the influx or efflux of cadmium, iron and zinc [Grass et al., 2005; Haney et al., 2005; Paulsen et al., 1997]. For this reason it has been suggested that MamB and MamM (and MamV in select magnetotactic bacteria) control the transport of iron to the magnetosome membrane prior to iron oxide or iron sulfide biomineralization [Grünberg et al., 2001]. A recent study by Uebe et al. [2011b] demonstrated that MamB and MamM form heterodimers and interact with other magnetosome proteins suggesting that magnetosome formation is a complex process that likely involves the coordinated interactions of many different proteins and genes. Suzuki et al. [2006] demonstrated that genes encoding ferrous iron transporter proteins are upregulated, whereas genes encoding ferric iron transporter proteins are downregulated in magnetotactic bacteria during biomineralization. Surprisingly, there was no change in the expression patterns of the cation diffusion facilitator transporter proteins described above.

Nucleation and controlled maturation of the magnetite crystal occurs within the magnetosome invagination/vesicle. Magnetite (Fe_3O_4) and greigite (Fe_3S_4) are both mixed-valence minerals, featuring Fe^{2+} and Fe^{3+} centers in a 1:2 ratio ($Fe^{2+}Fe^{3+}{}_2O_4$, $Fe^{2+}Fe^{3+}{}_2S_4$) [Chang et al., 2009]. Therefore, the synthesis of magnetite (or greigite) in magnetosomes is clearly a more complex process than just concentrating iron within the magnetosome invagination/vesicle. As with other steps involved in magnetite or greigite biomineralization in magnetosomes, the crystallization process is not well understood. It has been suggested that magnetite precipitation occurs through the reduction of hydrated ferric oxide(s) [Frankel et al., 1979, 1983; Schüler and Baeuerlein, 1998]. This seems unlikely since cells of *M. gryphiswaldense* shifted from iron-limited to iron-sufficient conditions showed no delay in magnetite production [Heyen and Schüler, 2003], indicating that no mineral precursors to magnetite exist in this organism [Faivre et al., 2007; Heyen and Schüler, 2003] during biomineralization or that they are unstable and transform to magnetite extremely quickly. Faivre et al. [2007] discovered a ferritin-like protein in the membrane fraction of *M. gryphiswaldense* produced during biomineralization. Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion [Theil, 1987]. It was suggested that iron contained within the ferritin-like protein coprecipitates soluble ferrous iron to form magnetite crystals within the cell membrane, which are then transported into the magnetosome invagination/vesicle [Faivre et al., 2007].

Among the proteins that may be involved in magnetosome magnetite crystal maturation, the cytosolic protein FtsZ is a ubiquitous tubulin-like protein in bacteria that polymerizes into an oligomeric structure that forms the initial ring at mid-cell and thus has an essential role in cytokinesis [Errington et al., 2003]. An *ftsZ*-like gene is present in the MAI of *Magnetospirillum* within the *mamXY* cluster. Like FtsZ, the FtsZ-like protein is able to form filaments in vitro that is GTP-dependent [Ding et al., 2010]. When the *ftsZ*-like gene was deleted in *M. gryphiswaldense*, cell division was unaffected but the magnetite crystals were significantly smaller than those of the wild-type resulting in nonmagnetotactic cells [Ding et al., 2010]. Interestingly, there was no detectable change in the magnetite crystals of *M. magneticum* when this gene was deleted [Murat et al., 2010].

Several magnetosome membrane proteins appear to be involved in controlling the morphology of magnetosome magnetite crystals. For example, MamC (Mms12 and Mms13), MamD (Mms7), MamF, MamG (Mms5), Mms6 are believed to be important in this role of magnetosome biomineralization [Arakaki et al., 2003; Fukuda et al., 2006; Grünberg et al., 2004; Taoka et al., 2006]. MamC, MamD and MamG account for about 35% of all magnetosome membrane proteins [Scheffel et al., 2008]. With the exception of MamC, these proteins contain glycine-leucine repeat motifs that are also present in the silk fibroin protein known to control the biomineralization of calcium carbonate minerals [Cheng et al., 2008]. Mms6 is an amphiphilic protein consisting of an N-terminal leu-

cine-glycine-rich hydrophobic region and a C-terminal hydrophilic region containing many acidic amino acids [Arakaki et al., 2003; Prozorov et al., 2007]. Mms6 has been shown to bind iron and control the morphology of magnetite crystals precipitated in vitro [Arakaki et al., 2003; Prozorov et al., 2007]. Experiments involving gene knockout mutants of *mms6* in *M. magneticum* resulted in elongated magnetite crystals, rather than the normal cuboctahedron typical of species of this genus, that were also 50% smaller compared to those of wild-type cells [Tanaka et al., 2011]. Interestingly, while the Mms proteins appear to be very important in controlling mineral morphology, these proteins have so far only been found in magnetotactic bacteria belonging to the Alphaproteobacteria. This suggests that other phylogenetic groups of magnetotactic bacteria, such as the Deltaproteobacteria (e.g. *D. magneticus*) and the Nitrospirae (e.g. *Magnetobacterium bavaricum*), which synthesize bullet- or tooth-shaped crystals, must rely on a different set of proteins to control crystal morphology. Lastly, for most magnetotactic bacteria, there is construction of the magnetosome chain.

One major protein in this role is MamK, a protein whose amino acid sequence is homologous to the bacterial actin-like protein MreB [Schübbe et. al., 2003]. Actin-like proteins are found in all prokaryotes and are important for maintaining proper cell morphology and elongation, peptidoglycan synthesis and plasmid DNA segregation [Dominguez-Escobar et al., 2011; Figge et al., 2004; Garner et al., 2011; Jones et al., 2001]. Taoka et al. [2007] showed that MamK associates with magnetosomes and forms filaments in an ATP-dependent manner similar to other bacterial actin-like proteins. The deletion of *mamK* in *Magnetospirillum* species did not inhibit magnetosome formation. However, in *M. magneticum* it resulted in cells containing magnetosomes that were scattered throughout the cell and not organized into a chain [Komeili et al., 2006]. Knockout mutants of *mamK* in *M. grysphiswaldense* resulted in magnetosome chains that were shorter than those of the wild-type strain and gaps were present within the chain where magnetosomes were missing [Katzmann et al., 2010]. In addition, the magnetosome cytoskeleton within the bacteria was no longer visible near the magnetosome chain [Katzmann et al., 2010, 2011]. These results suggest that MamK functions to recruit nascent magnetosomes to the growing chain structure or that MamK maintains proper positioning of the mature magnetosome chain in the cell after the chain has been fully formed and perhaps during cell division.

The *mamJ* gene, which is cotranscribed with *mamK*, is immediately upstream of *mamK* within the *mamAB* gene cluster and presently only found in *Magnetospirillum* species [Abreu et al., 2011; Jogler et al., 2009, 2011; Nakazawa et al., 2009: Schübbe et al., 2006, 2009]. MamJ is an acidic protein with a repeating glutamate-rich domain [Scheffel et al., 2006] that is typical of some proteins involved in the biomineralization of calcium carbonate [Endo et al., 2004]. When *mamJ* was deleted in *M. grysphiswaldense*, cells synthesized magnetosomes but failed to form linear chains and formed clumps of magnetosomes instead [Scheffel et al., 2006]. However, in *M. magneticum*, the phenotype of a codeletion of *mamJ* with the paralogous *limJ* gene resulted in interrupted, shorter magnetosome chains [Draper et al., 2011]. The MamJ protein appears to act as an adhesive-type protein that anchors magnetosomes to MamK filaments in *Magnetospirillum* species [Komeili et al., 2006; Scheffel et al., 2006; Scheffel and Schüler, 2007].

Once the Mam and Mms proteins are expressed within the cell, there must be a mechanism in place that physically sorts these proteins inside the cell and then arranges them within the magnetosome membrane. Recent studies suggest that three proteins, MamE, MamO and MamP, play important roles in arranging proteins into functional complexes within the magnetosome membrane [Murat et al., 2010; Quinlan et al., 2011; Yang et al., 2010]. These proteins exhibit sequence similarity to HtrA-like serine proteases, heat-shock induced serine proteases first discovered in *E. coli* [Lipinska et al., 1989] that function to degrade misfolded periplasmic proteins [Pallen and Wren, 1997]. These proteases may also act as chaperone proteins assisting in protein localization and processing [Fanning and Anderson, 1996]. Murat et al. [2010] demonstrated that a deletion of *mamE* in *M. magneticum* resulted in magnetosome proteins that were not properly localized to the magnetosome, suggesting that MamE is important for protein sorting and proper arrangement of magnetosome proteins within the magnetosome membrane. Individual deletions of *mamE* and *mamO* resulted in cells that were still capable of producing magnetosome chains within the bacteria; however, the individual magnetosomes did not contain mineral nanoparticles [Murat et al., 2010; Yang et al., 2010]. These results taken together show that MamE and MamO are essential for magnetite biomineralization probably by actively sorting magnetosome proteins to help localize specific proteins to the magnetosome membrane.

Concluding Remarks

The unique organelle, the bacterial magnetosome, has stimulated great interest over the years in numerous research fields including microbiology, geology, paleomagnetism, geochemistry, physics, geophysics, materials science and astrobiology providing insight and partial answers to questions regarding the origin of life on Earth, evidence for life on extraterrestrial bodies in meteorites [Thomas-Keprta et al., 2002], what represents a reliable prokaryotic fossil [Jimenez-Lopez et al., 2010], how proteins catalyze solid mineral crystals, and so on. In addition, cells of magnetotactic bacteria and especially magnetosomes have many unique properties as magnetic crystals partially due to their enveloping magnetosome lipid bilayer membrane. For this reason, their novel magnetic, physical and optical properties have been exploited in numerous different scientific, commercial and other applications [Arakaki et al., 2008; Lang and Schüler, 2006; Lang et al., 2007; Matsunaga and Arakaki, 2007; Xie et al., 2009] and the number of applications and patents involving magnetotactic bacteria continues to be ever increasing [e.g. Alphandéry et al., 2011a, 2011b]. One of the more recent interesting and perhaps controversial findings regarding the regulation of expression of some magnetosome membrane genes is that several, including *mamA*, appear to upregulated in cells exposed to static and pulsed magnetic fields [Wang and Liang 2009; Wang et al., 2009]. While we show that a great deal of progress has been made in the elucidation of the biologically controlled mineralization process involved in magnetosome synthesis, many questions and problems remain, surely enough to keep researchers in magnetotactic bacteria busy for a long time!

Acknowledgements

We are grateful to our students, especially Z. Oestreicher for electron microscopy, postdocs, and numerous collaborators, in particular R.B. Frankel and C.T. Lefèvre. We are supported by US National Science Foundation grants EAR-0920718 and B.H.L. also by EAR-0920299.

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