Magnetotactic Bacteria and Magnetosomes

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1. Introduction

Iron biominerals are formed by a broad host of organisms, in which they serve various functions. For example, iron oxides formed by organisms serve for strengthening of tissues[1] and hardening of teeth.[2] Iron biominerals are also associated with iron overload diseases and involved in intracellular iron storage and detoxification and sensing of magnetic fields,[3–6] and crystals of magnetic iron minerals have been found even in the human brain.[7–9]

One of the most intriguing examples for the biological synthesis of iron minerals, and biomineral formation in general, is represented by the biomineralization of magnetic minerals in bacterial magnetosomes. Magnetosomes are specialized organelles synthesized by magnetotactic bacteria (MTB) for geomagnetic navigation in their aquatic habitats. The magnetosomes comprise membrane-enveloped, nano-sized crystals of either the magnetic iron oxide magnetite, Fe_3O_4 or, less commonly, the magnetic iron sulfide greigite, Fe_3S_4. The magnetosomes are arranged in intracellular chains that enable the cell to align and swim along external magnetic fields, a behavior known as "magnetotaxis".[10] Magnetotaxis is thought to facilitate the dwelling of bacteria within their growth-favoring microoxic zones on the bottom of chemically stratified natural waters.[11]

The synthesis of bacterial magnetosomes is achieved by a high degree of control over the biomineralization of perfectly shaped and sized magnetic crystals, and their assembly into a highly ordered chain structure to serve most efficiently as a magnetic field sensor. The unique characteristics of magnetosome biomineralization have attracted a broad interdisciplinary interest and might be exploited for a variety of applications in diverse disciplines from microbiology, cell biology, and geobiology to biotechnology.[12,13] The biomineralization of magnetosomes and their assembly into chains is of great interest for the generation of bioinspired magnetic materials and has even been suggested as a biomarker to detect extraterrestrial life.[14] In addition, the formation of magnetosomes is a fascinating example of how supposedly primitive organisms can translate genetic blueprint information into complex inorganic and cellular structures. As many of the fundamental mechanisms of biomineralization are found in bacterial magnetosome biomineralization, magnetotactic bacteria may serve as an accessible and relatively simple model for studying and understanding biomineralization processes in general.

Although several early reports by Salvatore Bellini (and perhaps even earlier by J. Massart) described the observation of bacteria, in which the swimming direction was apparently

Damien Faivre studied physical chemistry at the University Claude Bernard (Lyon, France), before joining Prof. G. Peshlerbe's group in 1999 at Concordia University (Montreal, Canada) to study theoretical nanochemistry. He returned to France in 2000, where his interests in magnetite started during his doctoral studies at the University Denis Diderot (Paris). He studied the geochemical properties of abiogenic magnetite and their implications on the definition of biogenicity criteria under the supervision of Prof. P. Zuddas. He was awarded an IPGP Fellowship to spend a research semester at the California Institute of Technology (Pasadena, USA) working in Prof. D. Newman's group with Dr. A. Komeili, where he for the first time came into contact with the fascinating magnetotactic bacteria. In 2005, he left France again, this time with a Ph.D. and for Germany, where he worked first as a Max Planck then as a Marie-Curie postdoctoral fellow in Prof. D. Schüler's group at the Max Planck Institute of Marine Microbiology (Bremen), where he got involved in various projects, specifically focusing on unraveling the biomineralization pathway of magnetosome formation. Since August 2007, he has been a research group leader in the biomaterials department of the Max Planck Institute of Colloids and Interfaces (Potsdam, Germany). His research interests range from magnetic nanoparticles in biosystems, with a particular emphasis on the physicochemical aspects of the magnetosome biomineralization by magnetotactic bacteria, to the development of molecular biomimetics for the formation and assembly of hybrid magnetic nanomaterials.

affected by magnetic fields[15,16] (http://www.calpoly.edu/~rfrankel/SBellini1.pdf), it was Richard Blakemore's serendipitous discovery of magnetotactic bacteria[10] that initiated and stimulated a wealth of research activities during the last few decades. Since Blakemore's seminal paper in 1975, the subject of magnetosome biomineralization has evolved into an interdisciplinary and unique field of research. The aim of this review is to give a broad overview over the current state of knowledge on the biology of magnetotactic bacteria and the structure of the biominerals formed by them. Emphasis is given on recent research studying the processes of magnetosome biomineralization at the physicochemical, biochemical, and genetic levels. Further, we will summarize new insights into the cell biology of bacterial mineral formation, and potential applications of magnetosome biomineralization in bio- and nanotechnology will be presented. Finally, we will discuss new perspectives and possible directions for future studies.
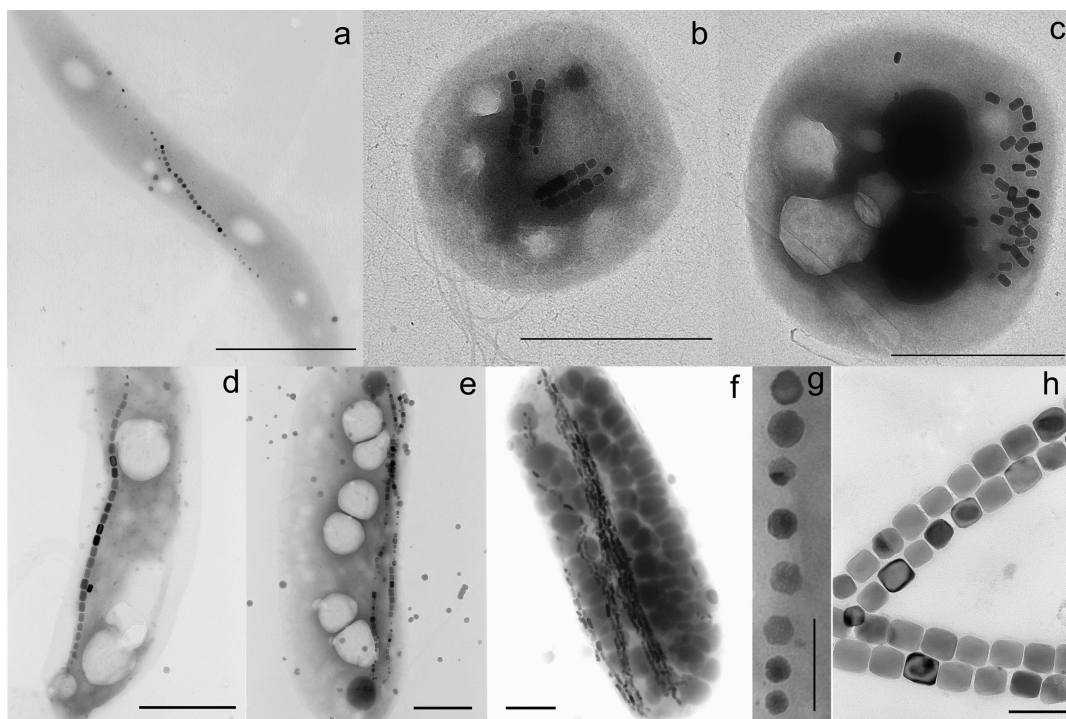
2. Magnetotactic Bacteria

2.1. Morphologic and Phylogenetic Diversity

MTB are aquatic prokaryotes that are diverse with respect to morphology, physiology, and phylogeny. Cell morphologies include rods, vibrios, spirilla, cocci, and ovoid bacteria as well as giant and multicellular MTB (Figure 1). All studied MTB are motile by means of flagella and have a cell wall structure characteristic of Gram-negative bacteria.[17] Despite the fact that only a minority of MTB can be grown in the

Dirk Schüler became fascinated by magnetotactic bacteria during his undergraduate studies at the Ernst Moritz Arndt University Greifswald (Germany), where he started working on the isolation of these microorganisms in the group of Manfred Köhler (1990). In 1991, he joined the laboratory of Edmund Bäuerlein at the Max Planck Institute of Biochemistry in Martinsried (Germany) to study biomineralization in *Magnetospirillum gryphiswaldense*, the bacterial strain he had isolated during his diploma thesis. In 1994, he finished his doctoral thesis, and in 1996, he joined the laboratory of Dennis Bazylinski at Iowa State University (USA). His second postdoctoral position was at the Scripps Institute of Oceanography (University of California, San Diego, USA), in the laboratory of Brad Tebo, where he started to do research on the molecular genetics of magnetosome formation. In 1999, he returned to Germany to join the Max Planck Institute for Marine Microbiology (Bremen) as the head of a research group. He completed his habilitation at the University of Bremen in 2004 and was appointed as an associate professor for microbiology at the Ludwig Maximilians University of Munich in October 2006. His research focus is on physiology, cell biology, and genetics of magnetosome biomineralization by magnetotactic bacteria, and their potential application in nanobiotechnology.

laboratory, their diversity can be studied by molecular methods without prior cultivation.[18] Based on 16S rRNA sequence similarity, MTB are polyphyletic, but all known MTB belong to the domain *Bacteria*.[18] Most of the cultured MTB and many of the so far uncultured phylotypes cluster within the *Alphaproteobacteria*, but MTB have also been affiliated to *Deltaproteobacteria*, to the phylum *Nitrospira*, and, tentatively, also to *Gammaproteobacteria*.[18] Within the alphaproteobacteria, MTB are closely related to the non-magnetic, photosynthetic, nonsulfur purple bacteria, with which they share the ability to form intracytoplasmic membranes. In this respect, it is interesting to note that the formation of magnet-sensitive inclusions could be induced by incubation in iron rich medium in some species of nonmagnetotactic photosynthetic purple bacteria; however, the intracellular iron-rich granules were distinct from magnetosome crystals.[19] Cultured species include *Magnetospirillum magnetotacticum* strain MS-1,[20] *M. gryphiswaldense* MSR-1,[20,21] and *M. magneticum* strain AMB-1,[22,23] isolated by R. Blakemore, D. Schüler, and T. Matsunaga and co-workers, respectively. Several cultured freshwater magnetotactic spirilla have not yet been completely described.[24,25] It has been noted that the genus *Magnetospirillum* also comprises a growing number of species, which resemble the magnetotactic strains with respect to morphology and phylogeny; however, they are unable to biomineralize magnetosomes.[26] Other cultured MTB of the *Alphaproteobacteria* are the marine vibrio strains MV-1 and MV-2,[27] a marine spirillum strain MMS-1,[28] and strain MC-1, all isolated by D. Bazylinski. The marine strain MC-1 represents the only cultured magnetotactic coccus,[29,30] which is representative of other, yet uncultured, magnetococci that are among the

Figure 1. Electron micrographs of various magnetotactic bacteria and magnetosome chains. These images show the diversity of the cell morphology, of the magnetosomes, and of the arrangement of magnetosomes in bacteria: (a) a spirillum with a single chain of cubooctahedral magnetosomes, (b) a coccus with two double chains of slightly elongated prismatic magnetosomes, (c) a coccus with clustered, elongated magnetosomes, (d) a vibrio with elongated prismatic or cubooctahedral magnetosomes arranged in a single chain, (e) a vibrio with two chains, and (f) a rod-shaped bacterium with bullet-shaped magnetosomes arranged in a multitude of chains. Scale bars in (a−f) represent 1 μm. Part g shows a chain from a similar type of MTB as in part a, and part h shows one from a bacterium as in part b. Scale bars in parts g−h represent 100 nm. Images d−f are courtesy of A. Isambert.

most abundant MTB in many freshwater and marine habitats.[25,31−35] The magnetite-containing sulfate reducing strain *Desulfovibrio magneticus* strain RS-1 affiliates with the genus *Desulfovibrio* of the *Deltaproteobacteria*.[36,37] Clearly, the phylogenetic and morphological diversity of naturally occurring, as yet uncultivated MTB goes beyond that of the cultivated strains. For instance, a "barbell" shaped bacterium was recently reported from a marine sulfidic pond, which was reported to affiliate with the genus *Desulforhopalus*.[38] One of the most unusual morphological types of MTB is a marine, greigite forming multicellular magnetotactic prokaryote ("MMP"), which consists of a compact assembly of flagellated cells that perform a coordinate biomineralization and magnetotactic motility.[39−43] The MMP, with one tentatively named representative Candidatus *Magnetoglobus multicellularis*,[44] is related to the dissimilatory sulfate-reducing bacteria within the *Deltaproteobacteria*, in particular to its cultured relative *Desulfosarcina variabilis*.[30,44] Preliminary evidence for the existence of magnetotactic *Gammaproteobacteria* was reported,[45] which suggests that the diversity of uncultivated MTB might be even larger than previously estimated. Uncultured MTB are not restricted to *Proteobacteria*. A very large, rod-shaped bacterium, Candidatus *Magnetobacterium bavaricum*, has been found by N. Petersen and colleagues in the sediments of calcareous freshwater lakes in Bavaria and was affiliated with the *Nitrospira* phylum.[45] The occurrence of this unusually large bacterium was first reported from oligotrophic sediments in various Bavarian lakes.[18,46−48] However, in more recent studies, MTB with morphologies similar to *M. bavaricum* were detected, for instance in the Seine river[49] and other freshwater habitats worldwide, and it will be interesting to be see if these represent similar phylogenetic lineages.

Recently, a related bacterium described as "Cand. *Magnetobacterium bremense*" or strain MHB-1 from the *Nitrospira* phylum was detected in a freshwater sediment.[18,25]

2.1.1. Physiology

The isolation and axenic cultivation of magnetotactic bacteria in pure culture have proven to be difficult.[50] All of the relatively few isolates biomineralize Fe_3O_4, but there are currently no greigite-producing strains available in pure culture. Thus, the metabolic characteristics of this group are largely unknown. One established fact is that MTB are metabolically versatile (for a detailed review, see ref 28). All known magnetotactic bacteria are microaerophiles, or anaerobes or facultative anaerobes, which means that growth and magnetite formation are repressed by higher concentrations of oxygen. However, it was determined that in cultured magnetospirilla the regulatory oxygen concentration for magnetite biomineralization is significantly lower than the maximum tolerated oxygen level for growth; that is, microaerophilic magnetospirilla can still grow nonmagnetically at oxygen concentrations that already suppress magnetite precipitation.[51−53]

Strains of *Magnetospirillum* can oxidize short-chained organic acids by respiration of oxygen or nitrate. The strain MV-1 can respire on nitrous oxide, whereas *Desulfovibrio magneticus* strain RS-1 and perhaps the uncultured greigite-producers are able to grow respiratorily by the oxidation of sulfate. Cells of strains MV-1, MC-1, and probably other MTB can utilize reduced sulfur compounds for growth.[28,29,54] The ability of chemoautotrophic growth seems to be a common trait among MTB and has been either demonstrated experimentally or predicted based on the presence of genes

encoding ribulose-1,5-bisphosphate carboxylase (RubisCO), which is a key enzyme of autotrophic carbon fixation.[28,55,56] In addition, many strains display nitrogenase activity and can fix atmospheric nitrogen.[57,58]

The fact that MTB handle and accumulate large amounts of iron has led to the suggestion that magnetosome biomineralization is the main result or byproduct of energy-yielding pathways.[59−61] The production and extracellular deposition of magnetic iron oxides or magnetic sulfide minerals as a result of energy-yielding metabolic reactions has been reported for a diverse range of ferrous iron-oxidizing, ferric iron-reducing, or sulfate-reducing nonmagnetic bacteria.[62] However, although apparently all cultivated MTB can either reduce or oxidize extracellular iron for growth and magnetosome synthesis,[28] no energy conservation associated with redox reactions of iron has been demonstrated unambiguously so far, and none of the cultured strains can utilize extracellular ferric iron or ferrous iron as electron acceptor or electron donor for growth. It has been calculated by Blakemore[59] that the amount of iron deposited within the ∼20−50 magnetosomes of most cultured strains is too small to contribute significantly to the energy metabolism of the cells. This situation, however, could be different for some of the as yet uncultured giant species, such as the Cand. *Magnetobacterium bavaricum*, which contains up to 1000 and more magnetite particles per cell.[63]

2.2. Ecology

MTB are ubiquitous in aquatic environments and cosmopolitan in distribution, although populations on the Northern and Southern hemispheres exhibit opposite magnetotactic polarity (North-seeking versus South-seeking; see section 5.2. on magnetotaxis). Beside a singular report of MTB from a waterlogged soil,[64] MTB have been found exclusively in water columns or sediments with vertical chemical stratification, and with the apparent exception of extreme environments such as acidic mine tailings and thermal springs, MTB occur ubiquitously in a wide range of different aquatic habitats.[10,25,27,31,32,42,45,49,63,65−71] The bacteria can be easily detected by separating them from mud or water samples by utilizing their directed motility in the presence of magnetic fields and subsequent microscopic observation in the hanging drop assay.[25,50,72] The occurrence and distribution of MTB is correlated with multiple vertical chemical gradients. Most MTB are typical gradient organisms, meaning both that they derive energy for growth from the proximity of reductants and oxidants at a chemical interface and that they occur in thin layers at particular locations relative to this interface.[73] These environmental conditions are difficult to reconstruct in laboratory cultures, which seems to be one of the reasons for the difficulties with the axenic isolation of many species.

2.2.1. Freshwater Habitats

Freshwater sediments were found to contain various morphological types of MTB, including rod-shaped, vibrio-like, coccoid, and helicoidal forms. In freshwater systems, only magnetite-producing MTB have been found. In contrast to what might be anticipated from their potential to accumulate large amounts of iron, habitats of MTB are typically characterized by low to moderate contents of iron (0.01−1 mg/L), and addition of iron does not increase the enrichment of MTB.[59,25,50] On the contrary, cultured MTB only tolerate extracellular iron concentration in the micromolar to low

millimolar range.[74] This implies that MTB are able to accumulate iron against a large concentration gradient. Remarkably, MTB were also detected in highly alkaline and saline environments such as in Mono Lake, California,[75] which is interesting, since these environments are iron limited due to the low solubility of the metal at high pH.

Different environments appear to select specific populations of MTB. It was reported by Flies et al. that mud sediments with a relatively high content of organic material are often dominated by populations of various magnetic cocci from the *Alphaproteobacteria*.[25] Upon prolonged storage of sediment samples in undisturbed laboratory scale microcosms, the MTB community composition underwent a drastic succession during incubation, ultimately resulting in the dominance of a single magnetococcus species. Consistent with earlier reports,[76] neither the amendment of the microcosms with various electron donors and acceptors, such as iron, sulfate, or nitrate, nor the variation of aerobic to anaerobic growth conditions resulted in increased numbers or diversity of MTB.[25] Remarkably, significant heterogeneities (i.e., succession and dominance of different MTB phylotypes) were observed between microcosms from the same environmental sources that were incubated under apparently identical conditions, indicating that the environmental parameters which select for particular species must be very subtle.

The highest numbers of MTB (10^5 to 10^6 mL^{-1}) were reported close to the oxic anoxic transition zone.[34,59,63] In a systematic study by Flies and co-workers, the occurrence of MTB in freshwater environments was correlated with various geochemical parameters.[32] It was demonstrated that the distribution of MTB in a microcosm was confined to a rather narrow sediment layer a few millimeters below the mud surface, whereas nearly no magnetotactic cells were found in deeper sediment layers or in the higher water columns. Different species showed different preferences within vertical gradients, but the largest proportion (63−98%) of MTB was detected within the suboxic zone and also below the maximum penetration depth of nitrate. In one microcosm, the community of MTB was dominated by one species of a coccoid *Alphaproteobacterium* in sediment horizons from 1 to 10 mm depth. Maximum numbers of MTB were up to 1.5×10^7 cells cm^{-3}, which corresponded to 1% of the total cell number in the upper sediment layer. The occurrence of MTB coincided with the availability of significant amounts (6−60 μM) of soluble Fe(II) and in one sample with hydrogen sulfide (up to 40 μM). Surprisingly, there was no clear relationship between the position of MTB within the sediment and the presence of either nitrate or oxygen, which are electron acceptors commonly used by many cultured species.[28] It therefore was discussed that MTB in these microcosms are metabolically inactive or use different, as yet unidentified electron acceptors.[32]

It seems that habitats that are low in nutrients are more diverse with respect to different morphotypes of MTB. At least 10 morphologically distinct MTB were found in oligotrophic lake sediment.[77] In certain sediment horizons of the same habitat, cells of the uncultured Cand. *Magnetobacterium bavaricum* were the dominant fraction of the microbial community and accounted for up to 30% of the biovolume.[63]
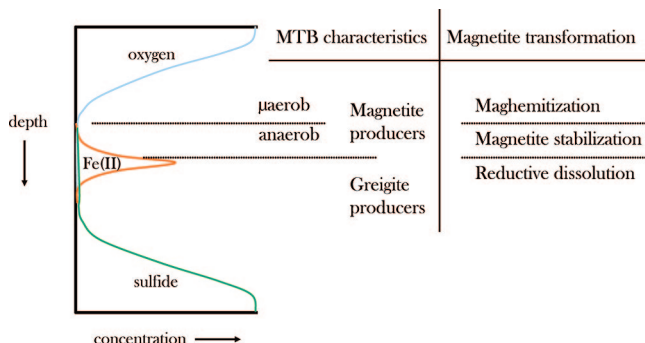
Figure 2. Schematic representation of geochemical gradients below the water−sediment interface. Magnetite producing bacteria can live in microaerobic to anaerobic conditions, whereas greigite producers require the presence of sulfide in anaerobic conditions. The diagenesis of magnetite after bacterial lyses strongly depends on the environmental conditions, as can be seen on the right-hand side, and thus influences the use of magnetite as biomarker (see the text for discussion).

2.2.2. Marine Habitats

In the marine environment, MTB have been detected so far mostly in coastal environments, although some studies indicate their apparently widespread occurrence in the ocean down to depths of 3000 m.[78,79] Generally, morphological types similar to freshwater MTB are found in marine habitats, although some sulfidic sediments are characterized by the presence of unique morphological forms, notably the MMP, which was identified in marine and brackish sediments, as mentioned above.[39,40,42,80] Both Fe_3O_4- and Fe_3S_4-producers have been found in marine environments. A wide variety of MTB are abundant in salt marshes, particularly in the surface layers of sulfidic sediments where they co-occur with photo- and chemosynthetic sulfide-oxidizing bacteria.[73] A virtually homogeneous population of ovoid−coccoid magnetotactic bacteria was found in the low tide subregion of a pond in the China Sea, and it was suggested that this population is specifically adapted to the marine tidal conditions.[81] As in freshwater environments, the occurrence of MTB is usually restricted to the upper layer of the sediment. However, in some chemically stratified estuarine basins, MTB were found to occur in the microaerobic layer of the water columns, as exemplified by Pettaquamscutt Lower Basin (RI, USA) and Salt Pond (MA, USA), which were analyzed in some detail with respect to the geochemistry and occurrence of MTB.[73,82] Since seawater contains high concentrations of sulfate (28 mM sulfate), hydrogen sulfide generated by the action of anaerobic sulfate-reducing bacteria diffuses upward from anoxic sediment layers into the water column, causing the oxic−anoxic transition to occur in the water column. This results in an inverse oxygen. sulfide concentration double gradient where O_2 diffuses downward from air at the surface and S^{2-} diffuses upward from the anaerobic zone.[28,73] Consistent with freshwater habitats, MTB were most abundant within and below the chemocline,[73,82] which corresponds to a boundary between magnetite and greigite producing MTB, with magnetite producers occurring higher in the water column than greigite producers[73,82] (Figure 2). Remarkably, an organism capable of coprecipitating magnetite and greigite was found in high numbers in the Pettaquamscutt River Estuary.[83] In a series of studies by Simmons et al., the dominant magnetite producers were uncultivated cocci closely related to the microaerophilic alphaproteobacterium MC-1. In a freshwater system,[32] their abundance peaked directly above the peak of the ferrous iron

concentration, a typical distribution pattern for sulfide oxidizers. Concentrations of 10^5 cocci mL^{-1} and 10^3 cocci mL^{-1} were observed in the Pettaquamscuttin and Salt Pond, respectively. An apparently new South-seeking MTB with a unique barbell-shaped morphology and unknown mineral content was found in higher numbers than all other MTB groups and in the late season comprised 2−10% of total eubacteria.[38] The distributional patterns of the barbell, along with the metabolic capabilities of coexisting species, suggest that the barbell either is a sulfate reducer with a low tolerance for sulfide or is capable of growth on intermediate sulfur compounds.

The MMP was also detected in the stratified Salt Pond and typically occurred at about 500 cells mL^{-1}.[38,70] The MMP comprised $1.9 \pm 1.4\%$ of all bacteria in Salt Pond sediments during early stratification in June, suggesting that MTB survive the oxic, mixed, water column during winter by retreating to the sediments. A large greigite-producing rod distantly related to *Thiomicrospira crunogena* was found at low cell abundances (100 cells mL^{-1} on average) from the base of the chemocline into the hypolimnion, where sulfide concentrations do not change much during seasonal stratification. Its distribution also indicates that it is not a gradient organism like the other three groups of MTB described here. Higher concentrations of unidentified large rods (10^5 cells mL^{-1}) were observed in sulfidic waters of the Pettaquamscutt, suggesting that greigite producers can occasionally reach higher concentrations.[38,45,69,70,73]

2.3. Geobiology

Given their high abundance, diversity, and metabolic versatility, MTB very likely play an important ecological role in marine and freshwater habitats, such as for instance in biogeochemical cycling of iron and other elements.

2.3.1. Iron and Sulfur Cycling

Considering the tremendous amounts of iron that MTB can accumulate (up to 4% dry weight,[52] 10^{-13} to 10^{-15} g of Fe per cell[73]) and their estimated population density, one can estimate that MTB substantially contribute to the flux of iron to the sediment, especially in chemically stratified marine environments where this contribution can reach 1−10%.[73,84] However, this contribution to iron cycling in the environment still has to be addressed quantitatively.

The potential contribution of MTB to sulfur cycling might also be very significant. In fact, the cultivated marine, and probably also many freshwater magnetite-producing, MTB are chemolithoautotrophic S-oxidizers.[85] Many form intracellular sulfur granules, probably for use as electron donors or intermediates during oxidation of reduced sulfur compounds.[31,55,63,86] In turn, greigite-producing MTB, which have not yet been isolated in axenic culture, should sequester a large amount of sulfur, which could also make the MTB a significant parameter in the sulfur geochemical cycle.

2.3.2. Fossil Record and Sediment Magnetization

Due to their magnetic alignment in the geomagnetic field, magnetosome chains potentially contribute after death to the natural magnetization of the sediment by their magnetosomes, which then become magnetofossils.[87] The single-domain particle size of the magnetosomes and thus of the magnetofossils make them ideal carriers of paleomagnetic

information.[88−90] However, as fossil bacterial magnetite usually occurs in sediments and soils as part of a mixture of different magnetic components, including detrital, authigenic, anthropogenic, and diagenetic phases, the biogenic signal can be blurred and thus other properties need to be used to differentiate the magnetic signals.[91−93] Moreover, due to their small particle size, magnetofossils tend to dissolve under reducing conditions or to undergo low temperature oxidation in the presence of oxygen; that is, the magnetosomes can be oxidized into maghemite in the so-called maghemitization process[73] (see Figure 2). Both processes will affect the magnetic properties of the sediment.[94] Based on the introductory work of Petersen et al.[95] and the subsequent extensive characterization of the MTB magnetic properties by Moskowitz et al.,[96,97] several groups have worked on the establishment of reliable criteria for the origin of magnetite crystals, based on magnetic,[98−100] isotopic,[101] or mineralogical[102−105] properties or combination of properties.[106−109] Using these, there is an ongoing debate about the origin of magnetite nanocrystals discovered in the Martian meteorite ALH 84001 and about the possibility of life on Mars,[14,103,110,111] based on the use of the magnetosomes as biomarkers. It has been shown that the magnetofossils can carry up to 10% of the total sediment magnetization, e.g., in Lake Chiemsee in Southern Germany.[47,93] Further research will be required to obtain a precise comprehension of the stability of magnetosomes in the sediment or other environments in order to use the magnetofossils as reliable paleotracers.
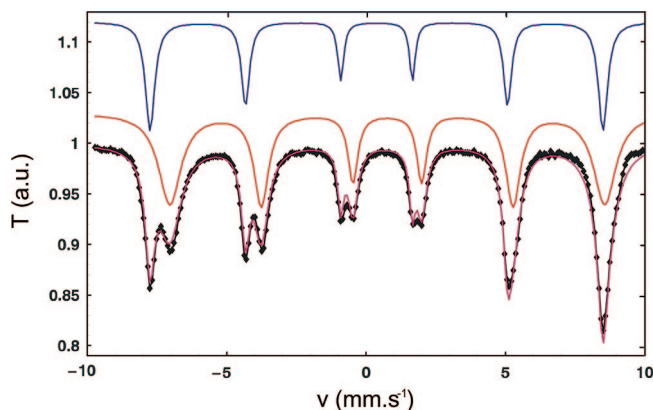
3. Magnetosomes

Magnetosomes are the key components of MTB, as they are dedicated organelles specific for the magnetotactic lifestyle. All MTB contain magnetosomes, which are intracellular structures comprising magnetic iron-bearing inorganic crystals enveloped by an organic membrane.[112,113] We will start this section by first describing the inorganic core and the organic membrane, then describe how the magnetosomes are biomineralized, and finally discuss how the organic phase of these specified organelles may control the mineral properties.

3.1. The Inorganic Core

As mentioned before, the mineral part of the magnetosome can be composed of either an iron oxide[114] or an iron sulfide mineral.[40,42] In the following, the physicochemical and mineralogical properties of the inorganic part of the magnetosomes will be described.

3.1.1. Structure of Magnetic Crystals

Magnetite. The structure of the mineral part of the magnetosomes was originally determined by electron diffraction[115−118] for the biogenic iron oxide mineral. The results of these studies identified the mineral as the inverse spinel magnetite (Fe_3O_4, $Fe^{2+}Fe^{3+}{}_2O_4$, $Fe^{3+}{}_{Td}.Fe^{2+}Fe^{3+}{}_{Oh}.O_4$, $Fd3m$ space group, cell dimension $a = 8.39$ Å). Magnetite is usually found in igneous and metamorphic rocks. The mineral is known for its magnetic properties (see section on magnetosomes chains). However, electron diffraction is not accurate enough to unambiguously reveal the unit-cell parameter of the mineral of interest, as magnetite and maghemite only present slight differences in their diffraction parameters and magnetite nanoparticles can be readily oxidized to form maghemite. For the purpose of magnetite/maghemite differentiation, ^{57}Fe Mössbauer spectros-

Figure 3. Mössbauer spectrum of isolated mature magnetosomes at 130 K. The experimental data are best fitted (green) by the superposition of the magnetite A site (blue) and the magnetite B site (red). For a comprehensive explanation of the spectrum, the reader is referred to ref 120 The spectrum is courtesy of L. Böttger and B. Matzanke, University of Lübeck.[120]

copy was used. However, due to the fact that a large amount of material is required, the use of the technique has been limited to cultured strains, including of *M. magnetotacticum*, the magnetic vibrio MV-1, and *M. gryphiswaldense*.[27,119,120] In every case, the spectrum of the magnetic mineral exclusively indicates magnetite (Figure 3). Small-angle neutron and X-ray scattering were also performed and confirm the presence of magnetite in MS-1 cells.[121] Finally, another technique that could convincingly differentiate magnetite from maghemite without requiring a large amount of magnetosomes is electron energy-loss spectroscopy (EELS). This technique can indeed unravel the oxidation state of iron in a sample analyzed in the TEM.[122] EELS was successfully employed for the study of abiotic magnetite[101] but to our knowledge has still to be performed on MTB. Microstructural characterization of the magnetosomes has indicated that they are essentially free of internal defects.[123,124] However, twinned and multiple-twinned crystals are frequently observed in several bacterial strains[125] (Figure 4d). The reported twins obey the spinel twin law[126] (the twin members share common (1 1 1) planes and are related by a rotation of 180° around the [1 1 1] direction) that does not affect the magnetic properties of the crystals because the easy axis of magnetization is ⟨1 1 1⟩, i.e., the direction of the twin axis.[127] In addition to twinnings, a few defects interpreted as stacking faults were also reported.[125] Finally, elongation along a different axis than the axis of easy magnetization (the [1 1 1] axis) was also reported by several groups, mostly for tooth- or bullet-shaped magnetosomes.[49,128−131]

Greigite. The structure of the iron sulfide mineral was so far only studied by electron diffraction in single cells, due to the unavailability of bulk methods, as no strains could be isolated in pure culture. It was a longer route than that for magnetite to arrive at a consensus, certainly because a larger variety of inorganic phases are present and thus the electron diffraction signals are more difficult to interpret. In fact, it was first proposed that the iron sulfide inclusions were pyrrhotite[42] or a combination of greigite and pyrite (FeS_2),[40] and the sphalerite-type cubic FeS was also tentatively identified.[132] It is now widely accepted that MTB form greigite (Fe_3S_4, a magnetic iron−sulfur mineral) by converting it from makinawite (FeS)[132,133] (Figure 4a). The sulfide magnetosomes typically show irregular contrast in HRTEM images. This feature likely results from defects that remain in the greigite structure as a result of the solid-state
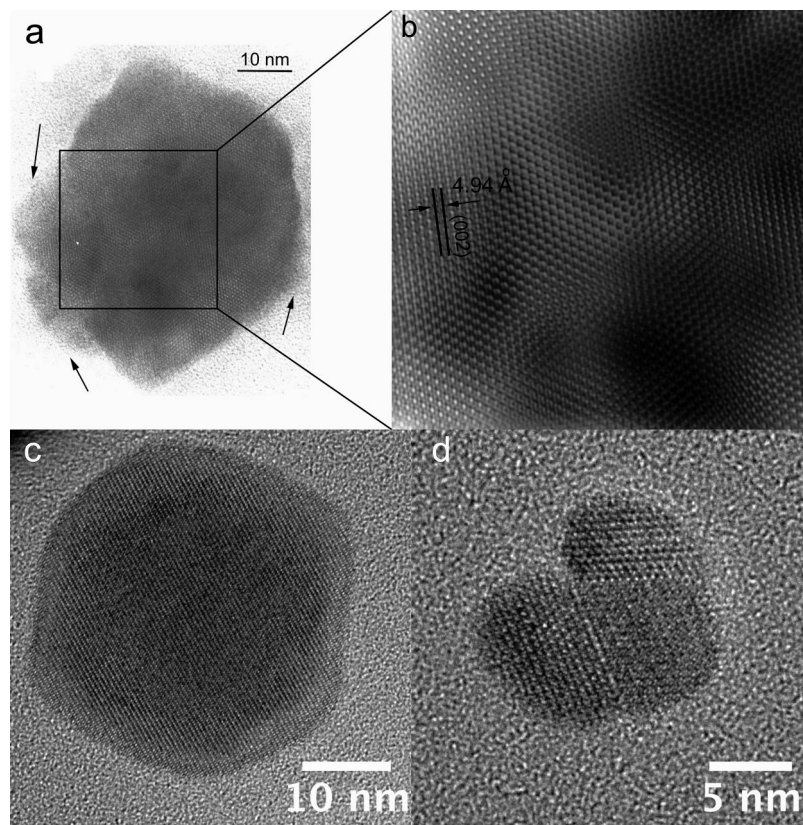
Figure 4. High-resolution transmission electron micrograph of (a) greigite magnetosomes from an environmental enrichment. The strong contrast variations, better seen in the Fourier-filtered image (b) of the boxed region in part a, likely result from changes in crystal thickness. The spacing of lattice fringes in part b, 4.94 Å, are consistent with the $d(0\,0\,2)$ of greigite. A magnetite magnetosome from *M. gryphiswaldense* with a typical cubooctahedral shape, viewed along [1 1 2], is shown in part c and a multiple twin is shown in part d. (a and b) Images courtesy of M. Pósfai. (c and d) Images courtesy of N. Menguy.

transformation from mackinawite to greigite.[134] Clearly, the isolation of a bacterial strain forming greigite magnetosomes would be of help for the better characterization of magnetosome structures.

3.1.2. Crystal Habits

The crystal habit or morphology of the magnetite crystals occurring in the magnetosomes varies significantly (Figure 1). However, it is widely accepted that the crystal shape is consistent for a given bacterial species or strain.[67,71,124] Magnetosome morphologies have extensively been studied, because of their extremely high crystallographic perfection.[115,116,118] Another interest resides in the understanding of their growth process or exceptional size,[135] especially for the elongated crystals.[29,117,123,131] Finally, morphology was proposed as part of the biogenicity criteria,[106] particularly for the question of life on Mars,[14] which has stimulated intensive research on the subject.[125,129] The use of electron tomography also helped to determine the unambiguous 3D morphology of the crystals.[102,136]

As viewed in electron micrographs, the magnetosomes can have square-like, rectangular, hexagonal, or bullet-shaped (or arrowhead- or tooth-shaped) projections[137] (Figure 1). By using high-resolution TEM, or better electron tomography, the morphology can be reconstructed. It was shown that all magnetosome morphologies are based on combinations of the {1 0 0} faces (those of the cube), the {1 1 0} faces (those of the dodecahedron), and the {1 1 1} faces (those of the octahedron) and all the possible distortions and elongations.[125] Of course, these are idealized morphologies, because

many magnetosomes display small irregularities that are difficult to analyze.

Specifically, the morphology of the magnetosomes from the cultured species is the following. Freshwater *Magnetospirillum* strains have a cuboctahedral morphology, which is composed of the {1 0 0} and {1 1 1} forms with nearly equidimensional development.[116,138] These strains are the only ones forming nearly equidimensional crystals. The marine spirillum MMS-1[28,139] (formerly known as MV-4[54]) also forms cubooctahedral but elongated crystals.[54] The magnetic vibrio MV-1 and the magnetococcus MC-1 produce elongated pseudohexagonal prismatic magnetosomes,[29,54,102,103] although with slightly different corner faces.[139] Finally, *Desulfovibrio magneticus* RS-1 forms bullet-shaped crystals,[140,141] as are found in some of the uncultured large freshwater rods. However, the exact habit remains to be determined.[34] These different types of elongations are striking in the sense that the cells are able to differentiate symmetry-equivalent faces. In this sense, the bullet-shaped magnetosomes are the most remarkable, as this particular shape does not exhibit a center of symmetry, in contrast to the case of the cuboctahedron or the elongated pseudohexagonal prism (Figure 1). This shows a biological control over crystal growth that is very strong and will be discussed below.

As mentioned above, the case of the greigite magnetosomes is more difficult to expose, as no axenic culture is available. Moreover, larger morphological variations and crystallographic imperfections can occur, as for example several particle morphologies have already been observed within a single cell.[142] However, greigite crystals in mag-

netotactic bacteria have the same general three morphologies as magnetite magnetosomes (cubooctahedral, pseudorectangular prismatic, and tooth-shaped),[133,134] and the morphology appears to be species-specific (although this remains to be proven once pure cultures will be available) except for the multicellular magnetic prokaryotes,[142] which can also form a combination of crystals of magnetite and greigite within a single cell, with different morphologies.[143,144]

3.1.3. Composition

Both magnetite and greigite minerals have a permanent magnetic moment at room temperature and can thus serve for magnetotaxis. The mineral and chemical compositions of the magnetosome appear to be strictly controlled by the organism. There are only sparse reports that some uncultured bacteria may incorporate trace amounts of the other elements into magnetosomes. For example, titanium was reported in magnetite magnetosomes of a coccus from a wastewater treatment pond.[145] Moreover, copper was detected in greigite magnetosomes from the uncultured multicellular prokaryote (MMP).[146] Other trace elements were found in MTB, but not associated to the magnetosomes; these include gold and silver,[147] and calcium and barium.[49] Finally, both magnetite and greigite were reported to be present in the same cell.[83]

Moreover, all tested cultured strains that are known to synthesize magnetite continued to do so even when incubated under reducing conditions, which should favor the formation of greigite (e.g., in the presence of hydrogen sulfide or titanium(III) citrate) (ref 29 and Faivre, unpublished results). Finally, the pathway of magnetosome formation seems to have a very high specificity for iron. Thus, the high chemical purity was used in the definition of biogenicity criteria.[106] Cells of magnetospirilla synthesize pure magnetite devoid of any contaminant even though they were grown in a medium where the iron was fully or partially replaced by other transition metals, which are known to easily get introduced in the magnetite structure (such as titanium), or that can be taken up and used by organisms for the synthesis of enzymes such as copper, zinc, nickel, or manganese (refs 148 and 149 and D. Schüler, unpublished results). In a recent study, it was reported that several strains of *Magnetospirillum* are apparently able to incorporate significant amounts of cobalt into their magnetite crystals, thereby substantially increasing the magnetic hardness of the magnetosome particles.[150] However, it has remained unclear why in these particular feeding experiments cobalt could be successfully incorporated. It is unknown which subtle modifications of the experimental conditions may have been responsible for this observation after numerous unsuccessful attempts from various laboratories to dope the magnetosome crystals with metals other then iron, using very similar experimental conditions.[151]

3.2. The Organic Part: The Magnetosome Membrane

3.2.1. Structure and Biochemical Composition of the Magnetosome Membrane (MM)

In all cultured magnetite-forming MTB, the magnetosome crystals are bounded by a protein-containing lipid bilayer membrane which was described as "magnetosome membrane (MM)" by Gorby et al.[113] Because of the lack of cultured species, it is currently u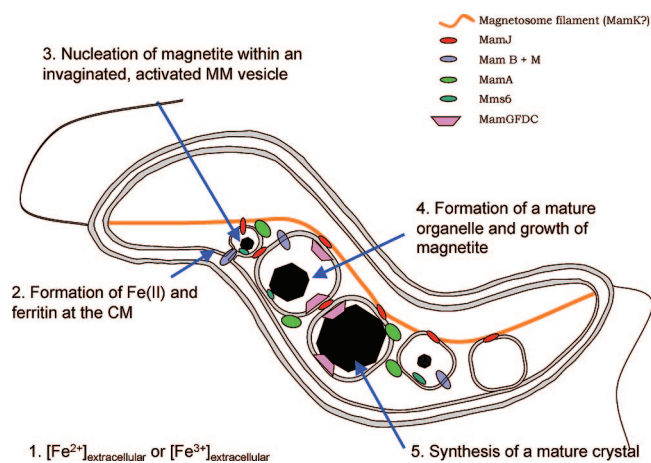ncertain if a membrane of similar composition is present in greigite magnetosomes. However, recent studies on the greigite magnetosomes in an uncultivated multicellular magnetotactic prokaryote suggested that the magnetosomes are enveloped by a MM whose staining pattern and dimensions are similar to those of the cytoplasmic membrane, indicating that the MM likely originates from the cytoplasmic membrane.[152] In cells of magnetospirilla, the MM forms vesicular structures visible by electron microscopy of thin sections or cryoelectron tomographic reconstructions (Figure 5A−C). The vesicles originate from the cytoplasmic membrane (CM) by invagination, and empty or partially filled MM vesicles harboring small immature crystallites of magnetite are present in iron-starved cells. Nascent magnetite crystals have been reported by Komeili et al. within invaginated structures still attached to the CM.[153] This has led to the notion that magnetosome vesicles are formed prior to magnetite formation and provide a "nanoreactor" in which conditions for magnetite synthesis can be strictly controlled by biochemical means. The number of empty MM vesicles present within a cell apparently exceeds the number of magnetite crystals, as suggested by studies in *M. gryphiswaldense*,[154,155] and thus seems not to limit the biomineralization of magnetite crystals. However, MM vesicles may require activation, possibly by the MM-associated tetratricopeptide-repeat MamA protein.[156]

Biochemical Composition. In *M. gryphiswaldense*, the lipid composition of the MM resembles that of the CM and contains a set of phospholipids including phosphatidylethanolamine, phosphatidylglycerol, ornithinamid lipid, and an unidentified amino lipid.[157] The protein composition, however, is very distinct from that of other subcellular compartments. Proteomic analysis by Grünberg et al. revealed that the MM of *M. gryphiswaldense* is associated with a specific set of more than 20 proteins present in various amounts[157−159] (Figure 5D). Similar studies in other MTB suggested that many magnetosome proteins are conserved between magnetite-forming MTB.[23,160−162] Magnetosome membrane proteins in *M. gryphiswaldense* and other MTB have been named either Mam (magnetosome membrane), Mms (magnetic particle membrane specific), Mtx (magnetotaxis), or Mme (magnetosome membrane) proteins and were assigned a letter in the order of their discovery or a number referring to their apparent molecular mass (e.g., MamA, Mms6). The magnetosome membrane proteins belong to characteristic protein families, which include TPR (tetratricopeptide repeat) proteins (MamA), CDF (cation diffusion facilitators) transporters (MamB and MamM), HtrA-like serine proteases (MamE, P, O), Actin-like proteins (MamK), generic transporters (MamH, N), and MTB-specific proteins with no homology to other proteins in nonmagnetic organisms (MamG, F, D, C, J, W, X, Y, Mms6, MmeA, MtxA).[158]

As mentioned above, the protein composition of the MM is very distinct, and it has been demonstrated by EGFP fusions and immunodetection that several MM proteins are targeted specifically to the MM but not other subcellular compartments. The molecular mechanisms controlling the sorting of MM proteins to the magnetosome compartment is currently unknown, and no sequence motifs or targeting signals universal to all MM proteins have been identified so far.[163,164] While many MM proteins display the characteristics of typical membrane proteins, others appear to be rather hydrophilic with a predicted cytoplasmic localization.[164] The different resistance of magnetosome proteins against proteases and detergents indicates that some proteins are very

Figure 5. (A) Transmission electron micrograph of a thin-sectioned cell of *M. gryphiswaldense* showing growing magnetite crystals within the vesicles of the magnetosome membrane (MM) (micrograph by Gerhard Wanner, München). (B) Isolated magnetosome particles are enveloped by the MM. (C) Treatment of isolated magnetosomes with SDS leads to the solubilization of the MM, resulting in the agglomeration of naked magnetite crystals. (D) SDS-PAGE of MM-associated proteins solubilized from isolated magnetosome particles. The positions of selected magnetosome proteins are indicated by characters, and the positions of their corresponding genes within the MAI in part D are shown by lines. Note that only a subset of MM proteins can be resolved and detected by one-dimensional SDS PAGE and that some of the magnetosome gene products form multiple bands. (E) Section of the genomic magnetosome island (MAI) from *M. gryphiswaldense* showing various magnetosome operons. Arrows in different colors represent different classes of genes.

tightly bound to the magnetosome crystals and/or embedded within the membrane. Others seem to be loosely attached and can be selectively solubilized by mild detergents.[157] It can be concluded from this that binding of MM proteins to the magnetosome is likely to involve specific protein—protein interactions or direct interaction with the mineral surface of magnetite crystals. This could be mediated for instance by the PDZ and TPR domains found in several MM proteins, which in other proteins are known to mediate protein—protein interactions, act as scaffolding proteins, and typically coordinate the assembly of proteins into multisubunit complexes at particular subcellular locations.[17,165] While the role of many MM proteins has remained obscure, several of them were already assigned functions in magnetosomal iron transport, magnetite nucleation and crystallization, and assembly of magnetosome chains, which will be described further below.

3.3. Genetics of Magnetosome Biomineralization

The genetics underlying magnetosome formation have remained mostly unknown for many years. However, substantial progress was made recently by establishing techniques for genetic manipulation of several strains of MTB[156,166—168] and the genome sequencing of as yet four MTB (refs 23 and 158 and http://genome.jgi-psf.org/mic_home.html). By comparative genome analysis of three *Magnetospirillum* strains and the magnetic coccus MC-1, a magnetobacterial core genome of only about 891 genes was found by M. Richter and co-workers to be shared by all four MTB.[158] In addition to a set of approximately 152 genus-specific genes shared only by the three *Magnetospirillum* strains, 28 genes were identified as group-specific; that is, they occur in all four analyzed MTB but exhibit no ("MTB-specific genes") or only remote ("MTB-related genes")

similarity to any genes from nonmagnetotactic organisms. These group-specific genes include all *mam* and *mms* genes which were previously implicated in magnetosome formation by proteomic and genetic approaches. The MTB-specific and MTB-related genes, which represent less than 1% of the 4268 ORFs of the *M. gryphiswaldense* genome, display synteny, i.e. a conserved gene order, and are likely to be specifically involved in magnetotaxis and magnetosome biomineralization, although many of them have as-yet unknown functions.[158]

3.3.1. Genomic Organization of Magnetosome Genes

In *M. gryphiswaldense*, most magnetosome genes are located within a 130 kb genomic magnetosome island (MAI), which in addition harbors a high proportion of transposase genes (>20% of the coding region) and many hypothetical genes[13,169,170] (Figure 5E). The MAI was found to be also conserved in other MTB,[139,171] including the marine strains MV-1 and MC-1.[172] From the conserved 28 group-specific (MTB-specific and MTB-related) genes mentioned above,[158] 18 are located within the MAI of *M. gryphiswaldense*, whereas 10 are located outside the MAI. It was speculated that genes required for the synthesis and biomineralization of magnetosomes are confined within the MAI, whereas the genomic organization of gene functions required for the "motility" and "signal transduction" part of magnetotaxis may display a wider genomic distribution.[158] The MAI undergoes frequent spontaneous rearrangements and deletions, perhaps even complete excisions, during subcultivation in the laboratory, which results in a nonmagnetic phenotype.[169—171] A compact, mobilizable genomic MAI that could have been distributed via horizontal gene transfer may also account for the widespread phylogenetic occurrence of the magnetic phenotypes,[172] although it remains to be demon-

Figure 6. Current model of the iron reaction pathway, and roles of the proteins that were so far shown to be necessary for magnetite biomineralization and chain formation.

strated whether remotely related magnetite-forming MTB, such as for example within the *Nitrospira* phylum or the *Deltaproteobacteria*, have magnetosome genes homologous to those found in the alphaproteobacterial MTB. Again, it needs to be stressed that nothing is known about the genetics of magnetosome formation in the greigite producing MTB.

In *M. gryphiswaldense* and other magnetospirilla, most magnetosome genes are comprised within the *mam* and *mms* operons, which are located within <65 kb of the MAI.[169,170,173] Cotranscription of long, single messengers was demonstrated for the *mamAB*, *mamGFDC*, and *mms6* operons, respectively, and promoters were mapped closely upstream of the first genes in the operons.[173] Expression of *mam* and *mms* genes was slightly up-regulated under magnetite-forming conditions, i.e. during microaerobiosis and in the presence of iron.[173,174] The *mamAB* operon encompasses 17 collinear genes extending over 16.4 kb of DNA. The 2.1 kb *mamGFDC* operon is located 15 kb upstream of the *mamAB* operon and comprises 4 genes. The 3.6 kb *mms6* operon is located 368 bp upstream of the *mamGFDC* operon and contains 5 genes. The four-gene *mamXY* operon is located about 30 kb downstream of the *mamAB* operon. Another magnetosome protein is encoded within the MAI by the monocistronic *mamW* gene.[170] Two further magnetosome proteins, MtxA and MmeA, are encoded outside the MAI. *mtxA* is part of a conserved operon-like cluster that was implicated in magnetotaxis.[158]

3.4. Pathway of Magnetosome Biomineralization

The current model for the pathway of magnetosome biomineralization and chain assembly, as well as the known cellular constituents controlling these processes, is summarized in Figure 6, which will be explained step by step in the following sections.
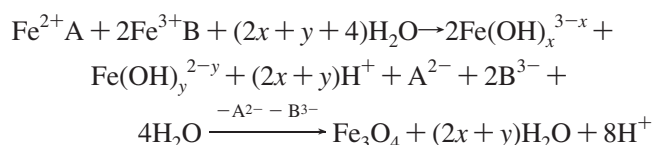
3.4.1. Iron Reaction Pathway

In the current section, we will focus on the species of iron that are found in the different cell compartments of magnetite-forming bacteria. As will be discussed below, both ferric and ferrous iron can be taken up by the cell from the growth medium, at least by *M. gryphiswaldense*[74,120] (Figure 6). There is also some evidence that Fe(II) is transported into the magnetosome vesicles, in *M. magneticum*,[175] in *M. magnetotacticum*,[119] and in *M. gryphiswaldense* MSR-1,[120]

mostly based on Mössbauer spectroscopic observations. However, two main and so far contradictory pathways have been proposed for magnetite biomineralization.[119,120] An early model proposed for *M. magnetotacticum*[119] assumes the presence of an intermediate ferrihydrite phase in the magnetosome compartment prior to the formation of magnetite. However, using controlled induction experiments in fermenter systems and precise biochemical separation, an alternative pathway was recently proposed for the MSR-1 strain as follows[120] (Figure 6):

(i) Iron is taken up from the environment either as Fe^{2+} or Fe^{3+}.

(ii) Iron is then converted into an intracellular ferrous high-spin species predominantly located in the membrane and to a membrane-associated ferritin.

(iii) Magnetite precipitation proceeds by fast coprecipitation of Fe^{2+} and Fe^{3+} ions within the magnetosome compartment, as no mineral precursor was detected. The magnetosome vesicle is likely alkaline to enable the thermodynamic stability of magnetite.[176] Thus, the following updated reaction pathway is proposed:

$$Fe^{2+}A + 2Fe^{3+}B + (2x+y+4)H_2O \rightarrow 2Fe(OH)_x^{3-x} +$$
$$Fe(OH)_y^{2-y} + (2x+y)H^+ + A^{2-} + 2B^{3-} +$$
$$4H_2O \xrightarrow{-A^{2-}-B^{3-}} Fe_3O_4 + (2x+y)H_2O + 8H^+$$

where Fe^{2+} and Fe^{3+} are ligated by organic substrates (A unknown and B = ferritin) at the cytoplasmic membrane level and are to be released at the magnetosome−compartment interface.

A recent study reported the observation of hematite in immature magnetite crystals.[177] However, its presence was observed only on the surface of the nanoparticles and in only one sample. Moreover, no possible mechanistic explanation was provided that could explain the formation of a completely different structural phase within such a short time and how the structure would change from the core to the exterior of the crystal. Thus, the observed hematite may be an artifact due to the surface oxidation of the magnetosomes while they were treated, as proposed by the authors.[177]

Thus, the presence of ferrihydrite or any other phase in the magnetosome vesicle remains unproven and must be considered with caution. In fact, all the analytical techniques associated with TEM failed to find any other mineral than magnetite or maghemite.

Moreover, the chemical stability of magnetite is restricted to a limited domain, as can be seen in the E_h−pH diagram (Figure 7). Magnetite typically forms at E_h values from −0.2 to −0.4 V, i.e., under slightly reducing conditions, requiring the presence of reducing agents, and at slightly basic pH. As magnetite formation releases protons into the solution,[101] there is a need for an effective buffer or proton pump to counterbalance the drop in pH due to the formation of magnetite. In the absence of such a system, the drastic drop in pH due to the release of 8 H^+ per formation of a single molecule of magnetite will create conditions that are non-favorable for magnetite synthesis. It has been speculated that the MamN protein, which has some similarity to proton transporting proteins, could play such a role in *M. gryphiswaldense*;[165] however, this requires experimental verification.

It was shown that the oxygen present in the magnetite crystals originates from the water and not from molecular
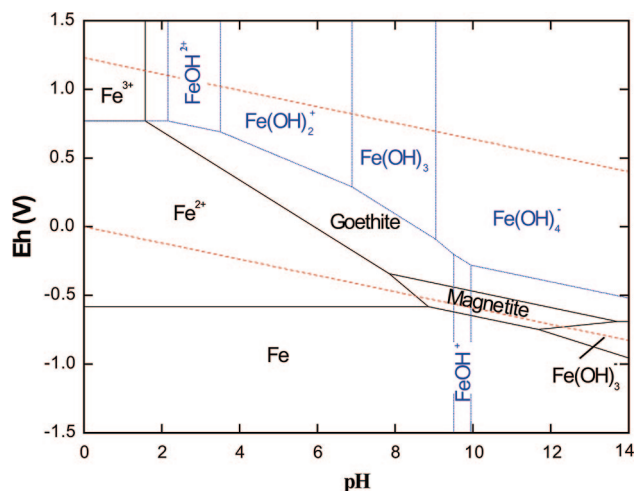
Figure 7. Pourbaix (E_h−pH) diagram for the iron−water system ($[Fe]_{tot} = 10$ μM). At this concentration, the thermodynamic stability domain of magnetite is restricted to a domain centered around a pH of 10 and an E_h of −0.5. Thermodynamic data are from Descostes.[278]

oxygen.[178] This shows that an oxidant other than oxygen is required for the biomineralization of magnetite. This is consistent with the observation that magnetite synthesis in MV-1 and other MTB can occur also in the absence of oxygen.[27] It is known that nitrate can play such a role in abiotic reactions,[179] but it was suggested that the dissimilatory nitrite reductase of *M. magnetotacticum* may also participate as an Fe(II)-oxidizing enzyme in magnetite synthesis under microaerobic and anaerobic conditions.[180] Other enzymes, such as ferric iron reductases, may also contribute to the establishment of the redox conditions required for the formation of magnetite.[181] Synthesis of magnetite crystals in *Magnetospirillum* strains depends on the prevalence of microoxic or anoxic conditions, whereas higher oxygen concentrations entirely suppress magnetite biomineralization or result in the formation of smaller and aberrantly shaped crystals.[51−53]

3.4.2. Systems for Iron Uptake and Transport

Because of the intracellular compartmentalization of the magnetite biomineralization within a membrane-enclosed compartment, specific mechanisms are required for active uptake and transport of iron into the cell and the magnetosome vesicle to accumulate oversaturating concentrations within the magnetosome vesicles. Both ferric and ferrous iron can be taken up actively from micromolar extracellular concentrations,[120,74] and the intracellular pathway for uptake and sequestration then has to be strictly controlled because of the potentially harmful effect of free intracellular iron levels.[182] It has been shown that bulk iron uptake is energy-dependent, regulated, and tightly coupled to magnetite synthesis in *M. gryphiswaldense*.[52,74] Iron supply was found close to saturation for a medium concentration of 20 μmol L^{-1}.[74] Iron concentrations above this threshold only slightly increased cell yield and magnetism, whereas iron concentrations above 100 μmol L^{-1} failed to show higher magnetosome production and iron concentrations above 200 μmol L^{-1} prevented cells from growing.[53,74]

The general iron metabolism in MTB is poorly understood at the molecular level. Despite their extreme intracellular iron accumulation and requirement for magnetosome formation, there is no evidence that MTB use unique systems for uptake

of iron. Genomic analysis and preliminary experimental data suggest that common constituents of the iron metabolism, such as uptake systems for ferrous and ferric iron, iron storage, iron reductases, as well as iron-regulatory elements, are present in MTB, although their significance for magnetite biomineralization is not fully understood.[23,183−187] A gene for the ferrous iron uptake protein FeoB (*feoB1*) that constitutes a putative operon with *feoA* was recently cloned from *M. gryphiswaldense*. Magnetosome formation was reduced but not abolished in a *feoB1* deletion mutant, indicating that FeoB1 protein plays an accessory, but not essential role in magnetite biomineralization, and other iron transport systems are presumed to be involved in this process.[188] Disruption of a *fur*-like gene in *M. gryphiswaldense* resulted in a dramatic inhibition of iron uptake and magnetosome formation, suggesting that this Fur-like protein may possess a novel function MTB.[189] In *M. magneticum, M. magnetotacticum*, and the marine magnetic vibrio MV-1, the synthesis of siderophores has been reported,[184,186,190] but it is not clear yet if siderophore-mediated iron uptake is associated with magnetite synthesis. In strain MV-1, a major copper-containing periplasmic protein (ChpA ("copper handling protein")) was found to be involved in iron uptake.[28,184] It was hypothesized by Dubbels et al. that ChpA is part of a three-component iron uptake system, which resembles the copper-dependent high-affinity iron uptake system in *S. cerevisiae* including an iron permease and an Fe(II) oxidase. Homologues of *chpA* are present in the genome of various other, mostly pathogenic, bacteria but also in magnetospirilla, suggesting that a similar pathway might be effective also in other MTB.[28,184]

The iron supply for magnetite biomineralization has to be integrated with the general and biochemical iron metabolism. On the other hand, there are some indications that uptake and intracellular processing of iron for magnetite synthesis proceeds through a distinct pathway, possibly through membrane-associated precursors involving a ferrous high-spin compound and a ferritin-like compound,[120] which have been identified by Faivre et al. using Mössbauer spectroscopy but not yet characterized at the genetic and biochemical level. Shift experiments, which involved the transfer of iron-replete but nonmagnetic cells to iron-deplete media under microoxic conditions that promote biomineralization, suggested that there are distinct intracellular pools of iron not readily interconvertible, one "biochemical" pool and another supplying iron specifically for magnetite synthesis[120] (Faivre and Schüler, unpublished data).

Iron Transport into the Magnetosomes. The accumulation of supersaturating quantities of iron into the magnetosomes requires specific routes for active iron uptake into the vesicles. As discussed above, it is currently not entirely clear if the transport proceeds via the cytoplasm or directly from the periplasmic space, although recent data from a Mössbauer study on *M. gryphiswaldense* seem to favor the latter scenario.[120] Whereas early genetic and biochemical studies suggested that the MagA protein may play a role in magnetosomal iron uptake in *M. magneticum*[175] and expression of MagA in human cell lines supposedly results in the intracellular precipitation of a magnetic iron biomineral,[191] this has not yet been verified in other MTB. In *M. gryphiswaldense*, the two abundant putative transport proteins MamB and MamM were identified within the MM of *M. gryphiswaldense*.[157] Both proteins are members of the cation diffusion facilitator (CDF) family of metal transporters,

which comprise proteins that function as efflux pumps of toxic divalent cations, such as zinc, cadmium, cobalt, and other heavy metal ions.[192] More specifically, MamB and MamM have the greatest similarity to the CDF3 subfamily.[192] FieF, a CDF protein from *E. coli* with high similarity to the MamB and MamM proteins, was recently demonstrated to export iron and zinc over the cytoplasm membrane.[192,193] Preliminary genetic evidence supports the assumption that MamB and MamM are involved in the magnetosome-directed uptake of iron (Junge et al., manuscript in preparation).

3.5. Chemical Control: Influence of Organic Components and Solution Chemistry on Inorganic Crystal Properties

In the previous sections, the critical role of the MM on magnetosome nucleation and growth, particularly the principal genes and proteins responsible for these processes, was described. In the following, we will focus on how the organic components might control the chemical aspects of these processes.

3.5.1. Nucleation and Growth of the Magnetic Mineral

The formation of vesicles occurs prior to the biomineralization event, as the presence of empty magnetosomes has now been confirmed in cells.[154,156] Moreover, the nucleation of magnetite appears to occur at the interface with the MM, as small magnetite crystals were only detected near the MM[113] or were MM associated.[120] Thus, the MM plays a critical role in magnetite formation. Magnetite nucleation will happen only if supersaturating concentrations of iron are achieved. Based on comparison of isotopic ratios in biogenic and abiotic magnetite, it was shown that at least 30 mmol L^{-1} of iron must be present in the magnetosome vesicle in order to trigger magnetite nucleation under basic conditions with an oxygen isotopic ratio of $1000 \ln \alpha = 0.3$.[101,108,137] The pumping of supersaturating amounts of iron into the vesicles could be performed *via* the MamB and MamM proteins, as explained previously.

Energetics is another aspect that favors magnetite nucleation at the surface of the MM, since interactions between ions in the crystal in *statu nascendi* and some charged proteins of the MM will reduce the surface energy of the entity. In many other biomineralizing systems, polyelectrolytic biological macromolecules are involved.[194,195] For example, acidic protein domains or amino acid residues are efficient in the generation of local supersaturation by complexing/binding cations[183,194,196] and help to concentrate the positively charged iron ions. In bacterial magnetite biomineralization, several proteins were implicated in a similar function. For example, the small Mms6 protein, which displays several acidic amino acid residues close to its C-terminus, may play this role in the magnetite crystallization process. The Mms6 protein was described in *Magnetospirillum magneticum* as a tightly bound constituent of the MM that exhibited iron binding activity and had a striking effect on the morphology of growing magnetite crystals *in vitro* by facilitating the formation of uniform 30 nm-sized, single magnetic domain particles in solution.[197,198] However, the significance of Mms6 for magnetosomal magnetite synthesis *in vivo* remains to be shown.

An acidic macromolecule could also have a crucial role in the site-directed nucleation of the nanoparticles. In fact,

the single-crystal nature of the biogenic crystals indicates that nucleation has to take place at a single nucleation site, with the crystal then growing at the expense of other potential sites. In the case of a multiple crystal nucleation, all the nuclei except one would have to rapidly redissolve. Such a configuration, however, was never observed in the TEM images except in the case of twinning and is thus very hypothetical. Apart from the unlikely option that a nucleation site provided by a polypeptide is present exactly in a single copy, a more probable *scenario* encompasses a spatially constrained organization in the MM and/or the temporary inhibition of the other sites by inhibitors or repressors.

Finally, because of the crystal orientation at the mature stage, i.e. at least in the magnetospirilla, crystals are preferentially oriented with the [1 1 1] parallel to the chain axis, it was also proposed that the membrane should exert a degree of crystallographic control on the magnetite faces that are nucleated adjacent to the organic surface of the MM.[71] However, the precise orientation and crystal morphology of magnetite nuclei could never be resolved, so this hypothesis still needs experimental evidence to be confirmed.[199]

The topic of crystal growth is intimately linked with those of crystal nucleation and habits. Once nuclei have appeared, growth will follow to eventually result in the final habit of the crystals. Based on the pathway suggested in ref 119, once a single nucleus per magnetosome is formed, the growth of the biogenic magnetite takes place by structural modification of a poorly crystalline nonmineral phase. These surface reactions are kinetically constrained and would result in a slow crystal growth with highly ordered and morphologically specific crystals.[71] However, in the hypothesis that does not assume the existence of a precursor,[120] magnetosome particles grow by the direct addition of iron ions, possibly from solution. This could explain the morphological characteristics of the magnetosomes from the spirilla, as those are cubooctahedral, similar to particles obtained by inorganic synthesis.[105] However, such a *scenario* requires additional components to explain the anisotropic morphology of magnetite of numerous strains, such as in MC-1 or MV-1, or the bullet shaped crystals. In the latter case, iron transport centers such the MamM and MamB proteins might be nonhomogenously distributed within the MM, thus providing transport of iron into a preferential direction; this could explain the high structural quality and the elongated morphology of the crystals.[71] At every stage of the nucleation and growth processes, iron specificity has to be ensured in order to form magnetite free of any other elemental inclusions.

3.5.2. Control of Particle Morphology

Figure 1 shows different strain- or species-specific morphologies for magnetosome crystals. Thus, although the function of the magnetic mineral is similar, the crystallochemical processes leading to the formation of those habits might be slightly different from those in the spirilla. This means that the different MTB have active systems to form and control the species-specific crystal habits.

As explained in a previous section, there is no need for invoking a biological intervention to explain the morphology of cubooctahedral crystals of the *Magnetospirilla* strains, as inorganic crystals have similar morphology.[105] However, the phenomenon leading to a common orientation remains to be unraveled. A more intriguing aspect concerns the bacterial strains that produce crystals with elongated morphologies.

In fact, anisotropic habits reflect some fundamental specificity of the biomineralization process. So far, the biological mechanism that controls the formation of elongated magnetosomes is unknown. Preformed elongated magnetosome vesicles might force the growth of crystals into a preferential direction, although this would not explain the elongation of the immature MV-1 crystals,[54] and thus, such an hypothesis remains unlikely.

The effect of several magnetosome proteins on the crystallization of magnetite particles of MTB was recently analyzed. For example, the *in vitro* effect of the Mms6 protein on the formation of magnetite was studied, and an effect on the crystals' morphology was observed.[197,198,200] However, these studies have to be complemented by genetic approaches to demonstrate the relevance of Mms6 function *in vivo.* Beside an effect on the size of the magnetosomes, Δ*mamA* and Δ*mamGFDC* mutant strains from *M. magneticum* and *M. gryphiswaldense,* respectively, apparently exhibit deviation from the cuboctahedral morphology, typical of the wild type strains.[155,156] These changes, however, will require further studies to be clearly understood. Beside an effect on the organization of the magnetosomes, these deviations possibly reflect subtle changes in the solution chemistry in the magnetosome vesicle, which thereby may affect the morphology of the magnetosome mineral particle.

In fact, besides biological and genetic control, it has also been demonstrated that the morphology of magnetite nanoparticles is very sensitive to changes in the solution chemistry and physical conditions so that supersaturation state, iron supply direction, concentration of activator and inhibitor ions or molecules, pH, redox potential, and temperature can all influence the final habits of the magnetosomes. For example, it was recently shown that changes in magnetosome morphology could be associated with changes in iron uptake rates,[199] as magnetosomes that were grown under conditions of high iron uptake rates presented cuboidal morphologies, whereas those grown with slower iron uptake rates exhibited the classical cuboctahedral morphology. Less recently, morphological changes were also attributed with changes in the medium composition and to the oxygen partial pressure, as it has been shown that cells of *M. gryphiswaldense* form small, aberrantly shaped magnetite crystals[29,54] near the regulatory oxygen concentration suppressing magnetite formation.

3.5.3. Control of Magnetosome Dimensions

Crystal size is most probably as strictly controlled as is the morphology of the crystals. Magnetosome sizes are optimized to bear optimal magnetic properties for magnetotaxis. The magnetic properties of magnetite nanocrystals are largely controlled by their domain states, which in turn depend upon grain size and aspect ratio.[93] The three domain states, by increasing size, are superparamagnetic (SP), single domain (SD), and multidomain (MD). In the case of an ideal SD particle, all the elementary magnetic dipoles are aligned parallel and thus form a uniform magnetization that is maximal for a given volume. The SD size in magnetite occurs in crystals with lengths less than about 100 nm.[201] Small particles (with particle edge length less than some 35 nm) are SP; that is, they cannot retain a temporally stable SD magnetization at room temperature because the magnetization is constantly buffeted by thermal fluctuations, leading to frequent spontaneous magnetization reversals in the particle that would minimize their strength for magnetotaxis. Larger

magnetite crystals in turn exhibit several uniformly magnetized domains, with the so-called multidomain structure with adjacent domains in the interior having possibly opposite polarity. Thus, while SD particles have the maximum magnetization for magnetite, the remanent magnetization is much reduced in MD particles. For a particle close to or above the SD/MD boundary, other spin configurations occur, resulting in a magnetization between SD and MD. For the purpose of magnetotaxis, it therefore is most efficient for a microorganism to produce SD particles. This is achieved by limiting the particle size and by increasing the particle elongation. Magnetosome crystals of magnetite and greigite are in fact from 30 to about 140 nm in size.[137] Within this range, the sizes of magnetosomes are under species-specific control. Magnetosomes from magnetospirilla typically range from 30 to 50 nm in length,[125,138] and the *Desulfovibrio magneticus* cells have magnetosomes about 40 nm long;[141] magnetosomes from vibrios (MV-1) are slightly larger, with average dimensions varying from 40 to 60 nm.[54,125] The largest magnetosomes from cultured strains occur in MC-1, with average sizes from 80 to 120 nm.[29,125] Exceptionally large (250 nm) magnetosomes have also been reported in uncultured coccoid MTB.[135] With the exception of the latter example, magnetite and greigite particles in this range of dimensions are stable single-magnetic domains and are thus permanently magnetic at ambient temperature.[202] The isolation of spontaneous *M. gryphiswaldense* mutants, which produce smaller and aberrantly shaped particles indicated that, in addition to the habits, the crystal dimensions are also under genetic control.[170,203]

Clearly, growth of magnetite crystals has to be regulated to generate the species-specific sizes of single-domain particles that are effective in magnetic orientation. However, it has remained unknown how this regulation is achieved. Until recently, it was assumed that the magnetosome crystal sizes were determined simply by spatial constraints exerted by the magnetosome vesicles. This was based on the observation that mature magnetite crystals in TEM micrographs entirely filled the lumen of the vesicles in membranes with no space between the crystal and the adjacent MM.[112,113,156] A recent study by Scheffel and co-workers has now indicated that the four small, hydrophobic magnetosome proteins MamG, MamF, MamD, and MamC are specifically involved in the size control of magnetite crystals in *M. gryphiswaldense.*[155] The MamGFDC proteins are encoded by a single operon in all magnetospirilla and altogether account for approximately 35% of all MM proteins. Except for *mamG,* which is a *Magnetospirillum*-specific gene with no orthologs in other MTB, the *mamD, mamF,* and *mamC* genes are part of the MTB-specific set of 28 "signature" genes.[158] The MamGFDC proteins are tightly bound to the MM, owing to various transmembrane helices, and their intracellular localization seems to be strictly confined to the magnetosome chains, as demonstrated by a recent GFP-based fluorescence microscopic study.[204] The 12.4 kDa MamC protein represents the most abundant MM protein followed by the 12.3 kDa MamF protein, which contains three predicted transmembrane segments and tends to form stable oligomers even in the presence of SDS.[157] The MamD (30.2 kDa) and MamG (7.7 kDa) proteins share a conspicuous motif containing a Leu-Gly-containing repeat. Based on their high abundance in the MM, their exclusive occurrence and high conservation in other MTB, MamC, and the further gene products of the *mamGFDC* operon were
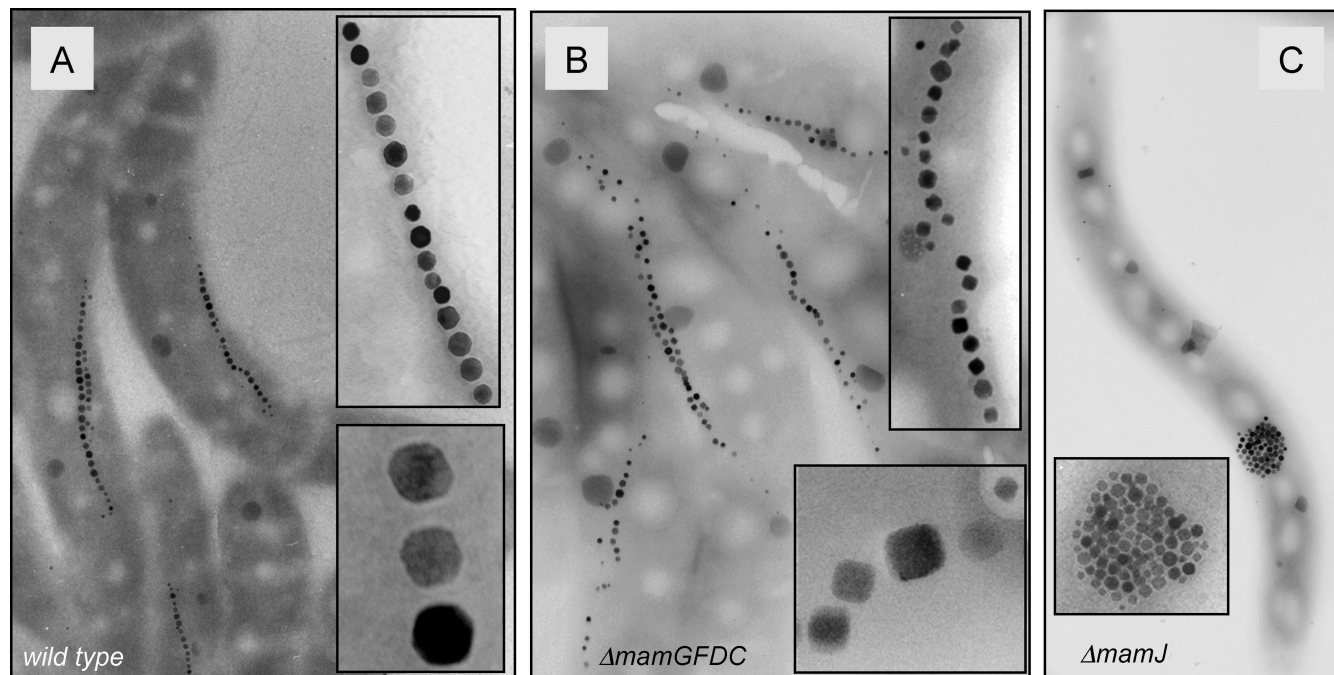
Figure 8. Phenotypes of mutant strains of *M. gryphiswaldense* in which genes for various magnetosome proteins were deleted. (A) Cells and magnetosomes of the wild type. (B) Cells in which the genes of the four major magnetosome proteins MamGFDC were deleted produce smaller, aberrantly shaped magnetite crystals that are predominantly in the superparamagnetic size range. Magnetosome chains are less regular and not as tightly spaced as in the wild type.[221] (C) A deletion mutant lacking the *mamJ* gene is not affected in the biomineralization of magnetosomes and forms wild type-sized and -shaped magnetite crystals.[154] However, particles are no longer arranged in regular chains; instead they agglomerate into clusters. Electron micrographs by André Scheffel, MPI Bremen.

assumed to play a key role in magnetite crystal formation. It therefore came as a surprise that mutants lacking either *mamC* or the entire *mamGFDC* operon continued to synthesize magnetite crystals, form intracellular MM vesicles, and align in magnetic fields. However, cells lacking *mamG-FDC* produced crystals that were only 75% of the wild-type size and were less regular with respect to morphology and chainlike organization[155] (Figure 8B). Apparently, growth of mutant crystals was not simply spatially constrained by the size of MM vesicles, as iron-starved cells lacking *mamGFDC* formed vesicles which, albeit slightly smaller than wild-type vesicles, still exceeded the size of mutant crystals. However, formation of wild-type-sized magnetite crystals was gradually restored by the *in trans* complementation with any combination of one, two, or three genes of the *mamGFDC* operon, respectively, whereas the expression of all four genes resulted in crystals even exceeding wild-type size. These observations suggested that the MamGFDC proteins have partially redundant functions and in a cumulative manner control the growth of magnetite crystals by an as-yet unknown mechanism, which is effective in addition to the spatial constraints provided by the boundaries of the MM vesicle.[155]

4. Intracellular Organization of Magnetosome Chains

4.1. Cell Biology of Magnetosome Chain Formation

The magnetic dipole moments of individual magnetite crystals are not sufficiently large to align a bacterial cell in the geomagnetic field against thermal disorientation. To most efficiently serve as a magnetic sensor, the cell has maximized its magnetic dipole by arranging the magnetosomes in chains, resulting in a single magnetic dipole which is the sum of the permanent magnetic dipole moments of the individual single-domain magnetosome particles. There are exceptions to this rule, however, as an agglomerated or clustered arrangement of magnetosomes has been found in some species of uncultured freshwater cocci[205] (Figure 1c). Yet, cells with these arrangements align in external fields and can be collected using simple bar magnets. In some magnetic bacteria complex, arrangements with two or multiple chains are found.[49,206,207] For example, in cells of Cand. *Magnetobacterium bavaricum*, the up to 1000 bullet-shaped magnetite crystals form 3−5 rope-shaped, twisted bundles of magnetosomes.[63,208] It has been reported that, in these cells and other uncultured bacteria with two or more chains, the intracellular arrangement of magnetosomes is such that the chains are positioned at maximum possible distances from each other and adjacent to the inner cell boundary. This can be understood in terms of magnetostatic repulsion forces between parallel magnetic dipoles driving the chains apart from each other.[208] In electron micrographs of MTB, magnetosome chains occasionally appear bent or kinked, which has been attributed to *artifacts* caused by shrinkage of cells during air drying of samples.[209] On the other hand, it has been demonstrated by electron tomography that in cryo-embedded cells of *M. gryphiswaldense* the chain of magnetosomes, which appears as a more or less straight line in air-dried cells in the TEM, is actually slightly bent and follows the curvature of the helical cell.[154,208] A string of magnetosome crystals, each representing a magnetic dipole, *per se* is not stable with respect to its magnetostatic energy, as this arrangement is out of dynamic equilibrium.[210,211] On the other hand, for crystals forced to remain in a chain, the lowest magnetic energy state is with all the individual moments parallel to each other along the chain. Thus, an
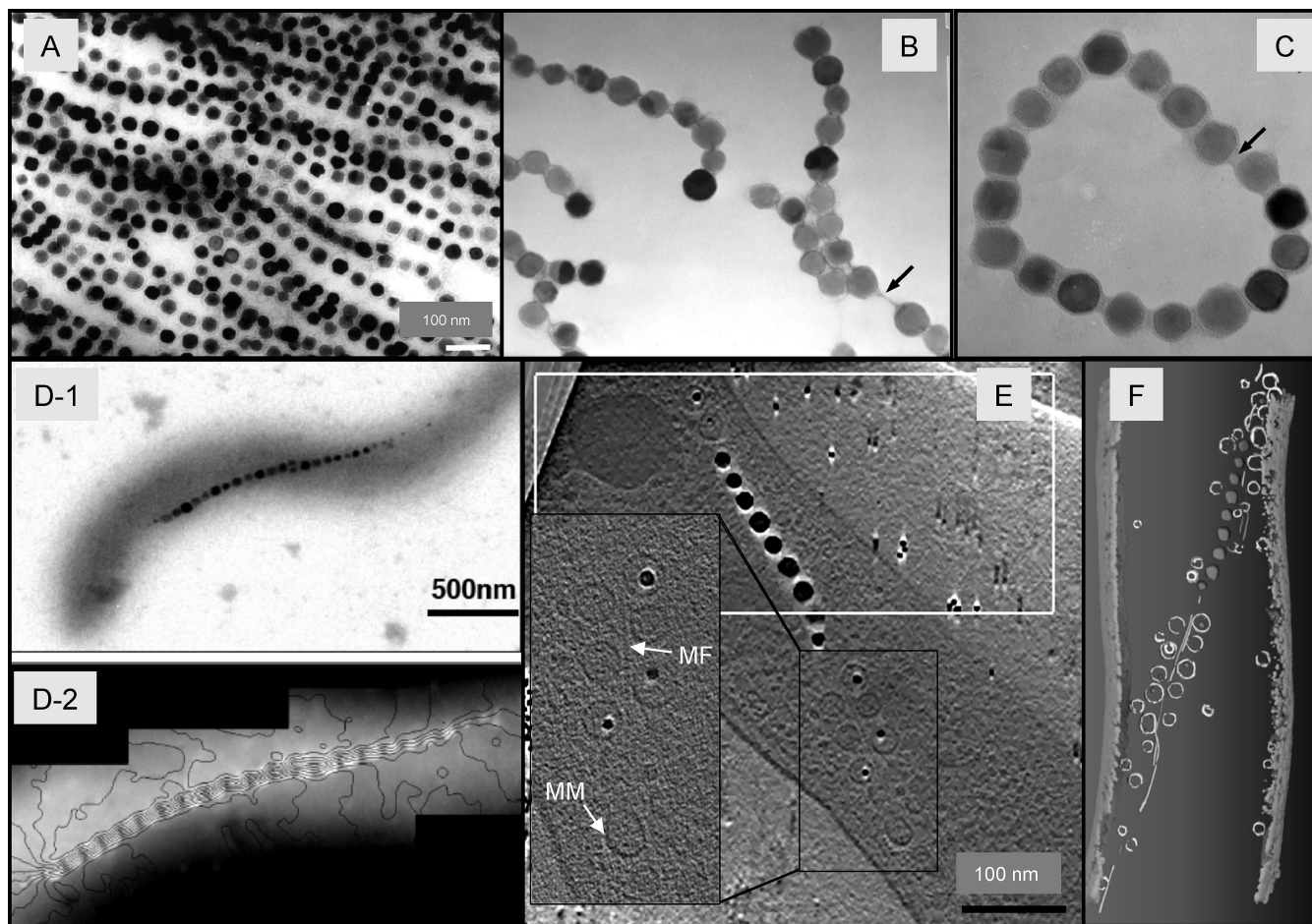
Figure 9. (A−C) Isolated magnetosome particles form straight chains in weak ambient magnetic fields (A) but form bent chains (B) or flux-closure rings in zero fields (C). Note that the MM-enveloped crystals adhere to each other by junctions of organic material (arrows). (D) Magnetic microstructure of a magnetosome chain. (D-1) TEM bright-field image of a single bacterial cell of *M. magnetotacticum*. (D-2) Magnetic induction map recorded using off-axis electron holography from the magnetosome chain of the same cell as shown in part D-1. Image by Rafal Dunin-Borkowski, adapted from ref 234. (E) Organization of the magnetosome chain and the cytoskeletal magnetosome filament (MF) in *M. gryphiswaldense*, shown by cryoelectron tomography.[154] Both empty magnetosome membrane vesicles and those that contain growing, immature magnetite crystals are closely attached to the cytoskeletal magnetosome filament. (F) Tomographic reconstruction of a magnetic cell showing the cytoplasmic membrane (blue), empty vesicles (yellow), growing and mature magnetite crystals (red), and the magnetosome filament (green). (E and F) Electron micrographs and reconstruction by Manuela Gruska and Jürgen Plitzko, MPI München.[154]

assembled chain spontaneously magnetizes itself. Therefore, intracellular chains have to be properly stabilized within the cells against their immanent tendency to collapse. Isolated intact magnetosome particles have a tendency to form chains *ex vivo*, as can be seen by TEM.[159] Strong dipolar attractions of particles are involved in intracellular chain formation but are not sufficient for maintaining straight chains, as isolated magnetosomes preferentially form structures such as flux-closure rings and folded chains (Figure 9A−C), which represent lower magnetostatic energy states for in-plane dipoles.[212] It was observed that isolated magnetosomes were tightly interconnected by organic material, which appeared to form junctions between individual particles, indicating that the presence of the MM is required for coherence within the chain.[154,162] Spontaneous chain formation *in vitro*, however, was abolished after the enveloping MM was removed by treatments with detergents[157,159] (Figure 5C). The resulting agglomerates of particles suggested that the formation of chains requires the presence of organic material that provides spacing and seems to mediate contact between adjacent particles.[154]

The assembly and maintenance of well-ordered chains *in vivo* has been recently demonstrated to be highly controlled

at the genetic and structural level. In cells of *Magnetospirillum*, a single chain is formed that is located adjacent to the cytoplasmic membrane. At least initially during formation, the nascent chain is connected to the CM by junctions with the invaginating MM vesicles.[163,213] In addition, some conspicuous fibrous structures were reported to connect the magnetosome chain to the cell boundary in a deep-etching electron microscopy study.[214] Further support structures, such as a "magnetosomal matrix" or similar sheath-like structures, were postulated,[131,162,211] the existence of which, however, has not been unambiguously demonstrated by high-resolution, artifact-free techniques such as electron tomography of cryo-fixed cells.

Recently, two complementary cryoelectron tomography studies led by Scheffel et al. and Komeili et al., respectively, directly demonstrated a network of filaments, 3−4 nm in diameter, which traverse cells of *M. gryphiswaldense* and *M. magneticum* closely adjacent to the CM. Magnetosomes were closely arranged along this cytoskeletal structure, which has been tentatively referred to as a "magnetosome filament" (MF)[215] (Figure 9E and F). There are a number of indications that this filament is formed by the magnetosome protein MamK. The MamK protein has homology with the cytosk-

eletal Actin-like MreB protein that is involved in a number of essential cellular processes in bacteria, such as cell shape determination, establishment of cell polarity, and chromosome segregation.[216−219] Although MamK represents a distinct lineage within the bacterial Actin-like proteins, its intriguing similarity to cytoskeletal constituents lead to speculations about a putative role in aligning and stabilizing the magnetosome chain.[164] In fact, in a mutant of *M. magneticum* in which the *mamK* gene was deleted, the magnetosome filament was no longer detectable.[153] The magnetosome chains were less regular and dispersed throughout the cell, which led to the notion that the magnetosome filament formed by MamK is involved in the proper stabilization and anchoring of the magnetosome chain within the cell.[153,213] The formation of filament-like structures seems to be an intrinsic property of MamK, because MamK of *M. magneticum* alone is sufficient to direct the synthesis of straight filaments in *E. coli*, which are structurally and functionally distinct from the known MreB and ParM filaments, as shown by Pradel and colleagues.[220] Also, the linear localization of MamK in *M. gryphiswaldense* mutants did not require the presence other magnetosome genes.[221] Recombinant MamK of *M. magnetotacticum* expressed in *E. coli in vitro* polymerized into long straight filamentous bundles, the formation of which was dependent on the presence of ATP.[222]

In addition to MamK, the acidic MamJ protein, which was identified in the MM of *M. gryphiswaldense*,[157] was implicated in the control of magnetosome chain assembly. The *mamJ* gene immediately neighbors *mamK* within the *mamAB* operon, and both genes are cotranscribed from a single promoter.[169,173] A deletion mutant of the *mamJ* gene no longer produces straight magnetosome chains, while the synthesis of magnetite crystals was not affected, as demonstrated by Scheffel et al.[154] Instead of regular chains, magnetite crystals were arranged in compact clusters[154,221] (Figure 8C). It was shown that empty vesicles and immature crystals were scattered throughout the cytoplasm and detached from the magnetosome filaments in Δ*mamJ* cells. The most conspicuous sequence feature of the MamJ protein is the <u>c</u>entral <u>a</u>cidic <u>r</u>epetitive ("CAR") domain, which, however, does not seem to be absolutely essential for its function in chain assembly.[221] Localization studies with GFP suggested that MamJ interacts with the magnetosome filament but is not required for its synthesis. A direct interaction between the putative filament-forming MamK protein and MamJ was experimentally confirmed by two-hybrid experiments.[221] One obvious model derived from these results is that the MamJ may connect magnetosomes to the cytoskeletal magnetosome filament composed of the MamK protein, which stabilizes the magnetosome chain and prevents it from collapsing.[221] In contrast, in cells lacking MamJ, mature magnetosome crystals are free to agglomerate once they are in close proximity.[154,163,213] However, so far it is unknown if additional proteins are involved in chain formation. Several conflicting observations between studies on MamJ and MamK in two different *Magnetospirillum* species seem to indicate that both proteins could perform slightly different or additional functions.[163,213]

4.2. Chain Assembly

One of the most intriguing questions in magnetosome formation is how newly biomineralized crystals are assembled into the nascent chains.[215,223] In cells of *M.*

gryphiswaldense that were permanently grown at iron concentrations saturating for magnetite synthesis, the chain is predominantly located at midcell, and growing crystals are preferentially found at the ends of the chain.[154] This ensures that the magnetosome chain is split more or less equally to newly divided cells. In contrast, empty vesicles are found along the entire length of the cell in iron-starved cells. Time course experiments, in which magnetite synthesis was induced in iron starved resting cells, revealed that immature crystallites were formed simultaneously at multiple sites along the entire length.[120,154] Later, growing crystals started to concentrate at midcell, first assembling into imperfect, loosely spaced chains that gradually developed into straight, tightly spaced chains of mature particles. In Δ*mamJ* cells empty vesicles were widely spaced and located along the entire length of cells, which suggested that agglomeration occurs only after the synthesis of strongly magnetic crystals.[154,199] This indicates that magnetosome particles undergo a dynamic cellular localization during maturation and chain assembly. It is not yet known what controls this process and what causes new magnetosomes to form at the ends of the inherited chain.[215] It is conceivable that MamK might function in establishing the chain by transporting newly formed magnetosomes with growing crystals to the ends of the nascent pre-existing magnetosome chains, similar to related bacterial MreB-like proteins that may function as intracellular motors. This notion is supported by observations that the assembly of the MamK filaments in *E. coli* is a highly dynamic and kinetically asymmetrical process.[220] Polymerization and depolymerization processes might generate forces sufficient for the transport and relocation of magnetosomes. It has been further speculated that the position and polarity of the magnetosome chain might relate to other cellular structures relevant for magnetotaxis, such as the flagellar motor,[223] and it was suggested that the inherent molecular polarity of MamK might be translated into a mechanism for controlling global cell polarity.[220]

Another open question in the complex cell biology of MTB is the mechanism that causes the nascent magnetosome chain to be precisely located at a particular cellular position, which is at midcell in the analyzed *Magnetospirillum* strains. This also relates to the problem of how magnetosomes are properly segregated to daughter cells during cell division.[215] Loss of MamJ resulted in an uneven segregation of magnetosome particles during cell division,[221] and there are indications for a controlled mechanism of magnetosome positioning and segregation. It was suggested that positional information for localization and segregation of the magnetosome chains could be provided by interaction with other positional determinants controlling cell division in bacteria, such as the FtsZ protein involved in divisome formation.[216,217,224]

5. Magnetic Orientation of Cells

5.1. Magnetic Properties of Magnetosome Chains

As magnetosomes are primarily devoted to magnetotaxis, they have to possess a magnetic moment sufficient to orient the bacteria in the geomagnetic field. Considering that the magnetic interaction energy has to be larger than the thermal energy for having magnetotaxis as an effective orientation mechanism, $mB_0 > k_BT$, where m is the magnetic moment of the cell, B_0 the magnetic induction of the geomagnetic field (from 25 to 60 μT), k_B the Boltzmann constant, and T

the temperature, the minimal magnetic moment of the cell for effective magnetotaxis can be determined as

$$m_{\text{min}} = \frac{k_{\text{B}}T}{B_0} = \frac{1.38 \times 10^{-23} \times 293}{25 \times 10^{-6}} = 0.16 \times 10^{-15}$$

So, at 20 °C, a minimal magnetic moment of 0.16×10^{-15} A m^2 is required. For an even better image, the number of magnetosomes aligned in a closely packed chain can also be estimated. Considering that the saturation magnetization of magnetite per unit volume is $M_s = 0.48 \times 10^6$ J m^{-3} T^{-1} and that the magnetic moment of one cell is the sum of n individual magnetosomes of volume v (for the spirilla species, the magnetosomes can be approximated by a sphere of 20 nm radius), the minimal number of such magnetosomes for an effective magnetotaxis is

$$n_{\text{min}} = \frac{m_{\text{min}}}{M_s \times V} = \frac{0.16 \times 10^{-15}}{0.48 \times 10^6 \times \frac{4}{3}\pi(20 \times 10^{-9})^3} = 11$$

This means that when at least 11 magnetosomes are aligned, the cells will passively align in the Earth's field. Of course, this is a very simple approximation to give an order of magnitude. Assessment of the partial forward movement can be given using the Langevin function (see for example calculations in refs 225 and 226). By this, it can be shown that MTB typically swim with 90% efficiency in the direction of the geomagnetic field for $mB_0/k_{\text{B}}T = 10$.

The first determination of the magnetic moments of environmental samples of MTB or of isolated strains was estimated based on TEM images,[227] following bacterial movement by optical microscopy,[227] by light scattering[228] or birefringence,[229] and later by direct magnetic measurements.[96] *M. magnetotacticum* MS-1 cells were reported to have a coercive force $H_c = 26.8$ mT for both aligned and randomly oriented cells[96] and an average magnetic moment per cell of $m = (0.22-2.4) \times 10^{-15}$ A m^2.[96,228,230-232] This is at least 50% larger than the minimum required moment calculated previously. Using a SQUID magnetometer, the average magnetic moment of MV-1 bacteria was determined to be $m = 0.16 \times 10^{-15}$ A m^2; however, while using a magnetic force microscope, a value of $m = 1.2 \times 10^{-15}$ A m^2 was determined for a single chain of 21 magnetosomes.[233]

Using off-axis electron holography, the magnetic microstructure of magnetosome chains in the TEM was studied in individual cells[234] (Figure 9D). This was performed on both axenic and environmental samples of MTB. In these micrographs, magnetic field lines associated with the magnetosomes are more or less parallel all along the chain, showing that the chain acts as a single dipole. Moreover, magnetic contours run closer in the crystals and are wider in between. MS-1 cells showed a magnetic moment $m = 0.5 \times 10^{-15}$ A m^2, whereas MV-1 bacteria exhibited a magnetic moment of 0.7×10^{-15} A m^2 and a coercive field of $30-45$ mT.[234,235] These values are of the same order of magnitude as the value determined using different magnetometers, although they are substantially larger than the usual average value ($2-5$ times). The difference can be explained by the fact that those values were recorded on a single bacterium that thus can have a larger moment than the average moment measured for many cells. Practically, the moment differences can be due to different chain lengths or the magnetosome volume. Electron holography was also used to determine the magnetization of single magnetosomes as

0.58 ± 0.02 T at room temperature[206] and to show the first experimental evidence that daughter cells are magnetotactic based on the fact that dividing the chain of magnetosomes of the parental cell between two daughters still should enable the daughter cells to be magnetotactic.[207] Finally, FORC diagrams but also electron holography can be used to assess the magnetostatic interaction between crystals.[236] As magnetic properties have been widely used for paleoenvironmental applications, magnetic criteria were defined for the detection of biogenic magnetite.[97] These criteria have been widely used over the years[46,47] and for the detection of a magnetic signature in meteorites.[98,99] A whole set of different magnetic properties of uncultivated MTB, i.e. temperature dependence magnetization, hysteresis loops, and magnetic relaxation, have also been studied.[227,229,237,238]

5.2. Magnetotaxis

Magnetotaxis refers to the orientation and migration of cells along the magnetic field lines.[10] As demonstrated in the previous sections, the biomineralization of magnetic single domain particles with optimum sizes and shapes, and their intracellular assembly, positioning, and anchoring result in maximum cellular dipole moments to serve as a magnetic sensor. Since the magnetosome chain is fixed within the cell, the entire cell is rotated into alignment with the geomagnetic field lines, thereby causing the bacterium to migrate along the field as it swims. Thus, a magnetotactic bacterium in effect has been depicted as a "self-propelled magnetic compass needle".[11] The classical model of magnetotaxis was based on the assumption that all MTB have a fixed polar preference to their swimming direction.[225] Since the geomagnetic field is inclined downward from horizontal in the Northern hemisphere and upward in the Southern hemisphere, North-seeking bacteria in the Northern hemisphere and South-seeking cells swimming southward in the Southern hemisphere would migrate downward toward the bottom of natural waters along the inclined geomagnetic field lines (Figure 10). This theory is supported by the predominant occurrence of North-seeking bacteria in the Northern hemisphere and South-seeking MTB in the Southern hemisphere, as well as by the presence of both polarities in equal numbers at the equator, where the inclination is zero.[10,239] This simple model, however, was later shown by Richard Frankel and colleagues to be strictly valid only under oxic conditions, e.g. during microscopic investigation in the hanging drop assay. It was demonstrated that, in cells of the magnetic coccus MC-1, the North-seeking swimming direction could be reversed to "South-seeking" by exposure to reducing conditions and that light of short wavelengths (<500 nm) could switch the cells back to "North-seeking". This led to Frankel's refined model of "polar magneto-aerotaxis", where the cells sense the direction of the field, and of "axial magneto-aerotaxis", where the organisms sense the axis of the field.[72] In "polar magneto-aerotaxis", a two-state sensory mechanism was proposed that determines the sense of flagellar rotation and consequently swimming direction in response to an upper and lower threshold value of oxygen concentration. The two-state sensing mechanism, which is clearly distinct from the conventional "run-and-tumble" temporal mechanism operative in *E. coli* and other nonmagnetic bacteria, results in an efficient aerotactic response in vertical oxygen gradients, as found in chemically stratified sediments or water bodies.[240] It remains to be proven whether this elegant model, which so far has been experimentally
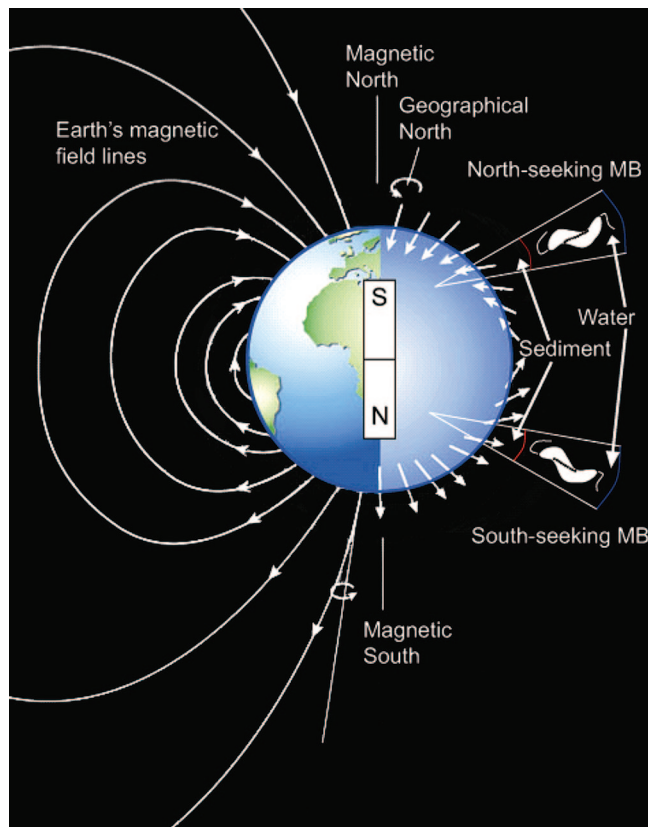
Figure 10. The intracellular magnetic dipoles of MTB enable the cells to align with the geomagnetic field lines while swimming. Due to the inclination of the Earth's magnetic field (white arrows), north-seeking bacteria are present in the Northern hemisphere and swim toward low oxygen concentrations (South-seeking bacteria in the Southern hemisphere swim in the other direction to fulfill the same goal). Figure adapted with permission from ref 279. Copyright 2003 Macmillan Publishers Ltd.

demonstrated only for the magnetic coccus MC-1, applies universally to other MTB. Cells of the cultivated *Magneto-spirillum* strains perform an "axial magneto-aerotaxis", which seems to be distinct from "polar magneto-aerotaxis" discussed above; that is, they are able to align along the magnetic field lines, but individual cells do not display a preferred North- or South-seeking polarity. However, there are indications that the apparent loss of polarity in cultured magnetospirilla might result from the absence of selective pressure under laboratory conditions.[24,25] Another possible deviation from the magnetotaxis model was recently described for an uncultivated magnetotactic prokaryote, whose swimming behavior in a magnetic field displays a characteristic "ping-pong" behavior, i.e., a more or less permanent forward movement (North-seeking on the Northern-hemisphere), which is interrupted by shorter intervals of backward excursions into the opposite magnetic direction.[44] Because the frequency of backward excursions increased with the magnetic field strength, it was suggested by Greenberg et al. that these organisms use a "magnetoreception" mechanism,[241] although this still awaits further experimental confirmation.

Recently, a population of polar MTB was identified from a marine stratified pond in the Northern hemisphere that responded to high oxygen levels by swimming toward the geomagnetic south, i.e. the opposite direction of all previously reported MTB behavior.[38] This seems to contradict a universal role of magnetotaxis, as discussed above, and

appears to argue for a different function of magnetic orientation and magnetosome biomineralization. Such functions could include the storage or detoxification of iron as well as yet unknown functions.[28,59] Whereas it is clear that the axial magnetic orientation of cells is passive by alignment of the cellular dipole with the ambient field lines (which can be easily demonstrated on killed cells), the active motility along the magnetic field and the decision on the direction of swimming is obviously not but has to involve the perception and intracellular transduction of extracellular signals. In the complex environments of MTB, magnetic orientation has to be integrated with the tactic responses to other environmental cues, such as multiple chemical gradients and light. It is currently not known how these aerotactic, chemotactic, and phototactic signals are processed and ultimately transduced toward the cellular flagellar motor. In *M. gryphiswaldense*, some components of the flagellar systems were identified, and the deletion of the *flaA* gene encoding the flagellin protein resulted in the loss of motility, magnetotactic reaction, and the absence of flagella.[167] An intriguing feature of all MTB is the genomic presence of unusually high numbers of chemotaxis transducers and other proteins potentially involved in cellular signaling and bacterial taxis, and it has been speculated that these might be related to the regulation and control of magnetotaxis.[23] In addition, a high number of genes putatively encoding bacterial hemerythrins are present in the magnetobacterial genomes, with some of them clustered in the genomic magnetosome island (MAI), and may be implicated in oxygen sensing and aerotaxis. Although none of these genes have been studied experimentally, the genomic data suggest the presence of extraordinarily complex, and potentially redundant, pathways of signal transduction in the MTB, which might reflect their adaptation to complex chemical gradients in their natural environments.

6. Application of MTB and Magnetosomes in Bio- and Nanotechnologies

Magnetosomes isolated from MTB represent magnetic nanoparticles (MNP) with unique characteristics. Compared to MNP that are chemically produced, magnetosome particles derived from MTB have a number of potential advantages. Magnetosome crystals display narrow size distributions and uniform morphologies. Typical sizes of the monocrystalline particles are in a range that is not easily accessible to chemical synthesis. As shown above, their magnetic characteristics are unique.[242−245] Whereas mature magnetosome crystals (>35 nm) fall into the ferrimagnetic, permanent-single-domain size range, as described in section 3.4.3, some mutants produce smaller particles with a narrower size distribution shifted predominantly toward the SP size range.[155,203] Particles of this size can not only diffuse through most tissues in the human body but also display SP properties, as they have high saturation magnetization values if an external magnetic field is applied, but upon removal from the magnetic field, the magnetic moment fluctuates freely in response to the thermal energy.[246] Moreover, the pathway of biosynthesis is genetically controlled and displays a morphological diversity that is unknown in inorganically produced magnetite crystals. The magnetite cores of bacterial magnetosomes have a low toxicity compared to some alloys used for the chemical synthesis of some MNP.[247] Finally, isolated magnetosome particles are enveloped by a biological membrane, which prevents suspensions of isolated particles
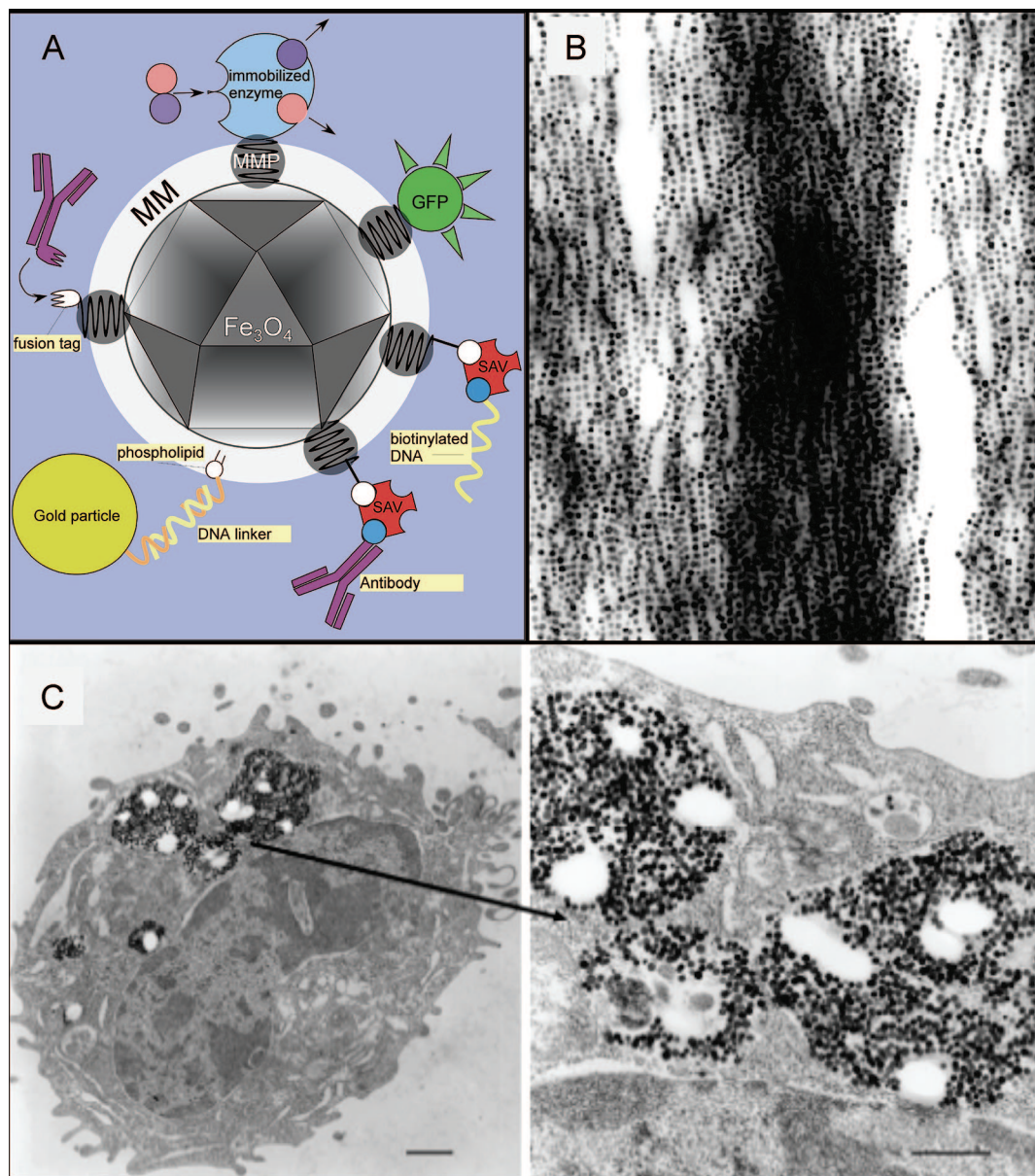
Figure 11. (A) Modifications of magnetosome particles by the chemical or genetic introduction of different moieties resulting in multifunctional hybrid magnetic nanoparticles (drawing by Claus Lang after ref 280): SAV, streptavidin; MMP, magnetosome membrane protein; GFP, green fluorescent protein. (B) TEM image of a purified preparation of magnetosome particles isolated from mass cultures of *M. gryphiswaldense*. (C) Ingestion of fluorescence-labeled magnetosomes by murine macrophages. Electron-dense vesicle-like structures (endosomes) inside the cytoplasm contain phagocyted magnetosomes (adapted from ref 258 with kind permission from I. Hilger and M. Lisy). Fluorescent bacterial magnetosome particles could be used as bimodal contrast agents for magnetic resonance imaging and near-infrared optical imaging.

from agglomeration and can be addressed by specific functionalization (Figure 11).

The unique and superior characteristics of magnetosome particles attracted interest in their use as magnetic nanoparticles. Potential applications of bacterial magnetosomes comprise their use in magnetic separation, diagnostics, and detection of analytes (for recent overviews of application studies, see refs 248−250). Reported applications include immunobinding and receptor binding assays, magnetic cell separation, and DNA extraction techniques,[251−256] and others. It was suggested that bacterial magnetosomes can be used as contrast agents for magnetic resonance imaging (MRI), and their usefulness for the detection of microtumors has been demonstrated.[257] Fluorochrome-coupled bacterial magnetic nanoparticles can be used as bimodal contrast agents for both MRI and near-infrared fluorescence optical

(NIRF) imaging of cultured macrophages.[258] Another technique that could make use of isolated magnetosome particles is magnetic hyperthermia, in which MNP are used for controlled tissue heating to promote cell necrosis. It was revealed in an *in vitro* study that the specific power loss determined for suspensions of bacterial magnetosomes significantly exceeded that obtained from synthetic MNP, and their broad hysteresis and high coercivity indicates that magnetosome particles are promising candidates for heating applications.[244]

Many of these applications require the functionalization of isolated magnetosome particles, e.g. by the magnetosome-specific display of functional moieties, such as enzymes, antibody binding proteins, protein tags, or oligonucleotides[250,259] (Figure 11A). This has been mostly achieved by chemical coupling of specific ligands to lipids or proteins of the

magnetosome membrane in vitro.[252,260−263] Alternatively, the use of integral magnetosome proteins was suggested as anchors for the magnetosome-specific display of heterologous proteins fused to them.[250,264−266] For example, luciferase was used as a reporter for magnetosome expression of genetic fusions to the Mms13 protein of *M. magneticum*.[264,265] Another fluorescent protein that is useful as a reporter for expression and intracellular localization of magnetosome proteins *in vivo* is the *green fluorescent protein* (GFP).[204] In addition to GFP, other functional moieties, such as enzymes, antibody binding proteins, receptors, peptide hormones, growth factors, autobiotinylation signals, and protein tags for "click chemistry" could be expressed on the magnetosome particles by use of magnetosome specific anchor proteins (Figure 11A).

Researchers from interdisciplinary fields haven been intrigued by the capability of MTB to build single or complex chains of magnetosomes, which are assembled and stabilized against their immanent tendency to collapse, and it was suggested to replicate this *in vitro* to build up complex nanostructures. In a study by Banerjee et al., isolated magnetosome particles were incorporated into a synthetic peptide matrix, resulting in magnetic nanotubes that were remotely reminiscent of bacterial magnetosome chains.[267] The formation of magnetic nanostructures, such as magnetic nanotubes and nanowires, would greatly benefit, however, from a deeper understanding of the cellular structures and mechanisms of magnetosome chain assembly, and their reconstitution *in vitro*.

The potential of MTB in nanotechnology is not restricted to isolated magnetosome crystals, but also includes suggested applications that use entire, living cells. It has been demonstrated that living MTB can be used for magnetic domain analysis in magnetic materials at the microscopic scale.[268−270] MTB were also proposed to be used in radionuclide recovery.[271] In another study, MTB could be manipulated and precisely positioned with microelectromagnets. The subsequent lysis and removal of the cellular body enabled the precise localization of the magnetosome in order to assemble a customized structure of magnetic nanoparticles.[272] Further applications of living MTB cells are in the field of nanorobotics. An external magnetic field can be used to manipulate MTB and to force them to push 3-μm-sized beads at an average speed of 7.5 μm s^{-1}.[273]

Both the use of isolated magnetosome particles and the application of entire living cells rely on the availability of large amounts of bacteria at reasonable costs. The fact that MTB are fastidious and rather difficult to handle hampered the realization of the applications described above at a commercial scale. However, increasing efforts in recent years resulted in the establishment of robust and reliable techniques for the mass cultivation of MTB. Initial attempts to scale up growth and magnetosome production were done with strain *Magnetospirillum* sp. AMB-1. Growth of *M. magneticum* in a 1000 L fermenter yielded a magnetosome production of about 145 mg L^{-1} of culture and a productivity of 1.85 mg L^{-1} day^{-1} of magnetite in a 10 L fermenter.[274,275] Fermentation of *M. gryphiswaldense* in an automated oxygen-controlled vessel was established, which allows the continuous maintenance of low pO$_2$ concentrations.[53] In a comparative study, a productivity of 6.3 mg L^{-1} day^{-1} of magnetite was reported for *M. gryphiswaldense* compared to 3.3 and 2.0 mg L^{-1} day^{-1} of magnetite for *Magnetospirillum magneticum* and *M. magnetotacticum*, respectively.[53]

Among these strains, *M. gryphiswaldense* exhibited the highest oxygen tolerance, and growth was unaffected by oxygen concentration over a wide range (0.25 up to 150 mbar). Recently, high-yield growth and magnetosome formation by *M. gryphiswaldense* MSR-1 in an oxygen-controlled fermentor supplied solely with air were reported by Sun and co-workers.[276] Under these conditions, the cell density (OD565) of strain MSR-1 reached 7.24 after 60-h of culture in a 42-L fermentor, and cell yield (dry weight) was 2.17 g L^{-1}, the highest yield so far reported. The yield of magnetosomes (dry weight) was 41.7 and 16.7 mg L^{-1} day^{-1}, which were 2.8 and 2.7 times higher than the previously reported yields.[276]

Magnetosome particles can be purified from disrupted cells using a straightforward protocol involving magnetic separation and ultracentrifugation.[157,159] The isolated particles form stable suspensions (Figure 11B) that are sensitive to treatment by detergents but stable over a wide range of pH and temperature.[204] In summary, the recent progress in cultivation and genetic manipulation of MTB will greatly facilitate their future application in various fields of biotechnology and nanotechnology.

7. Perspectives and Directions for Future Research

In his historical first review on magnetotactic bacteria, Richard Blakemore, who had discovered these organisms just a few years before, expressed his wish "to spark in some readers interest and activity in this new area of research".[59] As shown above, he and others certainly succeeded in fascinating an interdisciplinary crowd of researchers, who provided numerous contributions to make this a *bona fide* field of research, as stated by Dennis Bazylinski and Richard Frankel in a more recent overview.[17] Despite the tremendous progress in the elucidation of magnetosome biomineralization, we also hope to have shown that this field is still a rapidly evolving area of research, with many questions that remain to be answered.

For example, while many of the genetic and biochemical determinants of magnetosome formation have been identified lately, their precise roles in biomineralization as well as the molecular mechanisms by which they control the composition, shape, size, and alignment of the growing magnetosome crystals remain to be elucidated. Therefore, a systematic and concerted effort to study the function of the various magnetosome genes and proteins *in vivo* and *in vitro* is urgently required. A deeper understanding of the biomineralization process at the molecular level could in the future also be used for a rational design of magnetic nanoparticles with properties tailored by genetic engineering. Examples include the generation of size- and shape-adjusted magnetosome particles or, by modification of the pathways of metal transport into the magnetosome vesicles, to give rise to nanocrystals with modified chemical composition, for instance by doping them with metals other then iron. Both genetic and chemical modifications could also be used for functionalization of bacterial magnetosome particles, e.g. by the introduction and addition of functional moieties to the magnetosome membrane.[250]

A more complete knowledge of the molecular players and mechanisms controlling magnetosome biomineralization will also open the door to the replication of magnetosome biochemistry in the test tube, i.e. by the biomimetic generation of magnetosome-like magnetic nanoparticles. This has

the potential to generate advanced magnetic nanomaterials with innovative properties.[250] There are already a few encouraging studies of the *in vitro* effects of purified and overexpressed magnetosome proteins on the crystallization of magnetite particles,[197,198] and similar experiments could reconstitute individual or complete constituents of the magnetosome membrane in biocrystallization experiments. This approach could eventually be extended to the formation of highly organized, supramolecular magnetosome-like chain structures *in vitro*, e.g. by the inclusion of the newly discovered cytoskeletal constituents which control magnetosome chain formation within the bacterial cell.[213,222]

A major problem that needs to be solved by microbiologists is the fact that still only a minority of MTB can be grown. Unfortunately, many species with unique magnetosome morphology, such as bacteria producing bullet-shaped, anisotropic magnetite crystals, or with highly unusual cell biology, such as the MMP, have been recalcitrant to axenic cultivation. Up to today, none of the greigite-producing MTB can be cultivated in the laboratory! This makes our knowledge of the biology of this type of biomineralization rudimentary to nonexistent. Conventional isolation and cultivation approaches are still indispensable and should be performed with increased efforts. However, since uncultivated MTB can be collected relatively easily from environmental samples by magnetic separation,[24,25,50,277] there is hope that culture-independent approaches, such as the metagenomic analysis of MTB and magnetosome biomineralization, will generate insights into the biology and molecular processes underlying magnetotaxis.

8. Acknowledgments

9. References

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