MAGNETOSOME FORMATION IN PROKARYOTES

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Magnetotactic bacteria were discovered almost 30 years ago, and for many years and many different reasons, the number of researchers working in this field was few and progress was slow. Recently, however, thanks to the isolation of new strains and the development of new techniques for manipulating these strains, researchers from several laboratories have made significant progress in elucidating the molecular, biochemical, chemical and genetic bases of magnetosome formation and understanding how these unique intracellular organelles function. We focus here on this progress.

FASTIDIOUS PROKARYOTES
Bacteria that are difficult to cultivate owing to unusual or numerous growth requirements.

VIBRIOID
Used to describe a rod-shaped bacterium that is curved.

TACTIC
Displays movement towards (positive taxis) or away (negative taxis) from a stimulus.

OXIC–ANOXIC INTERFACE
The microaerobic boundary between oxygenated and anaerobic water in an aquatic environment that contains a vertical oxygen gradient.

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doi:10.1038/nrmicro842

Magnetotactic bacteria[1] are motile, mostly aquatic prokaryotes that swim along geomagnetic field lines. Some types of magnetotactic bacteria in water droplets swim persistently northwards in the northern hemisphere; this observation led to their serendipitous discovery by R. P. Blakemore, then a graduate student at the University of Massachusetts at Amherst. All magnetotactic bacteria synthesize unique intracellular structures called magnetosomes[2], which comprise a magnetic mineral crystal surrounded by a lipid bilayer membrane about 3–4 nm thick. In general, little is known about the methods by which bacteria synthesize these mineral crystals, although there has been a good deal of progress both in the isolation and mass-culturing of these microorganisms, and in our understanding of some of the specific features of magnetosomes and how they function within cells. We review this progress in this article, focusing mainly on the synthesis of the bacterial magnetosome.

The magnetotactic bacteria

The term 'magnetotactic bacteria' has no taxonomic significance and represents a heterogeneous group of FASTIDIOUS PROKARYOTES that display a myriad of cellular morphologies, including coccoid, rod-shaped, VIBRIOID, spirilloid (helical) and even multicellular[3,4]. They represent a collection of diverse bacteria that possess the widely distributed trait of magnetotaxis[3] — the term that is used to describe their magnetic behaviour.

Despite the great diversity of these microorganisms, they have several important features in common[4]: all that have been described are Gram-negative members of the domain Bacteria; they are all motile, generally by flagella; all exhibit a negative TACTIC and/or growth response to atmospheric concentrations of oxygen; all strains in pure culture have a respiratory form of metabolism (that is, none are known to ferment substrates); and they all possess magnetosomes (FIG. 1). It is possible that some Archaea or non-motile bacteria produce magnetosomes; however, none have been reported so far.

Magnetotactic bacteria are easy to detect in samples collected from natural habitats without isolation and cultivation[5]. They are cosmopolitan in distribution but, on a local basis, they are found in their highest numbers at, or just below, the OXIC–ANOXIC INTERFACE in aquatic habitats, where they can constitute a significant proportion of the bacterial population[4,6]. Physiological studies of several strains of magnetotactic bacteria show that they have the potential to participate in the biogeochemical cycling of several important elements, including iron, nitrogen[7–10], sulphur[11–15] and carbon[11], in natural environments.

The sensitivity of most magnetotactic bacteria to even relatively low concentrations of oxygen (they are OBLIGATE MICROAEROPHILES, anaerobes or both) and the fact that cells of most cultivated strains only produce magnetosomes in a narrow range of very low oxygen

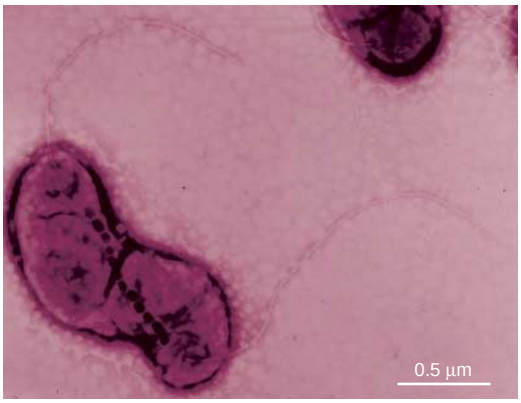


Figure 1 | **Transmission electron micrograph of a negatively stained cell of a typical magnetotactic bacterium.** This is a cell of strain MV-4, a marine magnetotactic spirillum. It has a flagellum at each end of the cell and a chain of electron-dense, magnetite-containing magnetosomes along the long axis of the cell.

concentrations were probably the primary limiting factors in this field of research for many years. Even now, there are relatively few species available in pure culture and even fewer working genetic systems in these organisms.

Bacterial magnetosome mineral crystals

Overall, magnetosome crystals have high chemical purity, narrow size ranges, species-specific crystal morphologies and exhibit specific arrangements within the cell[4,6,11]. These features indicate that the formation of magnetosomes by magnetotactic bacteria is under precise biological control and is mediated by a mineralization process, which is known as biologically controlled mineralization[16].

Magnetotactic bacteria usually mineralize either iron oxide magnetosomes, which contain crystals of magnetite (Fe_3O_4)[17], or iron sulphide magnetosomes, which contain crystals of greigite (Fe_3S_4)[14,15,18]. Several other iron sulphide minerals have also been identified in iron sulphide magnetosomes — including mackinawite (tetragonal FeS) and a cubic FeS — which are thought to be precursors of Fe_3S_4[19,20]. One organism is known to produce both iron oxide and iron sulphide magnetosomes[21,22], but has not yet been isolated and grown in pure culture. The mineral composition of the magnetosome seems to be under strict chemical control, because even when hydrogen sulphide is present in the growth medium, cells of several cultured magnetotactic bacteria continue to synthesize Fe_3O_4 and not Fe_3S_4[23,24]. Moreover, Fe_3O_4 crystals in magnetosomes are of high chemical purity[4,6,11], and reports of impurities, such as other metal ions, within the crystals are rare[25]. Additionally, no proteins are found within Fe_3O_4 magnetosome crystals[26].

Phylogenetic analysis of many cultured and uncultured magnetotactic bacteria shows that most of the Fe_3O_4-producing strains are associated with the α-subdivision of the PROTEOBACTERIA[6,11], whereas one uncultured Fe_3S_4-producing bacterium is associated with the sulphate-reducing bacteria in the δ-subdivision of the Proteobacteria[27]. As the different subdivisions of the Proteobacteria are considered to be coherent, distinct evolutionary lines of descent[28,29], DeLong *et al.*[27] proposed that the evolutionary origin of magnetotaxis was polyphyletic and that magnetotaxis that is based on iron oxide magnetosomes evolved separately from that based on iron sulphide magnetosomes. However, recent studies have shown that not all magnetotactic bacteria with Fe_3O_4 magnetosomes are associated with the α-Proteobacteria. *Desulfovibrio magneticus* strain RS-1 (REF. 13), which is a cultured, sulphate-reducing magnetotactic bacterium, has Fe_3O_4 magnetosomes, yet belongs to the δ-Proteobacteria[30], whereas another uncultured magnetotactic bacterium with Fe_3O_4 magnetosomes, *Magnetobacterium bavaricum*[31], is placed phylogenetically within the Bacteria in the newly formed Nitrospira phylum, not in the Proteobacteria[6]. These results indicate that magnetotaxis as a trait might have evolved several times and, moreover, could indicate that there is more than one biochemical/chemical pathway for the biomineralization of magnetic minerals by magnetotactic bacteria. Alternatively, these findings might also be explained by the lateral transfer of a group or groups of genes that are responsible for magnetosome synthesis between diverse microorganisms.

The particle morphology of Fe_3O_4 and Fe_3S_4 magnetosome crystals varies, but is consistent within cells of a single magnetotactic bacterial species or strain[32]. Three general crystal morphologies have been reported in magnetotactic bacteria on the basis of their two-dimensional projections in the electron microscope: roughly cuboidal[2,3,32,33]; elongated prismatic (roughly rectangular)[3,5,23–25,32]; and tooth-, bullet- or arrowhead-shaped[34–36] (BOX 1; FIG. 2).

Magnetosome Fe_3O_4 and Fe_3S_4 crystals are typically 35–120 nm long[32]. This size range is within the permanent, single-magnetic-domain (SD) size range[37,38] for both minerals. Smaller crystals are superparamagnetic, that is, not permanently magnetic at ambient temperature, and domain walls would form in larger crystals. In both cases, the MAGNETIC REMANENCE is less than that of SD crystals. Statistical analyses of crystal size distributions in cultured strains show narrow, asymmetrical distributions and consistent width-to-length ratios within each strain[39]. Whereas the size distributions of inorganic Fe_3O_4 crystals are typically log-normal tailing out to large crystal sizes[40], the size distributions of magnetosome Fe_3O_4 crystals have a sharp, high-end cutoff within the SD size range[39].

Magnetotaxis

In most magnetotactic bacteria, the magnetosomes are arranged in one or more chains[4,41]. Magnetic interactions between the magnetosome crystals in a chain cause their MAGNETIC DIPOLE MOMENTS to orientate parallel to each other along the length of the chain. In this chain arrangement, the total magnetic dipole moment of the cell is the sum of the permanent magnetic dipole moments of the individual SD magnetosome particles. Magnetic measurements[42], magnetic force microscopy[43]

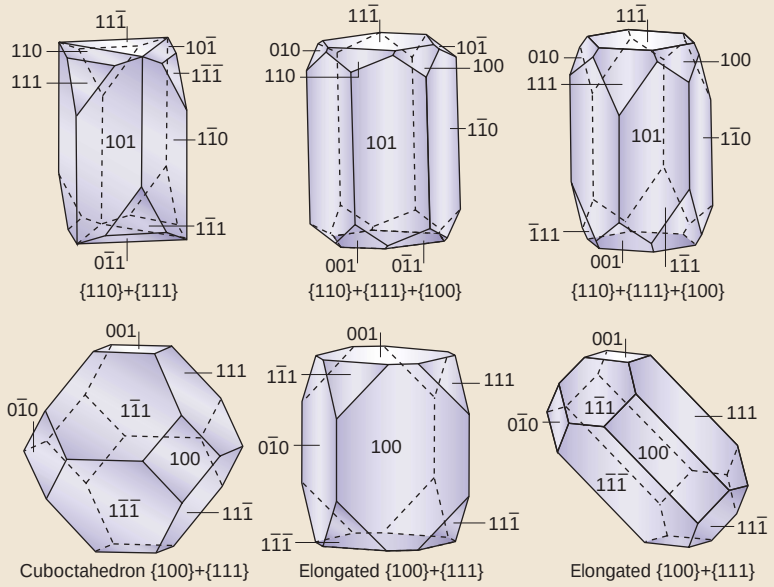
OBLIGATE MICROAEROPHILE
A bacterium that grows aerobically but only at low, less-than-atmospheric concentrations of oxygen.

PROTEOBACTERIA
An assemblage of metabolically diverse Gram-negative prokaryotes in the domain Bacteria that are separated into five subdivisions: α, β, γ, δ and ε.

MAGNETIC REMANENCE
The net magnetic dipole moment of a magnetic structure after the removal of an external magnetic field.

MAGNETIC DIPOLE MOMENT
An elementary magnetic structure, such as a compass magnet, with north and south magnetic poles that experiences a torque in a uniform magnetic field.

Box 1 | Magnetosome crystal morphology



Fe_3O_4 and Fe_3S_4 have face-centred, spinel crystal structures (Fd3m space group)[114]. Idealized crystal habits, derived from high-resolution electron microscopy studies, are based on combinations of {100} (cube), {110} (dodecahedron) and {111} (octahedron) forms (bracketed numbers represent specific crystal forms)[114]. Macroscopic crystals of Fe_3O_4 often display habits of the octahedral {111} form, and, more rarely, of the dodecahedral {110} or cubic {100} forms. The idealized habits of cuboidal magnetosome crystals are cuboctahedra, composed of {100} + {111} forms[33], with equal development of the six symmetry-related faces of the {100} form and the eight symmetry-related faces of the {111} form. The habits of the non-equidimensional crystals that are found in some magnetotactic strains can be described as combinations of {100}, {111} and {110} forms[39]. In these cases, as shown in the figure, the six, eight and 12 symmetry-related faces of the respective forms that constitute the habits do not develop equally. With the exception of the equidimensional cuboctahedron in the lower left panel of the figure, all the crystal habits shown have elongated projected shapes, which could result from ANISOTROPY during crystal growth. Anisotropy could derive from an anisotropic flux of ions through the magnetosome membrane surrounding the crystal, or from anisotropic interactions of the magnetosome membrane with the growing crystal[82]. In these cases, the growth process could break the symmetry of the faces of each form.

The most anisotropic crystal habits are those of the tooth-, bullet- or arrowhead-shaped Fe_3O_4 crystals (FIG. 2). Growth of these crystals seems to occur in two stages. The nascent crystals are cuboctahedra, which subsequently elongate along a [111] axis to form a pseudo-octahedral prism with alternating (110) and (100) faces, capped by (111) faces[34,35]. Tooth-shaped Fe_3S_4 crystals have also been observed[20].

Elongated crystals are so unusual that their presence in recent and ancient sediments and in the Martian meteorite ALH84001 has led to their designation as magnetofossils[115], and is cited as evidence for the past presence of magnetotactic bacteria in aquatic habitats and sediments[115–117] and life on ancient Mars[118–121]. However, elongated crystals of Fe_3O_4 have recently been synthesized in the laboratory[122]. Figure modified with permission from REF. 39 © (1998) Mineralogical Society of America.

and ELECTRON HOLOGRAPHY[44] (FIG. 3) studies confirm this conclusion, and show that the chain of magnetosomes in a magnetotactic bacterium functions as a single magnetic dipole. The cell has therefore maximized its magnetic dipole moment by arranging the magnetosomes in chains. The magnetic dipole moment of the cell is usually large enough such that its interaction with the Earth's geomagnetic field overcomes the thermal forces that tend to randomize the orientation of the cell in its aqueous surroundings[45]. Magnetotaxis results from the passive alignment of the cell along geomagnetic field lines while it swims. Cells are neither attracted nor pulled towards either geomagnetic pole. Dead cells also align along geomagnetic field lines but do not move. So, these living cells behave like tiny, self-propelled magnetic compass needles.

The term magnetotaxis, which has been used to describe the behaviour of magnetotactic bacteria, is in fact a misnomer. In contrast to a true tactic response, magnetotactic cells swim neither up nor down a magnetic field gradient. In water droplets, cells of each magnetotactic species or strain display either 'two-way' or 'one-way' swimming behaviour along local geomagnetic field lines. In the two-way swimming mode, which is exemplified by *Magnetospirillum* spp. grown in liquid culture, cells are equally likely to swim parallel and anti-parallel to the magnetic field with random abrupt changes in direction[46] (see online Movie 1). In the one-way swimming mode, which is typified by the marine coccus, strain MC-1, cells swim persistently in one direction along the magnetic field and accumulate on one side of a water droplet[46].

Bacteria from northern-hemisphere sites swim preferentially parallel to the magnetic field, which corresponds to a northward migration in the geomagnetic field; these bacteria are known as north-seeking (NS)[1]. Bacteria from southern-hemisphere sites swim preferentially anti-parallel to the magnetic field and are known as south-seeking (SS)[47] (FIG. 4a). The geomagnetic field is inclined downwards from the horizontal in the northern hemisphere and upwards in the southern hemisphere, with the magnitude of the inclination increasing from the equator to the poles. NS cells in the northern hemisphere and SS cells in the southern hemisphere therefore migrate downwards towards the sediments along the inclined geomagnetic field lines. The original hypothesis was that magnetotaxis helps to guide cells downwards to less-oxygenated regions of the habitat (the sediment), where they would presumably stop swimming and adhere to sediment particles. If displaced from the sediments up into the water column, they would use the magnetic field to migrate back down[1,3]. This theory is consistent with the predominant occurrence of NS cells in the northern hemisphere and SS cells in the southern hemisphere.

The discovery of large populations of magnetotactic bacteria at the oxic–anoxic interface in water columns of chemically stratified, aquatic habitats[22], and the isolation of obligately microaerophilic, coccoid magnetotactic bacterial strains[47], have led us to revise our view of magnetotaxis. The original model did not completely explain how bacteria in the anoxic zone of a water column benefit from magnetotaxis, nor did it explain how magnetotactic cocci form microaerophilic bands of cells in semi-solid, oxygen–gradient growth media. Experiments involving various strains of magnetotactic bacteria with Fe_3O_4 magnetosomes in oxygen-concentration gradients in thin, flattened capillary tubes showed clearly that magnetotaxis and AEROTAXIS work in conjunction in

ANISOTROPY
Magnetic properties that vary with the direction of an applied magnetic field relative to the crystallographic direction are said to exhibit anisotropy.

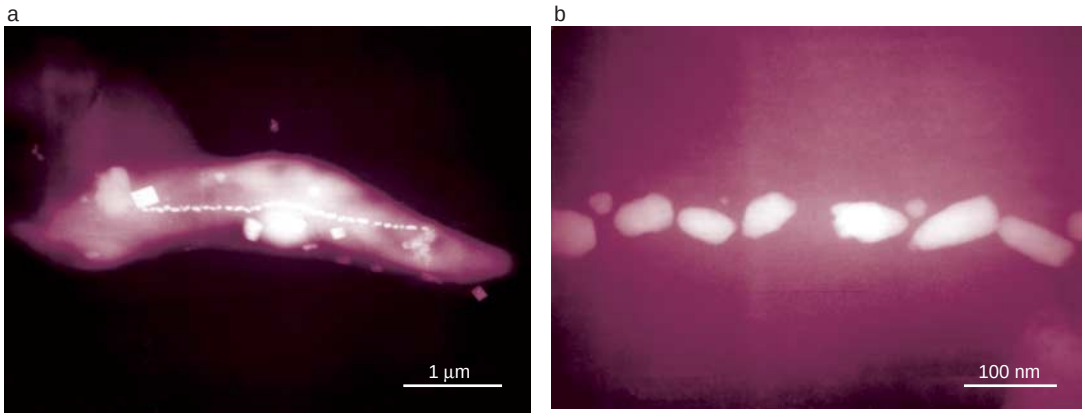


Figure 2 | **Anisotropic crystal habits of Fe_3O_4 crystals. a** | Dark-field scanning-transmission electron microscope (STEM) image of an uncultured spirillum collected from the Pettaquamscutt Estuary, Rhode Island, USA, containing a chain of tooth-shaped magnetite crystals that traverse the cell along its long axis. **b** | High-magnification STEM image of the crystals from the cell in part **a**.

these bacteria[47]. Aerotaxis is the response by which bacteria migrate to an optimal oxygen concentration in an oxygen gradient[48]. It has been shown that, in water droplets, one-way swimming magnetotactic bacteria can reverse their swimming direction and swim backwards under reducing conditions (less than optimal oxygen concentration), as opposed to oxic conditions (greater than optimal oxygen concentration). The behaviour that has been observed in these bacterial strains has been referred to as 'magneto-aerotaxis'[47].

Two different magneto-aerotactic mechanisms — known as polar and axial — are found in different magnetotactic bacterial strains[47] (FIG. 4b). Some strains that swim persistently in one direction along the magnetic field (NS or SS) — mainly the magnetotactic cocci —are polar magneto-aerotactic. Those that swim in either direction along magnetic field lines with frequent, spontaneous reversals of swimming direction without turning around — for example, freshwater spirilla — are axial magneto-aerotactic and the distinction between NS and SS does not apply to these bacteria (see online Movie 2). The magnetic field provides both an axis and a direction of motility for polar magneto-aerotactic bacteria, whereas it only provides an axis of motility for axial types of bacteria. In both cases, magnetotaxis increases the efficiency of aerotaxis in vertical concentration gradients by reducing a three-dimensional search to a single dimension[47]. It is possible, and perhaps likely (given that greigite producers also seem to occupy discrete depths in the anaerobic zone of chemically stratified ponds), that there are other forms of magnetically assisted chemotaxis in response to molecules or ions other than oxygen, such as sulphide, or magnetically assisted redox- or phototaxis in bacteria that inhabit the anaerobic zone below the oxic–anoxic interface.

Conditions that favour magnetosome synthesis

As there are no strains of magnetotactic bacteria with Fe_3S_4 magnetosomes in pure culture, very little is known about how, and under what conditions, these organisms synthesize Fe_3S_4. However, given the anaerobic, sulphidic conditions of the sites at which they are generally found[49–52], it is likely that Fe_3S_4 mineralization by magnetotactic bacteria occurs only in the absence of oxygen.

Several factors influence Fe_3O_4 magnetosome biomineralization, the most important being oxygen concentration and the presence of nitrogen oxides. Blakemore *et al.* first reported that microaerobic conditions (and therefore some molecular oxygen) are required for Fe_3O_4 production by *Magnetospirillum magnetotacticum*[53]. Cells of this organism could grow in sealed, unshaken culture vessels with 0.1–21% oxygen in the headspace; maximum Fe_3O_4 production and cellular magnetism occurred with an oxygen concentration of 1%, whereas oxygen concentrations >5% were inhibitory. Subsequent isotope experiments showed that molecular oxygen is not incorporated into Fe_3O_4, however, and that the oxygen in Fe_3O_4 is derived from water[54]. So, the role of molecular oxygen in Fe_3O_4 synthesis is unknown, although it clearly affects the synthesis of specific proteins. For example, Sakaguchi and co-workers[55] showed that the presence of oxygen in nitrate-grown cultures repressed the synthesis of a 140-kDa membrane protein in *M. magnetotacticum*, and Short and Blakemore[56] showed that increasing the oxygen tension in cultures from 1% saturation to 10% caused cells to show increased activity of a manganese-type superoxide dismutase relative to that of an iron-type. The addition of nitrate to the growth medium as an additional terminal electron acceptor also seems to stimulate Fe_3O_4 production — *M. magnetotacticum* is a microaerophilic denitrifier that converts nitrate to nitrous oxide (N_2O) and dinitrogen, but which cannot grow under strict anaerobic conditions with nitrate[8,53]. Guerin and Blakemore[57] reported anaerobic, Fe(III)-dependent growth of *M. magnetotacticum* in the absence of nitrate. Cells grown anaerobically with poorly ordered (amorphous) Fe(III) oxides, presumably as the terminal electron acceptor, were extremely magnetic and produced nearly twice as many magnetosomes when compared with nitrate-grown cells with 1% oxygen in the headspace[57]. However, the cells grew very slowly under these conditions and the growth

ELECTRON HOLOGRAPHY
An electron interference technique in a transmission electron microscope that is sensitive to magnetic fields in the sample. Analysis of the interference pattern allows visualization of the magnetic field lines.

AEROTAXIS
Motility towards or away from different concentrations of oxygen.

yields were poor compared with cells that were grown on nitrate and/or oxygen. They further showed that, in this bacterium, Fe(II) oxidation might also be linked to aerobic respiratory processes, energy conservation and Fe_3O_4 synthesis.

Schüler and Baeuerlein[58] showed that Fe_3O_4 formation in *Magnetospirillum gryphiswaldense* is induced in non-magnetotactic cells grown in a fermenter lacking a continuous oxygen-controlling system by a low threshold oxygen concentration of ~2–7 μM (1.7–6.0 mbar) at 30°C. *Magnetospirillum magneticum* strain AMB-1 synthesizes Fe_3O_4 either microaerobically or anaerobically using nitrate as the terminal electron acceptor[59,60]. The marine magnetotactic vibrio, strain MV-1, synthesizes Fe_3O_4 microaerobically in semi-solid agar oxygen-gradient cultures, and anaerobically under 1 atm of N_2O, which it uses as a terminal electron acceptor in respiration[10].

Recently, Heyen and Schüler[61] reported the effect of oxygen on the growth and magnetite magnetosome synthesis of *M. gryphiswaldense*, *M. magnetotacticum* and *M. magneticum* grown microaerobically in a continuous, oxygen-controlled fermenter. They found that for all three *Magnetospirillum* strains, magnetite synthesis was only induced when the oxygen concentration was below a threshold value of 20 mbar, and that the optimum oxygen concentration for magnetite biomineralization was 0.25 mbar.

Synthesis of the bacterial magnetosome

Synthesis of the bacterial magnetosome seems to be a complex process that involves several discrete steps, including magnetosome vesicle formation, iron uptake by the cell, iron transport into the magnetosome vesicle and controlled Fe_3O_4 (or Fe_3S_4) biomineralization within the magnetosome vesicle (FIG. 5). Although it is clear that the uptake, transport and mineralization steps are temporally ordered, it is unclear whether iron uptake precedes or follows vesicle formation, or if both steps occur simultaneously.

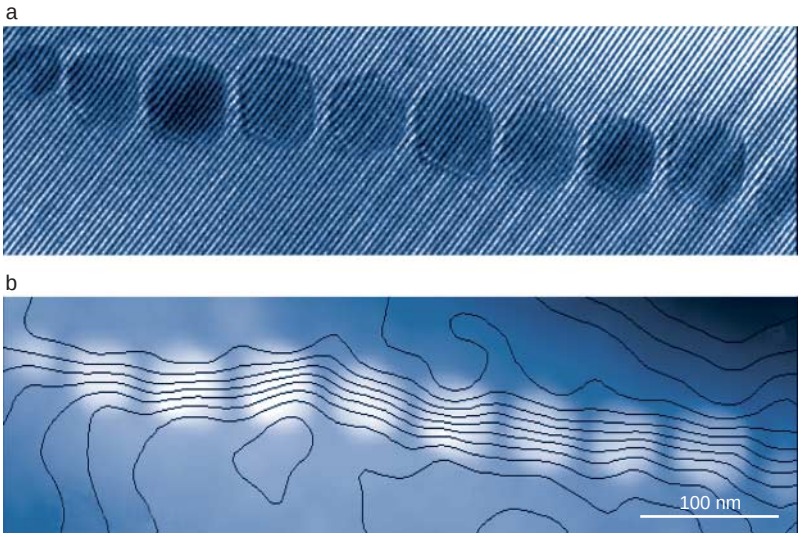


Figure 3 | **Electron holography of a region of the magnetosome chain in *Magnetospirillum magnetotacticum*. a** | Magnetosomes with the electron interference pattern. **b** | Magnetic field lines derived from the interference pattern superimposed on the positions of the magnetosomes. The confinement of the field lines within the magnetosomes is indicative of single magnetic domains and shows that the chain of magnetosomes acts as a single magnetic dipole.

Iron uptake in magnetotactic bacteria. Despite the fact that magnetotactic bacteria consist of up to 3% iron as measured by dry weight[3] — which is several orders of magnitude higher than non-magnetotactic species — at present there is no evidence to indicate that they use unique iron-uptake systems. Fe(II) is very soluble (up to 100 mM at neutral pH[62]), and is generally taken up by bacteria by nonspecific mechanisms. However, Fe(III) is so insoluble that most microorganisms produce and rely on iron chelators, known as siderophores (BOX 2), which bind and solubilize Fe(III) for uptake. Siderophores are low-molecular weight (<1 kDa), specific ligands that are generally produced by bacteria under iron-limited conditions, and their synthesis is repressed under high-iron conditions[63].

Frankel and co-workers[64] assumed that iron uptake by *M. magnetotacticum* was by a nonspecific transport system. In this study, iron was supplied as Fe(III) quinate, however, the growth medium also contained chemical reducing agents (for example, ascorbic acid) that are potent enough to reduce Fe(III) to Fe(II). So, both Fe(II) and Fe(III) were present in the growth medium and it is unknown which form was taken up by the cells; however, *M. magnetotacticum* was reported to produce a hydroxamate siderophore when grown under high (20 μM), but not under low (5 μM), iron conditions[65], which indicates that cells can take up Fe(III) (the siderophore production pattern here is the reverse of what is normally observed).

Schüler and Baeuerlein[66] have described two iron-uptake systems in *M. gryphiswaldense*. They showed that iron was mostly taken up as Fe(III) and that this is an energy-dependent process. Fe(II) was also taken up by cells, but by a slow, diffusion-like process, whereas Fe(III) uptake followed Michaelis–Menten kinetics, which indicates that Fe(III) uptake by *M. gryphiswaldense* is a low-affinity, but high-velocity transport system. Although they found no evidence for siderophore production, the use of spent culture fluid stimulated iron uptake by iron-depleted cells.

Nakamura *et al.*[67] reported molecular evidence for the involvement of a periplasmic-binding protein, SfuC, in the transport of iron by *M. magneticum* strain AMB-1. They did not detect siderophores in spent growth medium, although *M. magneticum* strain AMB-1 was recently found to produce both hydroxamate and phenolate siderophores[68]. Like *M. magnetotacticum*, *M. magneticum* strain AMB-1 produces siderophores under growth conditions that would be considered to be iron-sufficient, if not iron-rich, for the growth of most prokaryotes. This pattern of siderophore production might be explained by the fact that iron is taken up rapidly and converted to inert Fe_3O_4, which apparently cannot be used by cells. So, the concentration of iron that is available for growth probably decreases relatively quickly and the cells experience iron-limiting conditions, which stimulates siderophore production.

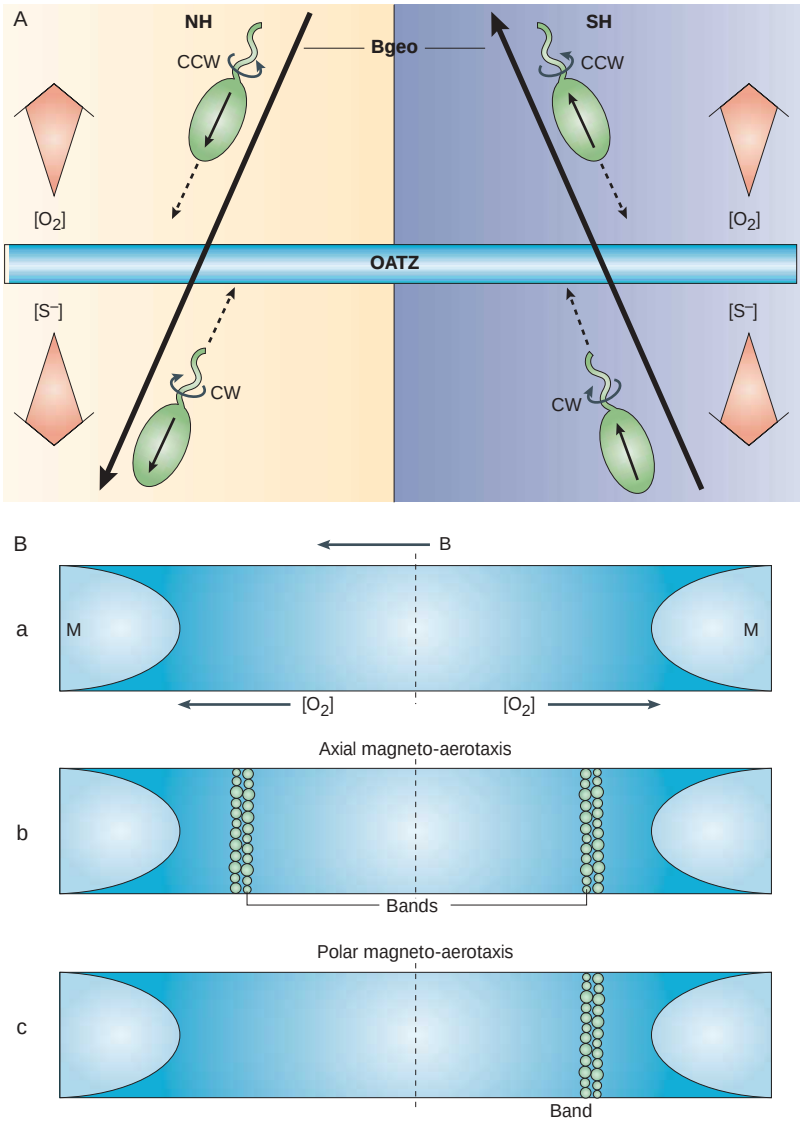


Figure 4 | **Magneto-aerotaxis. A** | Magneto-aerotaxis in the northern (NH) and southern (SH) hemispheres aids cells in efficiently finding their optimum oxygen concentration ($[O_2]$) at the microaerobic oxic–anoxic transition zone (OATZ) in water columns or sediments with horizontal chemical stratification (inverse concentration gradients of oxygen and hydrogen sulphide). In both hemispheres, cells on the oxic side of the OATZ swim down along the geomagnetic field lines (B_{geo}) by rotating their flagella counterclockwise (CCW), whereas those on the anoxic side swim up along B_{geo} by rotating their flagella clockwise (CW). This requires polar magneto-aerotactic cells in the NH and SH to have opposite magnetic polarity (shown by arrows inside cells). This means they exhibit north-seeking and south-seeking behaviour, respectively, when examined in oxic water droplets in a magnetic field. Axial magneto-aerotactic cells swim in both directions along the magnetic field. **B** | Determining axial and polar magneto-aerotaxis in bacteria. **Ba** | Schematic drawing of a thin, flat capillary (cross section 0.2 mm x 5 mm) that is used to distinguish between polar and axial magneto-aerotactic bacteria. Cell suspensions of magnetotactic bacteria in reduced growth medium are drawn up in the capillaries, resulting in a meniscus (M) at both ends. Oxygen diffuses into the medium from the open ends of the capillaries resulting in an oxygen-concentration gradient, $[O_2]$, that increases from the centre of the capillary towards each end. The tube is placed in a static magnetic field (B), which is of the order of a few gauss and is oriented along the tube. **Bb** | Band formation by axial magneto-aerotactic cells, such as *Magnetospirillum magnetotacticum*, occurs at both ends of the capillary. Rotation of the magnetic field by 180° after formation of the bands causes the cells in both bands to rotate 180°, but the bands remain intact. **Bc** | Band formation by polar magneto-aerotactic cells, such as the marine coccus, strain MC-1, occurs only at the end of the capillary for which the magnetic field and the oxygen concentration gradient are oriented opposite to each other. Rotation of the magnetic field by 180° after formation of the band causes the cells in the band to rotate 180° and swim away, resulting in dispersal of the band.

Recently, we have found that strain MV-1 also produces a siderophore (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation). It seems to be a hydroxamate type and the pattern of siderophore production under different iron concentrations is similar to that observed for the *Magnetospirillum* species. We have also found biochemical and molecular evidence for the presence of a copper-dependent, high-affinity iron-uptake system in strain MV-1 (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation), which is similar to the system that is found in the yeast *Saccharomyces cerevisiae*[69].

Magnetosome vesicle formation. The magnetosome membrane in several *Magnetospirillum* species has been shown to consist of a lipid bilayer about 3–4 nm thick[70], comprising phospholipids, fatty acids and some proteins that are similar to those found in the cytoplasmic membrane[70] (FIG. 6). This indicates that the magnetosome membrane vesicle originates from the cytoplasmic membrane and might be the reason why magnetosomes in almost all of the magnetotactic bacteria seem to be anchored to the cytoplasmic membrane, as shown by electron microscopy and electron tomography. However, there is no direct, unequivocal evidence for the contiguity of these two membranes. Nonetheless, the current belief is that the magnetosome membrane vesicle is created by invagination and 'pinching off' of the cytoplasmic membrane. An important question that is yet to be answered is whether the vesicle is produced before Fe_3O_4 nucleation and precipitation, or whether Fe_3O_4 nucleation takes place in the periplasm and the cytoplasmic membrane invaginates around the developing crystal. There is some evidence for the former as apparently empty and partially filled magnetosome vesicles have been observed in iron-starved cells of *M. magnetotacticum*[70] and strain MV-1.

Small GTPases, such as Sar1p, are known to be essential for the budding reaction in the production of membrane vesicles and vesicle trafficking in eukaryotes[71]. Okamura *et al.*[72] identified a 16-kDa protein that has GTPase activity — known as Mms16 — in the magnetosome membrane vesicle of *M. magneticum* strain AMB-1, where it was the most abundant of five proteins present. Cells that were grown in the presence of a GTPase inhibitor showed less overall magnetism and produced fewer magnetosomes than those grown in the absence of a GTPase inhibitor, which indicates that GTPase activity is required for magnetosome synthesis. A protein with high sequence similarity to Mms16 was recently shown to be involved in polyhydroxybutyrate depolymerization in the photosynthetic bacterium *Rhodospirillum rubrum*[73].

Iron transport into the magnetosome membrane vesicle. From an early stage in magnetosome research, electron microscopy has been used to show Fe_3O_4 crystals in various stages of maturity, and that these crystals increase in size within magnetosome vesicles. So, regardless of

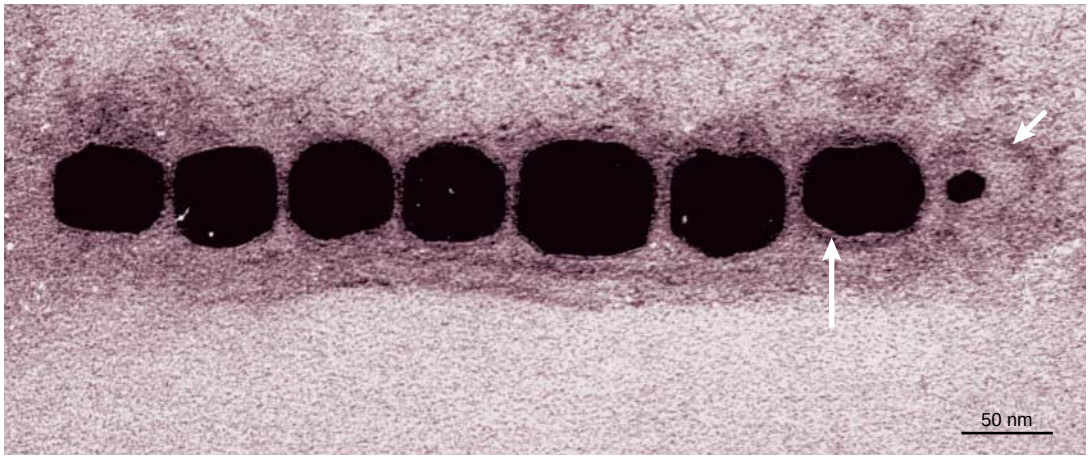


Figure 5 | **Transmission electron micrograph of a thin section of a magnetosome chain in a cell of the marine spirillum strain MV-4.** The magnetosome membrane is shown as an electron-dense coating surrounding the magnetite crystals. The membrane seems to be pulled away from the crystal at the long arrow, and the short arrow indicates a partially filled magnetosome vesicle. The magnetosome membrane is often difficult to visualize around all the magnetite particles owing to its close proximity to the crystal. Sometimes, the thin section must be tilted in the electron beam. Immature magnetite crystals are often observed at the ends of the chain in magnetotactic bacteria. Image courtesy of T. J. Beveridge.

when the magnetosome membrane vesicle is formed, additional iron must be transported into the vesicle for the crystal to grow.

It is not known which redox forms of iron are transported into the magnetosome vesicle in most magnetotactic bacteria, but there is evidence that Fe(II) is transported into vesicles of *M. magneticum* strain AMB-1 (REF. 74). Using transposon mutagenesis, Nakamura and co-workers[74,75] identified a gene, *magA*, that encodes a protein with significant sequence homology to the cation-efflux proteins, KefC, a K^+-translocating protein in *Escherichia coli*, and NapA, a putative Na^+/H^+ antiporter from *Enterococcus hirae*. The MagA protein is present in both the cytoplasmic and magnetosome membranes of *M. magneticum* strain AMB-1. *magA* was expressed in *E. coli* and inverted membrane vesicles prepared from these cells were shown to transport Fe(II) in an energy-dependent manner, leading to accumulation of Fe(II) in the vesicle, which indicates that MagA functions as a H^+/Fe(II) antiporter in *M. magneticum* strain AMB-1. However, *magA* expression was higher when cells of wild-type

Box 2 | Siderophores in bacteria

Siderophores are relatively low-molecular weight (most are between 0.5 to 1.5 kDa), Fe(III)-specific chelating compounds that are produced by most prokaryotes and fungi under environmental conditions where the concentration of biologically available iron is low[123]. Iron is a required nutrient for all but a few species of organisms and is used for many essential purposes. Despite the fact that iron is the fourth most abundant element on Earth, most iron is not biologically available because, under aerobic atmospheric conditions, Fe(III) exists predominantly in the form of Fe(III) oxyhydroxides, which are almost insoluble at neutral pH[62]. As a result, the maximum concentration of uncomplexed Fe(III) in solution has been estimated at 10^{-18} M (REF. 124). Microorganisms require a minimum effective iron concentration of ~0.01 μM for growth, but ~1 μM for optimal growth[125]. Siderophores are a mechanism by which microorganisms can scavenge iron from non-soluble sources, and some microorganisms can even use siderophores that are produced by different species.

Siderophores can be divided into four broad groups based on the chemical type of the chelating ligands in the molecule: catecholates, hydroxamates, hydroxypyridonates and aminocarboxylates[125]. There are approximately 200 recognized siderophores, and most are catecholates and hydroxamates. Some well-characterized examples include the catecholate enterobactin that is produced by *Escherichia coli* and the trihydroxamic acid desferriferrioxamine B that is produced by the Gram-positive actinomycete *Streptomyces pylosus*. The latter has been used in humans in iron-chelation therapy[126].

Much of the work involving the transport of Fe(III)–siderophore complexes has been done using *E. coli*. Despite the low-molecular mass of the siderophore itself, the Fe(III)–siderophore complex is too large to passively diffuse across the outer and inner membranes of a Gram-negative bacterium, or to be taken up by nonspecific methods. Uptake of the Fe(III)–siderophore complex is receptor- and energy-dependent. The binding of the complex to the outer membrane receptor is usually very specific. The transport of the Fe(III)–siderophore complex across the outer membrane into the periplasm requires an energy-transducing complex composed of the proteins TonB, ExbB and ExbD. In the periplasm, the Fe(III)–siderophore complex binds to its cognate binding protein and is actively transported by an ATP-transporter system across the cytoplasmic membrane to the cytoplasm[127]. It can be concluded that if most of the iron in magnetosomes is transported by cells through the use of siderophores, then the cell is probably expending a significant amount of energy on iron uptake.

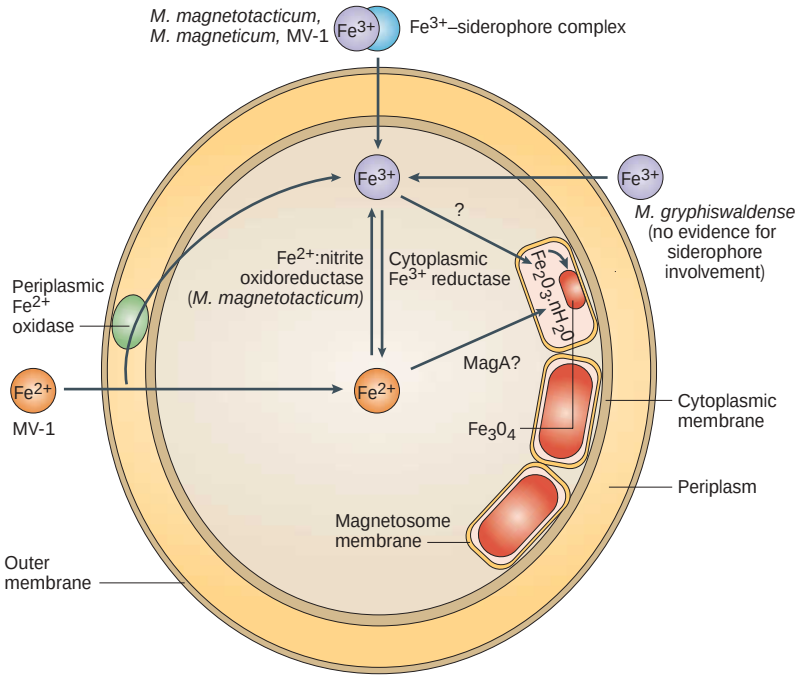


Figure 6 | **Schematic of possible reactions leading to magnetite biomineralization in known, cultured species of magnetotactic bacteria.** Fe(III) is actively taken up by cells of *Magnetospirillum gryphiswaldense*, with no evidence of the involvement of a siderophore[66]. It is likely that cells of the other *Magnetospirillum* strains and strain MV-1 also take up Fe(III) as it is thought that they form siderophores (REFS 65,68; B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation), although it is not known what proportion of the iron in the magnetosomes is taken up as Fe(III) by these organisms. The siderophore-bound Fe(III) is probably reduced to Fe(II). At least 70% of the iron in the culture medium that is used to grow strain MV-1 is present as Fe(II), so it is likely that much of the iron that is taken up by this strain is of the form Fe(II). A periplasmic, multicopper-containing Fe(II) oxidase has been purified from this organism that could be involved in the transport of iron into the cytoplasm (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation). A protein capable of Fe(II) oxidation[99] has also been found and purified from *Magnetospirillum magnetotacticum*. A cytoplasmic Fe(III) reductase activity is present in strain MV-1 (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation) and a cytoplasmic Fe(III) reductase has been purified from *M. magnetotacticum*[103]. There is some evidence that in *Magnetospirillum magneticum*, MagA — a protein that is found in the cytoplasm and the magnetosome membranes of *M. magneticum* — functions as an H^+/Fe(II) antiporter[74,75]. Other proteins possibly responsible for, or involved in, iron transport into the magnetosome membrane vesicle are MamB and MamM — proteins that are abundant in the magnetosome membranes of *M. gryphiswaldense*[77]. Iron that is transported into the magnetosome membrane vesicle in *M. magnetotacticum* is then thought to form a high-density hydrous Fe(III) oxide (ferrihydrite; $Fe_2O_3{\cdot}nH_2O$); reduction of one-third of the Fe(III) ions and adjustment of the pH results in nucleation of the magnetite crystal within the magnetosome vesicle[64]. The nucleation step might involve the adsorption of aqueous Fe(II) ions onto the surface of the hydrous iron oxide[82,83].

M. magneticum strain AMB-1 were grown under iron-limited conditions, rather than under iron-sufficient conditions where they produce more magnetosomes. So, even though MagA seems to be involved in iron transport, it is not solely responsible for magnetosome synthesis. Genes that share significant sequence homology with *magA* are present in *M. magnetotacticum* and the unnamed magnetotactic coccus, strain MC-1 (REF. 76).

Two other proteins that are abundant in the magnetosome membrane of *M. gryphiswaldense* — MamB and MamM — might also be involved in the transport of iron into magnetosome vesicles[77]. Both proteins seem to belong to a group of heavy-metal-ion-transporting proteins that is known as the cation diffusion facilitator (CDF) family[78,79] (BOX 3). A spontaneous non-magnetotactic mutant of *M. gryphiswaldense*, which lacks *mamB* and *mamM*, as well as numerous other magnetosome membrane proteins and other genes, and which does not biomineralize magnetosomes (discussed below), was found to be deficient in iron uptake[77]. Iron has not been unequivocally shown to be bound and transported by any known CDF protein, although in the yeast *S. cerevisiae*, the overexpression of two genes that encode mitochondrial membrane proteins affects Fe(II) concentrations in the mitochondria and cytosol[80]. Although these genes have been referred to as mitochondrial iron transporters (MFT; *MFT1* and *MFT2*)[80], deletion of either or both genes did not affect essential Fe^{2+}-dependent processes in the mitochondrion, leaving the specific role of these genes unclear[81].

Controlled Fe_3O_4 biomineralization within the magnetosome vesicle. Frankel *et al.*[64] have proposed a model in which Fe(III) is taken up by the cell, reduced to Fe(II) and then transported to the magnetosome membrane vesicle. It is then thought to be reoxidized to form hydrous Fe(III) oxides, which are similar to the mineral ferrihydrite. In the final step, one-third of the Fe(III) ions in the hydrous oxides are reduced and, with further dehydration, Fe_3O_4 is produced. The crucial step in the transformation of hydrous iron oxides to magnetite *in vitro* involves the adsorption of aqueous Fe(II) ions onto the surface of the hydrous iron oxide[82,83]. Schüler and Baeuerlein[58] have subsequently shown that, in *M. gryphiswaldense*, Fe(III) is taken up and rapidly converted to Fe_3O_4 without any apparent delay, indicating that there is no significant accumulation of an Fe_3O_4 precursor inside the cell, at least under the microaerobic conditions of the experiment, which were apparently optimal for Fe_3O_4 production by *M. gryphiswaldense*.

The size and shape of the magnetosome mineral phase has long been thought to be controlled by the magnetosome membrane vesicle, although the exact mechanism by which this occurs is unclear. Perhaps certain proteins are distributed asymmetrically in the magnetosome membrane, thereby facilitating crystal growth in certain directions but retarding it in others. It is also possible that the magnetosome membrane vesicle places physical constraints on the growing crystal, thereby limiting its size. Arakaki and co-workers[26] partially characterized several magnetosome membrane proteins that were tightly bound to Fe_3O_4 crystals in *M. magneticum* strain AMB-1, including Mms5, Mms6, Mms7 and Mms13. Mms6 was overexpressed in *E. coli*, purified and found to bind iron. More importantly, Fe_3O_4 crystals that were formed chemically in the presence of Mms6 had a size range of about 20–30 nm and a cuboidal morphology similar to those produced by intact cells (FIG. 7). Those produced in the absence of Mms6 were non-homogeneous in shape and were 1–100 nm in size. All four Mms proteins contain hydrophobic amino-terminal and hydrophilic carboxy-terminal regions, the latter being rich in carboxyl and hydroxyl groups that are thought to bind iron. All

Box 3 | Cation diffusion facilitator proteins

The cation diffusion facilitator (CDF) protein family is a diverse family of heavy metal ion transporters (metal/H^+ antiporters[81]) that is found in both prokaryotes and eukaryotes[79,81]. Many CDF genes were discovered through overexpression experiments that resulted in the resistance of an organism to the toxicity of a specific metal[81]. They show an unusual degree of size variation, sequence divergence and polarity, and can catalyze the influx or efflux of metal ions[79]. In addition, there are differences in the cellular localization of the protein (they can be localized in specific organelles in eukaryotes) and some have been shown to affect the cellular distribution of specific metals[81]. All recognized CDF proteins contain six putative transmembrane-spanning domains, with the highest amino-acid sequence conservation in the four amino-terminal spanning domains[79]. Eukaryotic CDF proteins also possess histidine-rich cytoplasmic loops between transmembrane-spanning domains 4 and 5 (REF. 79). Heavy metals that have been shown to be transported by CDF proteins include cobalt, cadmium and zinc[79].

four Mms proteins also have the common amino-acid sequence LGLGLGLGAWGPXXLGXXGXAGA. As Mms7 and Mms13 show very high sequence identity to the MamD and MamC proteins in *M. gryphiswaldense*, respectively, they should be considered as identical, equivalent proteins and therefore should retain the names MamD and MamC (see next section).

Other proteins and genes

Magnetosome membrane proteins. Okuda *et al.*[84] identified three magnetosome membrane proteins with molecular weights of 12, 22 and 28 kDa in *M. magnetotacticum*. The gene encoding the 22-kDa protein was cloned and sequenced, and the amino-acid sequence showed significant homology to proteins of the tetratricopeptide repeat protein (TPR) family (BOX 4), including mitochondrial and peroxisomal protein import receptors. It was proposed that this protein functions as a receptor that interacts with associated cytoplasmic proteins. The *mam22* gene was expressed in *E. coli* and the resultant protein partially characterized[85]. A structural model of Mam22 was proposed, which contains five TPR repeats and a putative sixth repeat.

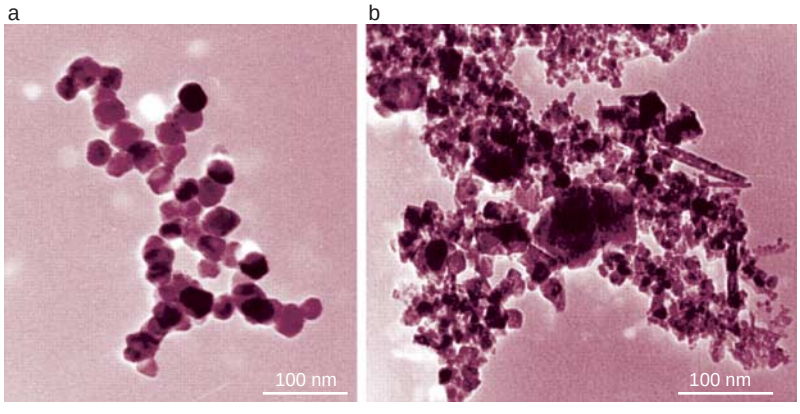


Figure 7 | **The effects of a magnetosome membrane protein, Mms6, on magnetite formation. a** | Fe_3O_4 crystals produced in the presence of Mms6 had a size range of 20–30 nm, with a cuboidal morphology similar to those produced by intact cells. **b** | Fe_3O_4 crystals produced in the absence of Mms6 are non-homogeneous in shape and range in size from 1 to 100 nm. Image courtesy of T. Matsunaga. Reproduced with permission from REF. 26 © (2003) American Society for Biochemistry and Molecular Biology.

A similar protein, called MamA, and/or genes encoding similar proteins have been found in *M. gryphiswaldense* and strain MC-1 (REF. 76).

Grünberg *et al.*[76] cloned and sequenced the genes encoding several Mam proteins in *M. gryphiswaldense* that had been assigned to two different genomic regions. These proteins exhibited the following homologies: MamA to TPR proteins (BOX 4); MamB to CDF proteins (BOX 3); and MamE to HtrA-like serine proteases (BOX 5). The sequences of MamC and MamD only show homology to some of the Mms proteins described in the previous section. Other putative genes in these genomic regions were also conserved and might encode other magnetosome membrane proteins. Similar gene clusters containing homologues to *mamA* and *mamB* and additional genes with no homology to known genes or proteins in established databases have been found in *M. magnetotacticum* and strain MC-1. However, it was not shown whether the homologous genes in strain MC-1 actually encode magnetosome membrane proteins in this organism, although we recently confirmed that the *mamC* gene encodes a magnetosome membrane protein in strain MC-1.

Several research groups have observed the frequent formation of spontaneous non-magnetotactic mutants of several magnetotactic bacterial strains, including *M. gryphiswaldense* and strain MV-1, which do not synthesize magnetosomes. These mutants do not contain intracellular vesicles and neither take up nor accumulate the large amounts of iron that are observed in the wild-type strains. Schüler and co-workers[77] have recently shown that one of these mutants has a chromosomal deletion of an ~80 kb region of DNA, which contains several insertion sequences, the *mam* genes and additional genes that encode magnetosome membrane proteins. The *mam* genes are localized in a 35-kb region of the deletion, so this area could represent a magnetosome genomic island. Genomic islands are large regions of DNA that contain genes linked to a metabolic activity or function and that are capable of horizontal gene transfer. We have preliminary evidence to show that a similar situation exists in strain MV-1.

Genomic islands often have a different G+C content to the rest of the genome[86]. They are also known to be flanked by several types of mobile genetic elements, including direct repeats, insertion sequences, integrases, transposases, proximal transfer RNAs and areas of atypical G+C content, which are responsible for the mobilization of the island. Owing to these flanking elements, genomic islands not only have a tendency to delete from genomes with high frequency, they can also undergo duplications, amplifications and rearrangements[87,88]. The possibility that the genes that are responsible for magnetite magnetosome synthesis are part of a genomic island could be the reason why this trait is observed in many diverse bacteria and could also explain why this trait seems to be easily lost in several strains of magnetotactic bacteria.

Matsunaga *et al.*[89] identified three important magnetosome membrane proteins with molecular masses of approximately 24.8, 35.6 and 66.2 kDa in

Box 4 | Tetratricopeptide repeat proteins

The tetratricopeptide repeat (TPR) is a degenerate sequence of 34 amino acids that is known to be present in >25 proteins of varying function in both prokaryotes and eukaryotes[128–130]. Sequence alignment of TPR domains reveals a consensus sequence consisting of a pattern of small and large hydrophobic amino acids[131]. TPRs are usually arranged in tandem arrays of 3–16 motifs, although occasionally, in some proteins, individual motifs or blocks of motifs can be dispersed throughout the protein sequence. Multiple copies of TPRs form scaffolds within proteins to mediate protein–protein interactions. They are known to coordinate the assembly of proteins into multisubunit complexes[132]. TPRs were first recognized in the eukaryotic cell-division protein subunits CDC16, CDC23 and CDC27, which comprise the anaphase-promoting complex[129]. Proteins containing TPRs are now also known to be involved in other processes, including protein folding, mitochondrial and peroxisomal protein transport, protein kinase inhibition, Rac-mediated activation of NADPH oxidase, neurogenesis, transcriptional control and protein phosphatase activity[128,130,131]. Recently, a model for TPR-mediated protein recognition was reported for the enzyme serine/threonine phosphatase PP5 (REF. 128).

M. magneticum strain AMB-1. The gene encoding the 35.6-kDa protein, *mpsA*, was cloned and the protein sequenced. MpsA was found to have homology with the α-subunits of acetyl-CoA carboxylases and the CoA-binding motif. At present, the function of this protein is unknown.

A series of non-magnetotactic mutants of *M. magneticum* strain AMB-1 was generated by mini-Tn*5* transposon mutagenesis[90]. One of these, designated strain NMA21, was recently isolated and characterized[91]. The transposon was found to have disrupted a gene encoding a protein with high sequence homology to a tungsten-containing aldehyde ferredoxin oxidoreductase from the hyperthermophilic archaeon *Pyrococcus furiosis*. The protein was produced under microaerobic conditions and was cytoplasmic. Cells of NMA21 did not produce magnetosomes and the rates of iron uptake and growth of this mutant strain were lower than those of the wild-type strain.

Nitrogen oxide, iron reduction and oxidation. To understand the relationship between nitrate and oxygen utilization and Fe_3O_4 synthesis in *M. magnetotacticum*, Fukumori and co-workers examined electron transport and cytochromes in this organism. Tamegai *et al.*[92] purified and characterized a novel 'cytochrome a_1-like' haemoprotein that was found to be present in greater amounts in magnetic cells than non-magnetic cells. There was no evidence for the presence of a cytochrome a_1, once reported to be one of the terminal oxidases, or an *o*-type cytochrome in *M. magnetotacticum*[93]. The 'cytochrome a_1-like' haemoprotein was composed of two different subunits with molecular masses of 41 kDa (subunit I) and 17 kDa (subunit II), and exhibited very little cytochrome *c* oxidase activity. The genes encoding this unusual cytochrome were identified and sequenced[94]. Three open reading frames preceded by a putative ribosome-binding site were found in the sequenced region and designated *mcaII*, *mcaI* and *hosA*. *mcaI* and *mcaII* were shown to encode subunits I and II of the 'cytochrome a_1-like' haemoprotein, respectively. *hosA* showed significant sequence homology to the gene encoding haem *o* synthase (protohaem IX farnesyltransferase), an essential enzyme for the biosynthesis of haem *o* and haem *a*[95]. Although six histidine residues that were predicted to associate with prosthetic cofactors of the haem-copper oxidase superfamily were conserved in the 'cytochrome a_1-like' haemoprotein, none of the amino acid residues that were proposed to participate in the oxygen-reducing and the coupled proton-pumping reactions in cytochrome *c* oxidase in *Paracoccus denitrificans*[96] were conserved in subunit I. The latter finding probably explains the observed poor cytochrome *c* oxidase activity of the protein.

A new *ccb*-type cytochrome *c* oxidase[97], a cytochrome *c*-550 that is homologous to cytochrome c_2 in some photosynthetic bacteria[98] and a cytochrome cd_1-type nitrite reductase[99] were identified and purified from *M. magnetotacticum*. The latter protein might be important in Fe_3O_4 biomineralization as it has a novel Fe(II):nitrite oxidoreductase activity that might be linked to the oxidation of Fe(II) in the cell and, therefore, to Fe_3O_4 synthesis. Recently, a soluble periplasmic nitrate reductase was purified from *M. magnetotacticum*[100]. The enzyme comprises two subunits of 86 and 17 kDa and contains molybdenum, non-haem iron and haem *c*. Molybdenum starvation of cells resulted in little periplasmic nitrate reductase activity in cell-free extracts, but the magnetosome fraction still had almost half the iron that was present in the same fraction of cells grown with molybdenum. These results indicate that nitrate reduction in this organism is not essential for Fe_3O_4 synthesis.

Several species of magnetotactic bacteria reduce or oxidize iron either as intact whole cells, as cell-free extracts or both. Cells of *M. magnetotacticum* reduce Fe(III)[57] and translocate protons when Fe(III) is introduced anaerobically[101], indicating that cells conserve energy during the reduction of Fe(III). Growth yields on Fe(III) indicate that iron reduction is also linked to growth, as is found in the dissimilatory iron-reducing bacteria[57]. Fe(III) reductase activity has also been shown in cell-free extracts of *M. magnetotacticum*[102] and strain MV-1 (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B. manuscript in preparation), and an Fe(III) reductase was purified from *M. magnetotacticum*[103]. The enzyme seems to be loosely bound to the cytoplasmic face of the cytoplasmic membrane, has an apparent molecular weight of 36 kDa, and requires reduced nicotinamide adenine dinucleotide and flavin mononucleotide as an electron donor and cofactor, respectively. Enzyme activity was inhibited by zinc, which also reduced the number of magnetosomes when included in the growth medium as $ZnSO_4$.

Genetic systems in the magnetotactic bacteria

It is unknown how many, or which, genes and proteins are required for Fe_3O_4 magnetosome synthesis, or how these genes are regulated. Establishing a genetic system with the magnetotactic bacteria is an absolute necessity to answer these questions. In many ways, progress in the elucidation of the chemical and biochemical pathways

Box 5 | The HtrA family of serine proteases

HtrA (also known as DegP) is an envelope-associated serine protease that was first discovered in *Escherichia coli* and is induced by heat-shock[133]. The enzymatic activity of HtrA occurs in the periplasm, where its main role seems to be in the degradation of misfolded proteins[134]. Although HtrA has a significant role in 'cellular cleaning', these proteases are also involved in non-destructive protein processing and modulation of signalling pathways by degrading important regulatory proteins. Homologues of HtrA have now been discovered in diverse bacteria and in some eukaryotes, including yeasts, plants and humans[134]. All have at least one PDZ domain — a region of sequence homology that has been found in a large number of diverse signalling proteins[134]. PDZ domains are known to be involved in a range of protein–protein interactions and mediate the assembly of specific multi-protein complexes by recruiting downstream proteins in a signalling pathway[134,135]. The *htrA* gene has practical significance and can be used in several commercial and medical applications[134]. For example, *htrA* mutants of several Gram-negative pathogens become attenuated in animal models, so cells of these mutant strains could potentially be used as live vaccines. These mutants might also have potential biotechnological applications as they show improved expression of envelope-associated proteins.

that are involved in Fe_3O_4 magnetosome synthesis, particularly in determining the function of specific proteins, has been limited by the general absence of a workable genetic system in the magnetotactic bacteria. There are still many problems in establishing genetic systems in the magnetotactic bacteria, including the lack of a significant number of magnetotactic bacterial strains. In addition, their fastidiousness and general microaerophilic nature require elaborate growth techniques, and they are difficult to grow on the surface of agar plates, which would enable the screening for mutants. Moreover, there is a lack of effective methods of DNA transfer in these microorganisms. However, this situation is improving rapidly.

Waleh and co-workers initiated the first studies in the establishment of a genetic system in magnetotactic bacteria. They showed that some of the genes from *M. magnetotacticum* can be functionally expressed in *E. coli* and that the transcriptional and translational elements of the two microorganisms are compatible, a feature that is necessary for a genetic system[104]. They cloned, sequenced and characterized the *recA* gene from *M. magnetotacticum*[105,106]. Focusing on iron uptake in *M. magnetotacticum*, they also cloned and characterized a 2-kb DNA fragment that complemented the *aroD* (biosynthetic dehydroquinase) gene function in *E. coli* and *Salmonella enterica* serovar Typhimurium[107]. *aroD* mutants of these strains cannot take up iron from the growth medium. When the 2-kb DNA fragment from *M. magnetotacticum* was introduced into these mutants, the ability to take up iron from the growth medium was restored. However, it did not mediate siderophore biosynthesis.

If a magnetotactic bacterial strain forms colonies, the selection of non-magnetotactic mutants that do not produce magnetosomes is a relatively easy task. Generally, cells that produce magnetosomes form dark coloured, even black, colonies, whereas mutants that do not produce magnetosomes form lighter-coloured, usually white to pink, colonies. Techniques for growing several magnetotactic bacterial strains including *M. magnetotacticum* strain AMB-1 (REF. 59), *M. magnetotacticum*[59,108], *M. gryphiswaldense*[108] and strain MV-1 (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation), on the surface of agar plates have now been developed. However, when cells are grown aerobically, the oxygen concentration of the incubation atmosphere must be decreased to 0.5–2%, depending on the strain. Strain MV-1 forms colonies not just microaerobically, but also anaerobically under 1 atm of N_2O (B.L. Dubbels, A.A. DiSpirito, J.D. Morton, J.D. Semrau & D.A.B., manuscript in preparation). The ability to grow cells on plates facilitates the selection of non-magnetic mutants that do not produce magnetosomes. For example, non-magnetic mutants of *M. magneticum* strain AMB-1, which were obtained following the introduction of Tn*5*, were easily detected using this screening technique[109]. Using these Tn*5*-derived mutants, Nakamura *et al.*[74] found that at least three regions of the chromosome of *M. magneticum* strain AMB-1 were required for the successful synthesis of magnetosomes. One of these regions, which consists of 2,975 base pairs (bp), contained two putative open reading frames, one of which, *magA*, was discussed above.

The presence of a cryptic 3.7-kb plasmid, pMGT, was reported in *M. magneticum* strain MGT-1 (REF. 110). Recombinant plasmids were constructed that were capable of replicating in both *Magnetospirillum* spp. and *E. coli*. These plasmids could be introduced into cells using a newly developed electroporation procedure, although the authors report that cells containing magnetosomes were killed during electroporation and they therefore had to use aerobically non-magnetotactic cells.

Schultheiss and Schüler[108] recently reported the development of a genetic system in *M. gryphiswaldense*. Colony formation on agar surfaces by this strain was achieved at a plating efficiency of >90% by adding activated charcoal, dithiothreitol and elevated concentrations of iron compounds that were known to decompose inhibitory, toxic oxygen radicals produced during respiration in the growth medium. The cells even formed colonies (white) on agar plates that were incubated under air, although cells from these colonies were non-magnetotactic. Protocols were also developed for the introduction of foreign DNA into cells by electroporation and high-frequency conjugation. Several broad-host-range vectors of the IncQ, IncP and pBBR1 groups containing antibiotic-resistance markers were shown to be capable of replicating in *M. gryphiswaldense*.

Genomics of magnetotactic bacteria

As a prelude to genomic studies involving magnetotactic bacteria, the genome arrangement and size of several different species were determined by pulsed-field gel electrophoresis (PFGE). The genomes of the marine vibrios, strains MV-1 and MV-2, consist of a single circular chromosome of ~3.7 and 3.6 Mb, respectively[111]. The coccus, strain MC-1, also has a single circular chromosome, of ~4.5 Mb[111]. There is

no evidence for the presence of extrachromosomal DNA, such as plasmids, in these strains. The genome of *M. magnetotacticum* is arranged as a single, circular chromosome of ~4.3 Mb[112].

Several magnetotactic species have recently been selected as part of a genome project in the United States, and the partially sequenced genomes of two magnetotactic bacteria, *M. magnetotacticum* and the marine coccus strain MC-1, are available for examination at the Joint Genome Institute web site (see Online Links). Schüler and co-workers have examined the organization of magnetosome membrane protein genes in *M. gryphiswaldense* and found that most of the *mam* and *mms* genes that encode most of the magnetosome membrane proteins are clustered within several operons[113] in an unstable region of the genome that constitutes a putative magnetosome gene island (discussed above). There are significant similarities in the conservation and organization of these genes in other magnetotactic bacteria, including other *Magnetospirillum* species and strain MC-1. However, many of these genes have not yet been shown to encode magnetosome membrane proteins, for example, in strain MC-1. Most of these findings are summarized in a recent paper[113].

To the future...

There is no doubt that the number of researchers involved in the study of magnetotactic bacteria has now reached a critical mass, while the subject has become a *bona fide* field of research in microbiology. It is also clear that research progress in the elucidation of magnetosome synthesis has increased tremendously over the past five years. We have highlighted much of this progress and its significance in this review. Owing to the numerous proteins that are present in the magnetosome membrane and the lack of information about their function, we can expect to see many studies focused on the characterization of these proteins, as well as site-directed mutagenesis studies to determine the role of these proteins in magnetite synthesis. This assumption is bolstered by the fact that several workable genetic systems are now available for many magnetotactic bacterial strains. In addition, now that we recognize the fact that many strains use siderophores for iron uptake, we can expect to see studies that examine the molecular mechanisms of iron uptake in magnetotactic bacteria and, hopefully, also studies that address one of the most important issues: why do these microorganisms take up so much iron in the first place?

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Acknowledgements

We acknowledge our students, postdoctoral researchers and numerous collaborators, and are particularly grateful for the support of the US National Science Foundation and the National Aeronautics and Space Administration. We thank Y. Fukumori for valuable discussions and suggestions; T. Matsunaga and Y. Okamura for the use of Figure 7; and D. Moyles and T. J. Beveridge for superb electron microscopy.

Competing interests statement

The authors declare that they have no competing financial interests.

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