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Formation of Magnetosomes in Magnetotactic Bacteria

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Abstract

The ability of magnetotactic bacteria to orient and migrate along geomagnetic field lines is based on intracellular magnetic structures, the magnetosomes, which comprise nano-sized, membrane bound crystals of magnetic iron minerals. The formation of magnetosomes is achieved by a biological mechanism that controls the accumulation of iron and the biomineralization of magnetic crystals with a characteristic size and morphology within membrane vesicles. This paper focuses on the current knowledge about magnetotactic bacteria and will outline aspects of the physiology and molecular biology of magnetosome formation. The biotechnological potential of the biomineralization process is discussed.

Introduction

The serendipitous observation of certain mud bacteria, whose swimming direction could be manipulated by magnetic fields, led to the discovery of unique intracellular structures of small magnetic particles known as magnetosomes (Blakemore, 1975). The magnetosomes, which were identified as membrane-bound particles of a magnetic iron mineral (Balkwill *et al.*, 1980), enable the bacteria to orient themselves and swim along the lines of a magnetic field, a behavior referred to as magnetotaxis (Blakemore and Frankel, 1981). The formation of well-ordered, nano-sized particles with perfect magnetic and crystalline properties in magnetotactic bacteria is an intriguing example for a highly controlled biomineralization process, which stimulated an interdisciplinary research following Blakemore's discovery. The mechanisms of magnetosome formation might be relevant for the synthesis of advanced biomaterials with designed properties (Mann, 1993). Commercial uses of bacterial magnetosome particles have been suggested including the manufacture of magnetic tapes and printing inks, magnetic targeting of pharmaceuticals, cell separation and the application as contrast enhancement agents in magnetic resonance imaging (Mann *et al.*, 1990a; Matsunaga, 1991). Recently, ultrafine-grained magnetite particles from a Martian meteorite, which resembled the magnetosome crystals of recent bacteria, have been cited as putative evidence for ancient extraterrestrial life (Frankel *et al.*, 1998; McKay *et al.*, 1996). Moreover, bacterial magnetosome formation might serve as a model system for the biomineralization of magnetic minerals in other organisms, as similar crystals of ferromagnetic material, mainly magnetite, has been found in a wide range of higher organisms and even humans (Kirschvink, 1989; Kirschvink *et al.*, 1992). Thus, an understanding of the structures and mechanisms involved in bacterial biomineralization of magnetosomes is of crucial interest.

This paper will focus on the current knowledge of the ecology, phylogeny and physiology of magnetotactic bacteria from marine and freshwater habitats. Since most of what we know about the mechanism of magnetosome formation comes from studies of *Magnetospirillum* species, emphasis will be given on the biomineralization of magnetite in these bacteria.

Ecology, Occurrence, and Phylogeny of Magnetotactic Bacteria

Magnetotactic bacteria (MTB) are a heterogeneous group of procaryotes which are ubiquitous in aquatic environments and cosmopolitan in distribution. Given their high abundance and variety in marine and freshwater habitats, MTB very likely play an important ecological role in many sediments, as for instance in biogeochemical cycling of iron and other elements. However, their role remains to be fully evaluated. Furthermore, magnetosome particles remain preserved after the bacterial cells die and thus can be deposited as magnetofossils, which significantly contribute to the magnetization of sediments (Petersen *et al.*, 1986; Stolz, 1990).

Freshwater Habitats

Freshwater communities of MTB have been studied by several microscopic, cultural and molecular-biological approaches. Highest numbers of MTB (10^5 -10^6/ml; Blakemore *et al.*, 1979; Spring *et al.*, 1993) are usually found at the oxic-anoxic transition zone generally located at the sediment-water interface, which is consistent with their microaerophilic to anaerobic lifestyle. Freshwater sediments were found to contain various morphological types of MTB, including rod-shaped, vibrio-like, coccoid, and helical forms (Figure 1). With the apparent exception of extreme environments like acidic mine tailings and thermal springs, MTB occur ubiquitously in a wide range of different habitats (Blakemore, 1982; Mann *et al.*, 1990a), and have been even reported from water-logged soils (Fassbinder *et al.*, 1990). Despite their ubiquitous occurrence, different environments appear to support the development of specific populations of MTB. For example, in a survey of several freshwater habitats, the population of MTB in an eutrophic pond was found to contain only three different morphological forms dominated by a species of a magnetic coccus occurring in high numbers (Schüler, 1994). MTB of this type were usually abundant in habitats with high content of organic nutrients (Moench and

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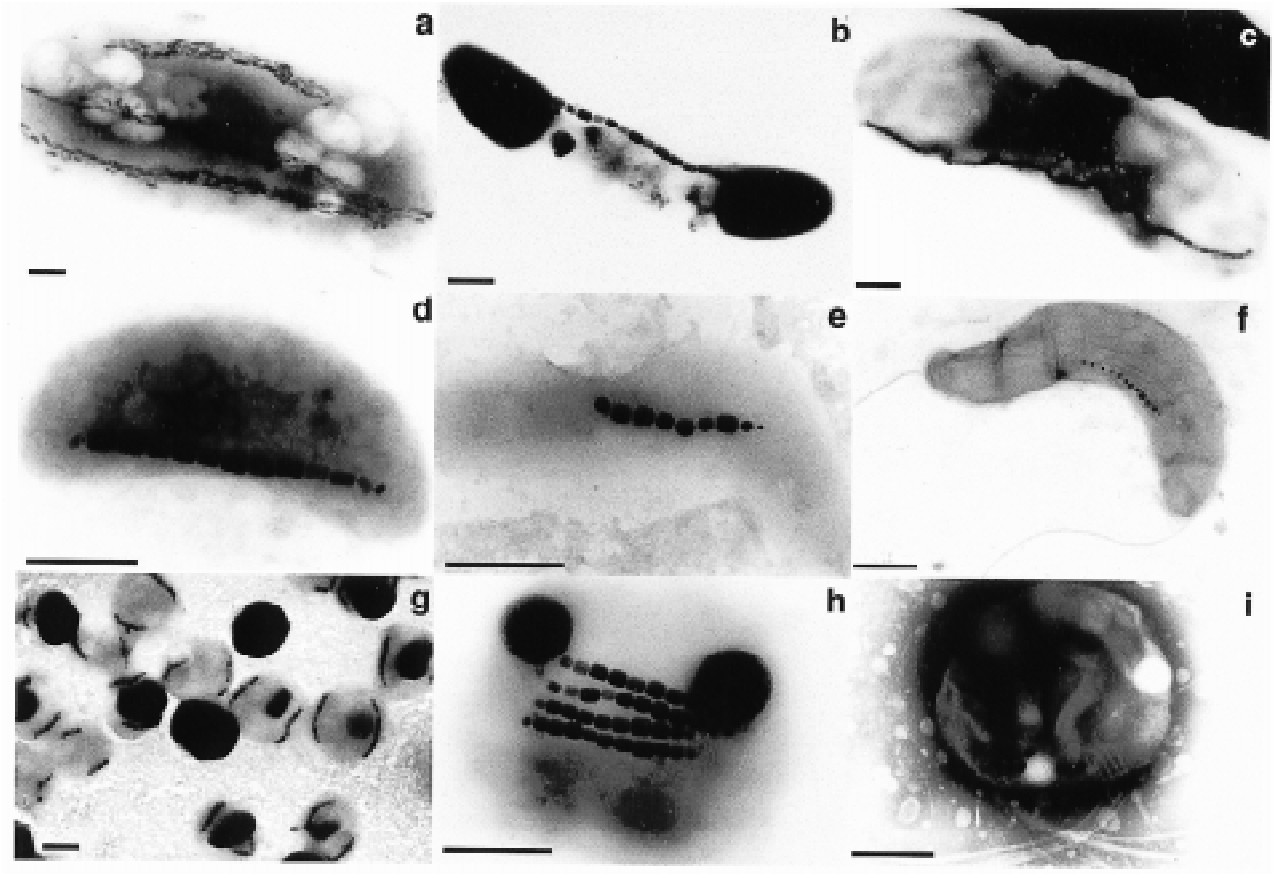

Figure 1. Electron Micrographs of Magnetotactic Bacteria
Transmission electron micrographs showing whole cells of various magnetotactic bacteria collected from different natural habitats. The diversity of morphological forms include large rods (a, b, c), vibrios (d), spirilla (e, f), and coccoid (g, h, i) cell forms. The bar is equivalent to 0.5 μm.

Konetzka, 1978; Mann *et al.*, 1990a). In contrast, MTB from an oligotrophic lake sediment were phylogenetically and morphologically more diverse with at least 10 morphologically distinct forms of MTB (Schüler, 1994). In certain microhabitats, cells of a single rod-like species of MTB were the dominant fraction of the microbial community and accounted for up to 30% of the biovolume (Spring *et al.*, 1993). The occurrence of this unusually large bacterium up to 20 μm long and with more then 1000 magnetosome particles, seems to be restricted to certain types of freshwater sediments (Spring *et al.*, 1993). In contrast to what might be anticipated from their remarkable potential to sequester 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 their enrichment (Blakemore, 1982; Oberhack and Süßmuth, 1986). This implies that MTB are able to accumulate iron against a large concentration gradient.

Marine Habitats

In the marine environment, MTB have been mostly found in coastal environments like marshes and estuaries, although some studies are indicating their apparently widespread occurrence in the ocean up to depths of 3000 m (Petermann and Bleil, 1993; Stolz *et al.*, 1986). Like 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 (Bazylinski, 1995). 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 a many-celled magnetotactic prokaryote which was identified in marine and brackish sediments (Farina *et al.*, 1990; Mann *et al.* 1990b). Several MTB from reducing environments with high concentrations of H_2S contain particles of iron-sulfide (greigite and pyrite) instead of iron oxides found in most MTB (Bazylinski *et al.*, 1993; Posfai *et al.*, 1998; Mann *et al.*, 1990b). It was demonstrated that a freshwater sulfate-reducing bacterium could intracellularly form magnetite (Sakaguchi *et al.*, 1993), suggesting that marine counterparts are most likely abundant given the importance of sulfate reduction in marine systems.

Phylogeny

Examinations of natural communities by molecular phylogenetic techniques revealed that the morphological diversity of MTB is matched by a remarkable phylogenetic diversity and there is likely a large number of different magnetotactic species. MTB occur in different phylogenetic groups of bacteria, so it has been argued that the ability to form magnetosomes has been evolved from separate evolutionary origins (DeLong *et al.*, 1993). MTB from marine habitats are generally found in the same phylogenetic groups as their freshwater counterparts, although they

apparently form slightly separate lineages. So far, there are no known examples of Archea or Gram-positive bacteria capable of forming magnetosomes. The majority of magnetotactic bacteria including cocci, rods, as well as cultivatable vibrios and spirilla, are members of the α-*Proteobacteria* (DeLong *et al.*, 1993; Schleifer *et al.*, 1991; Spring *et al.*, 1992; 1994; 1998). Interestingly, based on comparative sequence analysis of 16S ribosomal RNA, some MTB from the α-subclass are closely related to the nonmagnetic, photosynthetic, non-sulfur purple bacteria, with whom they share the feature of intracytoplasmatic membrane formation. A morphologically distinct large magnetic rod was assigned to the *Nitrospira* phylum, whereas a magnetotactic, many-celled procaryote and a magnetic sulfate-reducing bacterium were found to belong to the δ-subclass of *Proteobacteria* (De Long *et al.*, 1993; Kawaguchi, 1995; Spring and Schleifer, 1995).

Isolation, Cultivation, and Physiology of MTB

Despite their ubiquitous occurrence and high abundance, cultivation of MTB in the laboratory has proven difficult. Problems in isolation and cultivation of these bacteria arise from their lifestyle, which is adapted to sediments and chemically stratified aquatic habitats. As typical gradient organisms, MTB depend on complex patterns of vertical chemical and redox gradients, which are difficult to mimic under laboratory conditions. Since no strictly selective media and growth conditions are known for the cultivation of MTB, the effective separation of magnetotactic cells from non-magnetic contaminants is crucial in their isolation. This can be achieved by exploiting their active migration along magnetic field lines in a capillary "racetrack" method (Wolfe *et al.*, 1987). Growth media involving sulfide and redox gradients have proven useful in the isolation of MTB (Schüler *et al.*, 1999).

Only a limited number of MTB has been isolated in pure culture so far and most of the isolates are poorly characterized in terms of growth conditions and physiology. Examples of isolates which can be grown under laboratory conditions include several freshwater species of *Magnetospirillum* (Blakemore *et al.*, 1979; Burgess *et al.*, 1993; Schleifer *et al.*, 1991; Schüler and Köhler, 1992; Schüler *et al.*, 1999). Two strains of a marine magnetic vibrio were isolated that are facultative anaerobes and can use either oxygen or nitrous oxide as terminal electron acceptors (Bazylinski *et al.*, 1988; Meldrum *et al.*, 1993a). The only cultivatable magnetic coccus was grown microaerobically in gradient cultures (Meldrum *et al.*, 1993b). Sakaguchi *et al.* (1993) isolated an obligate anaerobic, sulfate-reducing magnetotactic bacterium.

Magnetosomes

The hallmark feature of all MTB is the presence of unique intracellular structures, known as magnetosomes, which consist of magnetic iron mineral particles enclosed within membrane vesicles (Balkwill *et al.*, 1980). With the exception of the aforementioned MTB producing iron-sulfide crystals, the iron mineral particles generally consist of magnetite (Fe_3O_4). Magnetite is an inverse spinel ferrite of structural formula Fe^{3+} [Fe^{2+}, Fe^{3+}]O_4, which has ferrimagnetic properties (Banerjee and Moskowitz, 1985). Unlike magnetite found in inorganic systems or produced extracellularly by the metabolic activities of dissimilatory iron-reducing bacteria (Moskowitz *et al.*, 1989), the intracellular magnetosome crystals are characterized by narrow size distributions and uniform, species-specific crystal habits (Figure 2). All habits can be derived from various combinations of the octahedral {111}, dodecahedral {110}, and cubic {100} forms compatible with magnetite (Fd3m) symmetry (Devouard *et al.*, 1998). The particle sizes are typically 35-120 nm, which is within the permanent, single-magnetic-domain-size range for magnetite (Moskowitz, 1995). The magnetosomes are usually organized in chains, resulting in a permanent magnetic dipole sufficiently large so that it will orient the entire bacterium along the geomagnetic field at ambient temperature. This passive orientation results in the migration of the cell along the magnetic field lines as it swims ("Magnetotaxis"; Blakemore and Frankel, 1981).

Function of Magnetotaxis

Because all known magnetotactic bacteria are either microaerophilic or anaerobic, a widely accepted hypothesis regarding the function of magnetotaxis is that they seek to avoid high, potentially toxic oxygen tension. That is, their navigation along the geomagnetic field lines facilitates migration to their favored position in the oxygen gradient (Blakemore and Frankel, 1981). The preferred motility direction found in natural populations of magnetotactic bacteria is northward in the geomagnetic field in the northern hemisphere, whereas it is southward in the southern hemisphere. Because of the inclination of the geomagnetic field, migration in these preferred directions would cause cells in both hemispheres to swim downward. Recent findings indicate that this process is more complex and apparently involves interactions with an aerotactic sensory mechanism (Frankel *et al.*, 1997). Besides magnetotaxis, other possible explanations for the intracellular iron deposition have been considered, including functions in iron homeostasis, energy conservation, or redox cycling (Mann *et al.*, 1990a; Guerin and Blakemore, 1992; Spring *et al.*, 1993).

Physiology, Biochemistry, and Molecular Biology of Magnetite Biomineralization in Members of the Genus *Magnetospirillum*

Most of our knowledge of the mechanism of magnetite biomineralization comes from studies involving several strains of the genus *Magnetospirillum*, although recently efforts were made to address magnetosome synthesis in other systems (Dubbels *et al.*, 1998). Strains of *Magnetospirillum* can be grown microaerobically on simple media containing short organic acids as a carbon source (Blakemore *et al.*, 1979; Matsunaga *et al.*, 1990; Schleifer *et al.*, 1991; Schüler and Köhler, 1992) and have a oxygen-dependent respiratory type of metabolism, although nitrate can be used microaerobically as an electron acceptor in some strains (Bazylinski and Blakemore, 1983). *M. magnetotacticum* was the first magnetotactic bacterium available in pure culture and was used in initial studies. One of the reasons for oxygen sensitivity in this organism may be the lack of the oxygen-protective enzyme catalase, as addition of catalase to the growth medium resulted in increased oxygen tolerance (Blakemore *et al.*, 1979). *M.*

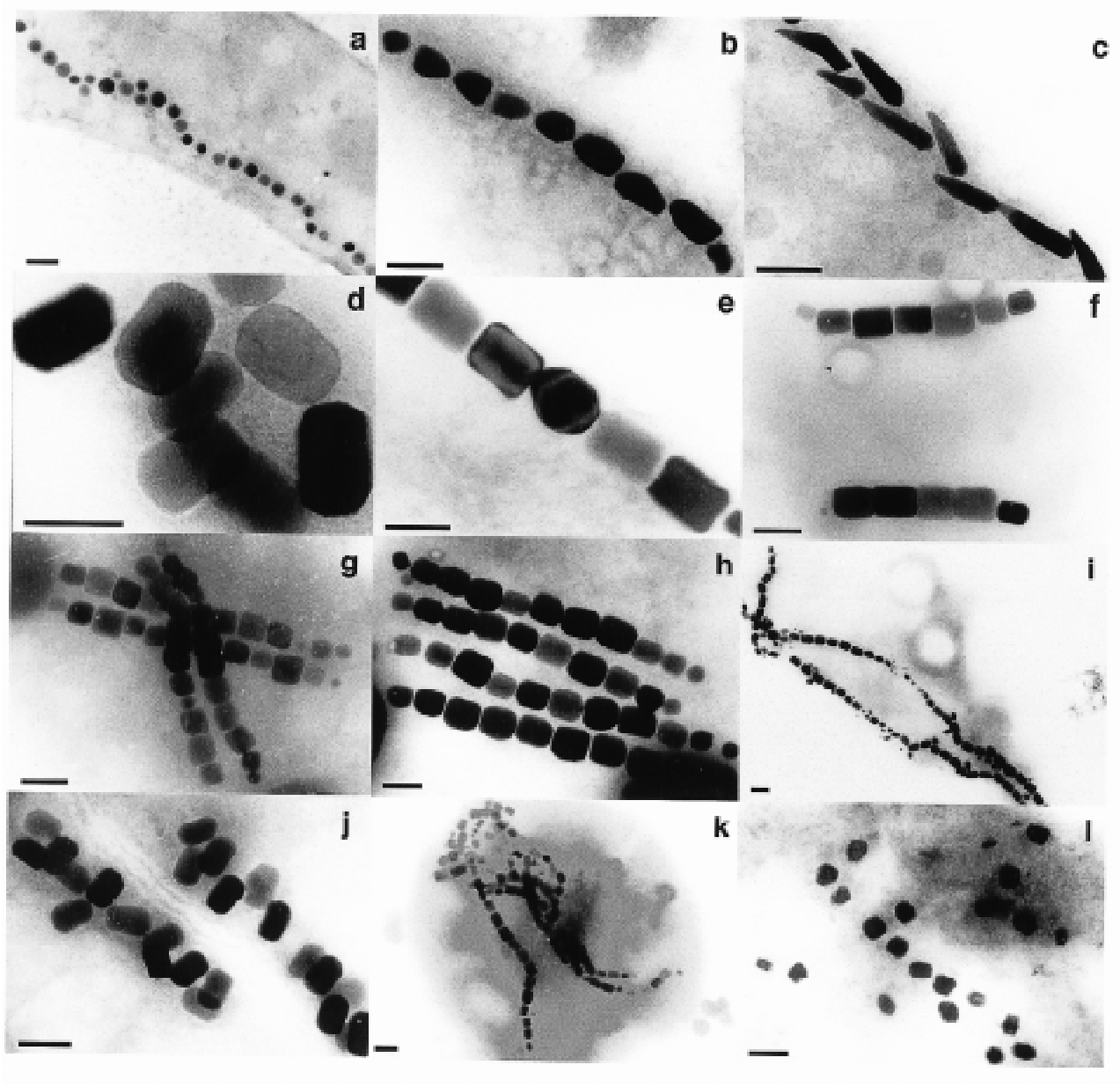

Figure 2. Electron Micrographs of Magnetosomes
Crystal morphologies and intracellular organization of magnetosomes found in various magnetotactic bacteria. Shapes of magnetic crystals include cubo-octahedral (a), bullet-shaped (b, c) elongated prismatic (d, e, f, g, h, i, j, k) and rectangular morphologies (l). The magnetosome particles can be arranged in one (a, b, c, e), two (f, i) or multiple chains (g, h) or irregularly (j, k, l). The bar is equivalent to 0.1 μm.

gryphiswaldense and *Magnetospirillum* AMB-1 are more oxygen tolerant, which obviates the needs of elaborate microaerobic techniques and facilitates the mass cultivation of magnetic cells (Schüler and Baeuerlein, 1997a; Matsunaga *et al*., 1990).

Cells of *Magnetospirillum* species form cubo-octahedral magnetite crystals, which are 42-45 nm in size and arranged in a chain (Figure 2a). On the basis of high resolution electron microscopy, Mößbauer spectroscopy and biochemical results, a model for magnetite biomineralization was proposed (Frankel *et al*., 1983; Mann *et al.* 1990a), in which Fe(III) is taken up by the cell, possibly via a reductive step (Paoletti and Blakemore, 1988). Iron is then thought to be reoxidized to form a low-density hydrous oxide which is dehydrated to form a high-density Fe(III) oxide (ferrihydrite). In the last step, one-third of the Fe(III) ions are reduced, and with further dehydration, magnetite is produced within the magnetosome vesicle (Figure 3).

Because of the large amounts of iron required for magnetite synthesis, magnetotactic bacteria can be anticipated to use very efficient uptake systems for the assimilation of iron. Although several studies have focused on iron uptake, the exact mechanisms and components involved are not well understood. The initial finding of Paoletti and Blakemore (1986) that cells of *M. magnetotacticum* produced a hydroxamate type siderophore under high iron condition, has not been

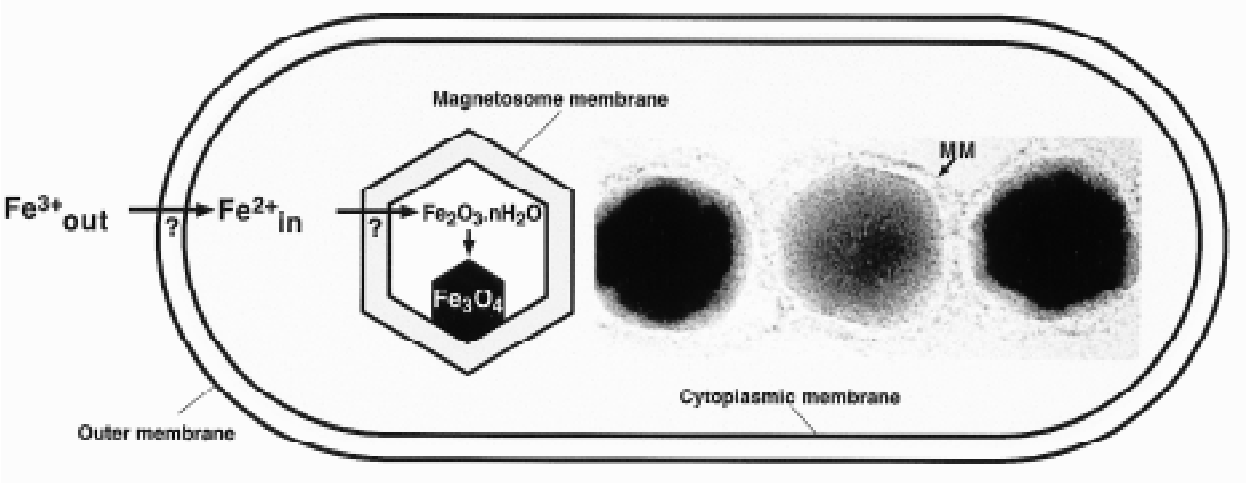

Figure 3 Proposed Model for Magnetite Biomineralization in *Magnetospirillum* species
Fe(III) is actively taken up by the cell, possibly via a reductive step. Iron is then thought to be reoxidized to form a low-density hydrous oxide which is dehydrated to form a high-density Fe(III) oxide (ferrihydrite). In the last step, one-third of the Fe(III) ions are reduced, and with further dehydration, magnetite is produced within the magnetosome vesicle. The magnetosome membrane contains specific proteins, which are thought to have crucial functions in the accumulation of iron, nucleation of minerals and redox and pH control. The electron micrograph shows magnetosome particles from *Magnetospirillum gryphiswaldense*.

replicated in other studies and no unequivocal evidence for the involvement of siderophores in the formation of magnetite has been found so far. Nakamura *et al.* (1993a) hypothesized that ferric iron was taken up in *Magnetospirillum* AMB-1 by a periplasmic binding protein-dependent iron transport system. In *M. gryphiswaldense*, the major portion of iron is taken up as Fe(III) in an energy-dependent process with a V_{max} and K_m of 0.86 nmol min^{-1} (mg dry weight)$^{-1}$ and 3 μM, respectively (Schüler and Baeuerlein, 1996). The high rates of ferric iron uptake may reflect the extraordinary requirement for iron in these bacteria. Both the amount of magnetite formed and the rates of iron uptake were close to maximum at extracellular iron concentrations of 15-20 μM Fe, indicating that this bacterium is able to accumulate copious amounts of iron from relatively low concentrations.

Indirect evidence suggests that ferritin-like iron storage proteins may be involved in the biomineralization of magnetite. Magnetite in the radula of Polylplacophoran mollusks is always formed by conversion of pre-existing ferritin (Kirschvink and Lowenstam, 1979). Studies of *M. magnetotacticum* have revealed the presence of a high-density hydrous ferric oxide (probably ferritin) within intact cells (Frankel *et al.*, 1983). Hence, it was concluded that these bacteria may use a biochemical pathway in magnetite formation similar to that of the Polyplacophoran mollusks. Recently, evidence for the presence of two overlapping bacterioferritin genes was found in *M. magnetotacticum* (Bertani *et al.*, 1997), but its relevance for magnetite formation requires further study.

While size and morphologies of mature magnetite crystals are largely unaffected by environmental conditions, the number of magnetosome particles per cell can vary considerably and strongly depends on the growth conditions. Besides the availability of micromolar amounts of iron, microaerobic conditions are required for magnetite formation in *Magnetospirillum* species (Blakemore *et al.*, 1985; Schüler *et al.*, 1995; Schüler and Baeuerlein, 1998). Cells of *M. gryphiswaldense*, which are non-magnetic during aerobic growth, start to produce Fe_3O_4 when shifted to microaerobic growth conditions corresponding to an oxygen concentration of about 2-7 μM O_2. The accumulation of iron during growth is tightly coupled to the induction of magnetite biomineralization. While the intracellular iron content in pre-magnetic cells is relatively low and in the same range as reported for other, non-magnetotactic bacteria, magnetic spirilla can accumulate more then 2 % iron on a dry weight basis (Blakemore *et al.*, 1979; Schüler and Baeuerlein, 1998).

In an attempt to understand the relationship between respiratory electron transport in nitrate and oxygen utilization and magnetite synthesis, cytochromes in *M. magnetotacticum* have been examined. Tamegai *et al.* (1993) reported a novel cytochrome a_1-like hemoprotein that was present in greater amounts in magnetic cells than nonmagnetic cells. A new ccb-type cytochrome c oxidase (Tamegai and Fukumori, 1994) and a cytochrome cd_1-type nitrite reductase (Yamazaki *et al.*, 1995) were isolated and purified from the same organism. The latter protein is of particular interest since it showed Fe(II):nitrite oxidoreductase activity. It was proposed by Fukumori *et al.* (1997) that the dissimilatory nitrite reductase of *M. magnetotacticum* could function as Fe(II) oxidizing enzyme for magnetite synthesis under anaerobic conditions, which is consistent with the observation that magnetite formation is enhanced in microaerobically denitrifying cells of *M. gryphiswaldense* (Blakemore *et al.*, 1985).

In strains of *Magnetospirillum*, the magnetosome mineral phase is enveloped by a trilaminate membrane (Gorby *et al.*, 1988; Matsunga, 1991; Schüler and Baeuerlein, 1997b). Compartmentalization through the formation of the magnetosome vesicle enables the processes of mineral formation to be regulated by biochemical pathways. The membrane may act as potential gate for compositional, pH and redox differentiation between the vesicle and the cellular environment. Although the magnetosome membrane (MM) does not appear to be contiguous with the cell membrane in electron-microscopic

images, it can be speculated that some sort of physical connection with the cytoplasmic membrane exists, which could explain the biosynthetic origin of the magnetosome compartment and the anchoring of the magnetosome chain in the cell. The MM vesicles apparently exist prior to the biomineralization of the mineral phase, because empty and partially filled vesicles have been observed in iron starved cells of *M. magnetotacticum* and *M. gryphiswaldense* (Gorby *et al.*, 1988; Schüler and Baeuerlein, 1997b). The MM is composed of phospholipids and proteins, at least several of which appear unique to this membrane. Although the protein patterns of the magnetosome membrane are different between several species of *Magnetospirillum*, at least one major protein with a molecular weight of about 22-24 kDa appears to be common to all strains tested so far as revealed by sequence analysis and antibody cross-reactivity (Okuda *et al.*, 1996; Schüler *et al.*, 1997; Schüler and Tebo, unpublished). The exact role of the magnetosome-specific proteins has not been elucidated, but it has been suggested that these have specific functions in the accumulation of iron, nucleation of minerals and redox and pH control (Gorby *et al.*, 1988; Mann *et al.*, 1990a).

Attempts to address the molecular genetics of magnetosome formation in MTB have been hampered by several problems including the lack of a significant number of magnetotactic bacterial strains and the elaborate techniques required for the growth of these bacteria. Therefore, our knowledge of the genetic determination of magnetosome formation is still limited. In initial studies, Berson *et al.* (1989) demonstrated that genes (e. g. *recA*) of *M. magnetotacticum* can be functionally expressed in *Escherichia coli*. They also cloned a 2 kb DNA fragment from *M. magnetotacticum* that complemented iron uptake deficiencies in *E. coli* and *Salmonella typhimurium* mutants lacking a functional *aroD* gene (biosynthetic dehydroquinase). This suggests that the 2 kb DNA fragment may have a function in iron uptake in *M. magnetotacticum* (Berson *et al.*, 1991).

Okuda *et al.* (1996) used the N-terminal amino acid sequence from a 22 kDa protein specifically associated with the MM to clone and sequence its respective gene. Based on the amino acid sequence, the protein exhibits significant homology with a number of functionally diverse proteins belonging to the tetratricopeptide repeat family, although its function in magnetosome synthesis is not clear.

Because of their microaerophilic nature, colony formation on plates is not easy to obtain in MTB and the poor ability of most strains to form colonies on plates has been a major obstacle for the establishment of genetic experiments. It has been demonstrated that strains of *Magnetospirillum* and related bacteria are otherwise accessible by genetic manipulation (Matsunaga *et al.*, 1992; Eden and Blakemore, 1991). Transfer of DNA has been achieved by conjugal transfer of broad host range IncP and IncQ plasmids, which formed autonomous replicons in *Magnetospirillum* sp. AMB-1. Cells of this strain were reported to form magnetic colonies on the surface of agar plates when grown under an atmosphere containing 2% oxygen (Matsunaga *et al.*, 1991). This facilitated the selection of non-magnetic mutants generated by Tn5-mutagenesis (Matsunga *et al.*, 1992). It was concluded that at least three regions of the chromosome are required for the synthesis of magnetosomes. One of these regions contained a gene, designated *magA*, encoding a protein with homology to cation efflux proteins, in particular the *E. coli* potassium ion-translocating protein KefC (Nakamura *et al.*, 1995a). Membrane vesicles prepared from *E. coli* cells expressing *magA* took up iron when ATP was supplied indicating that energy was required for the uptake of iron. The expression of *magA* was enhanced when wild-type *Magnetospirillum* AMB-1 cells were grown under iron-limited conditions rather than iron-sufficient conditions in which they would produce magnetosomes (Nakamura *et al.*, 1995b). Thus, the role of the *magA* gene in magnetosome formation requires further clarification.

Biotechnological Applications

Magnetosome formation in bacteria has the potential to yield useful biomaterials. The synthesis of a nano-sized magnetite particles by a biological process promises advantages in terms of controlling crystal growth and structural properties. By comparison, synthetic magnetic particles are non-uniform, often not fully crystalline, compositionally nonhomogenous, and in an agglomerated state which imposes problems in processing (Sarikaya, 1994). Moreover, biomineralization provides a way to produce highly uniform magnetite crystals without the drastic regimes of temperature, pH and pressure which are often needed for their industrial production (Mann *et al.*, 1990a). Accordingly, numerous biotechnological applications of the small magnetic crystals have been contemplated (Schüler and Frankel, 1999).

The small size of isolated magnetosome particles provides a large surface-to-volume ratio, which makes them useful as carriers for the immobilization of relatively large quantities of bioactive substances, which can then be separated by magnetic fields. In several studies, bacterial magnetosomes were used for immobilizing enzymes and antibodies (Matsunaga and Kamyia, 1987; Nakamura *et al.*, 1991; 1993b). The presence of the magnetosome membrane resulted in dispersion and handling properties superior to those of synthetic magnetic particle conjugates (Matsunga, 1991). Using phagocytosis and polyethylene glycol fusions, bacterial magnetite particles were incorporated into eucaryotic cells, which could be manipulated by magnetic fields (Matsunaga *et al.*, 1989).

Another application of bacterial magnetosomes may lie in their potential use as a contrast agent for magnetic resonance imaging and tumor-specific drug carriers based on intratumoral enrichment. Synthetic liposomes containing superparamagnetic iron oxide particles have been already used in biomedical applications of this type (Päuser *et al.*, 1997). Other suggested applications of MTB involve the use of living, actively swimming cells. Proposed examples include the application of MTB in the analysis of magnetic domains in magnetic materials (Harasko *et al.*, 1993; 1995) and the use of MTB for the removal of heavy metals and radionuclides from wastewater (Bahaj *et al.*, 1994; 1998).

As yet, no application of MTB has attained commercial scale, however. This situation is in part due to the problems related to mass cultivation of these bacteria. Another reason for the limited practicability of many systems is the lack of an basic understanding of bacterial magnetite biomineralization at a biochemical and molecular level. Thus, more research in this field is clearly required. Genetic approaches are offering promising ways for engineering

the biomineralization process and providing new materials. Questions in further research could be addressed in relatively well-studied systems such as members of the genus *Magnetospirillum*, while at the same time increased effort needs to be spent to exploit the metabolic potential of the vast biodiversity of natural occurring MTB, including largely unexplored habitats such as the marine environment.

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