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REVIEW ARTICLE

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The biomineralization of magnetosomes in *Magnetospirillum gryphiswaldense*

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Abstract Magnetotactic bacteria (MTB) are major constituents of natural microbial communities in sediments and chemically stratified water columns. The ability of MTB to migrate along magnetic field lines is based on specific intracellular structures, the magnetosomes, which, in most MTB, are nanometer-sized, membrane-bound magnetic particles consisting of the iron mineral magnetite (Fe_3O_4). A broad diversity of morphological forms has been found in various MTB. The unique characteristics of bacterial magnetosomes have attracted a broad interdisciplinary research interest. The magnetosome membrane (MM) in *Magnetospirillum gryphiswaldense* contains a number of specific Mam proteins. Several *mam* genes were analyzed and assigned to different genomic regions. Many of the Mam proteins are highly conserved in other MTB but display low sequence similarity to any proteins from nonmagnetic organisms.

Keywords *Magnetospirillum gryphiswaldense* · Magnetotactic bacteria · Magnetite · Magnetosomes · Mam proteins

Introduction

If a drop of mud sampled from a pond is placed onto a microscopic slide, an intriguing behavior of some bacteria can be observed: As soon as a magnet is brought close to the edge of the drop, various highly motile, conspicuous bacteria immediately start to migrate apparently unidirectional along the magnetic field lines (Fig. 1). Within a few minutes, the whole population is found accumulated at the "north" edge of the drop, but they instantly turn and rush away if the magnetic polarity is reversed. The reason for the magnetic sensitivity of the bacteria ("magnetotaxis") becomes easily obvious upon electron microscopic examination: The magnetotactic cells contain a number of intracellular crystals of a magnetic mineral, the so-called magnetosomes [2], which are aligned in chain-like structures, thereby exerting a magnetic moment on the cell.

Although magnetotactic bacteria (MTB) occur in high numbers in the sediments of many freshwater and marine habitats, their existence remained overlooked for many years before their serendipitous discovery by the American microbiologist Richard Blakemore [6]. As an undergraduate student at Greifswald University, I was so fascinated by Blakemore's breathtaking description of how he had discovered these intriguing organisms [7] that I rushed to the closest pond in order to get mud samples to experience for myself these spectacular bacteria. Once I had observed them under the microscope, I entered into an ambitious project, attempting to isolate my "own" magnetotactic bacterium, so that I could grow and study it in the laboratory. After many more sampling trips and a number of fruitless and rather frustrating months of work in the lab, I finally isolated a magnetotactic bacterium from a mud sample collected from the little river Ryck, near the town of Greifswald, Germany [39]. It was described as *Magnetospirillum gryphiswaldense* and, at that time, was one of the few magnetotactic bacteria available in pure culture [32]. (The epithet "*gryphiswaldense* " is the Latin derivative of the name of the town.) Since then, my fascination in these bacteria has continued to grow, and I have been lucky enough to be able to continue my research on several aspects of their biology.

The formation of well-ordered, nano-sized particles with perfect magnetic and crystalline properties in MTB is an intriguing example of 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

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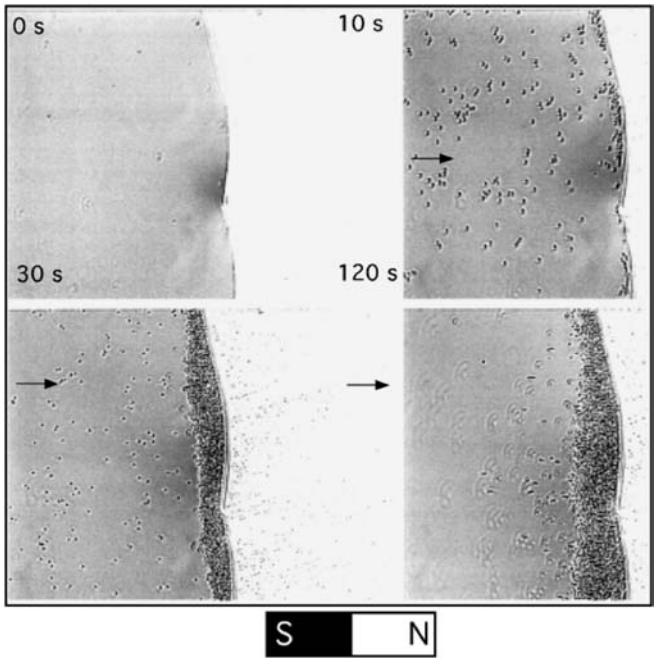

Fig. 1 Micrographs of magnetotactic bacteria (MTB) in a hanging drop at different points of time after application of a magnetic field. Cells swim in random directions in the absence of a magnetic field (0 s). If a magnetic field is applied, MTB immediately orient and swim aligned to the field lines. After a few minutes, bacteria are found accumulated at the "north" edge of the drop (next to the south pole of a magnet)

custom-designed properties [21] and several 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 their application as contrast enhancement agents in magnetic resonance imaging [38]. 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 [12, 14]. 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, have been found in a wide range of higher organisms and even humans [17, 19]. Thus, an understanding of the structures and mechanisms involved in bacterial biomineralization of magnetosomes is of crucial interest. Despite interdisciplinary efforts, however, not much is known about the biology of MTB, mainly because of the problems that have been associated with the isolation and cultivation of most species.

Ecology and diversity of MTB

High numbers of MTB (10^5–10^6/ml) [8, 43], are usually found at the oxic-anoxic transition zone, generally located at the sediment-water interface. Although MTB occur ubiquitously in diverse aquatic environments, different habitats have been found to contain various morphological types of MTB, including rod-shaped, vibrio-like, coccoid, and helical forms. A great diversity was also noted in the number, arrangement, and shape of magnetosome particles in different MTB (Fig. 2).

MTB occur in different phylogenetic lineages of bacteria [1]. Therefore, it has been argued that magnetosome biomineralization evolved from separate evolutionary origins [11]. The majority of MTB affiliate with the α-Proteobacteria [11, 32, 42, 44, 45]. A morphologically distinct, large magnetic rod was assigned to the *Nitrospira* phylum, whereas a magnetotactic many-celled prokaryote and a magnetic sulfate-reducing bacterium belong to the δ-subclass of Proteobacteria [11, 18].

Despite their ubiquitous occurrence and high abundance, cultivation of MTB in the laboratory has been difficult. Problems arise from their lifestyle, which is adapted to sediments and chemically stratified aquatic habitats that are difficult to mimic under laboratory conditions. Since no selective 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 [46]. Growth media containing sulfide and redox gradients also facilitate the isolation of MTB [41]. Nonetheless, only a few strains of MTB have been isolated in pure culture so far and most of the isolates are poorly characterized in terms of growth conditions and physiology [4, 8, 22, 24, 25].

It is generally accepted that magnetotaxis functions as a navigational mechanism under environmental conditions. The orientation of MTB along geomagnetic field lines is thought to facilitate their migration to their favored position in the oxygen gradient [9], as all known MTB are either microaerophilic or anaerobic. The preferred motility direction found in natural populations of MTB is northward in the geomagnetic field in the Northern hemisphere, whereas it is southward in the Southern hemisphere [10]. 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 [13].

Magnetosome biomineralization

Common to all MTB is the presence of specific intracellular structures, the magnetosomes, which in most MTB consist of particles of the magnetic iron mineral magnetite (Fe_3O_4) that are enclosed within membrane vesicles [3, 15]. Most of our knowledge of the mechanism of magnetite biomineralization comes from studies involving strains of different *Magnetospirillum* species (Fig. 3), which form cubo-octahedral crystals that are

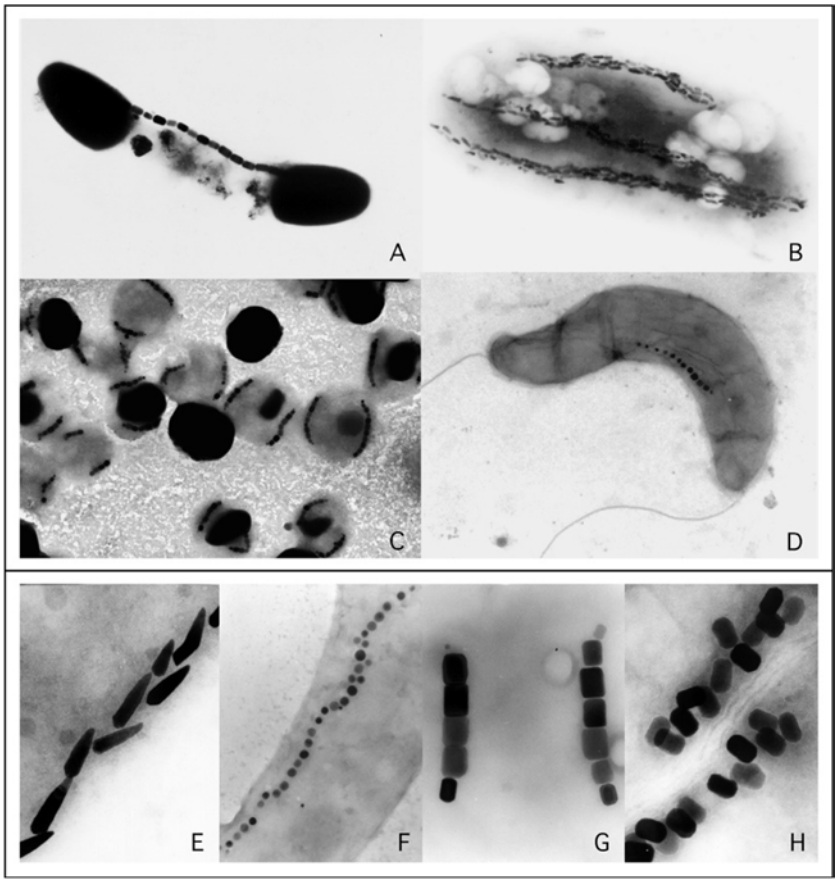

Fig. 2A–H Transmission electron micrographs showing cells of various magnetotactic bacteria and magnetosome crystals. The morphological forms include large rods (**A, B**), coccoid cells (**C**), and spirilla (**D**). Morphologies of magnetosomes include bullet-shaped (**E**), cubo-octahedral (**F**) and prismatic crystals (**G, H**)

42 nm in size and arranged in a chain. *Magnetospirillum gryphiswaldense* produces up to 60 magnetosome particles that strongly resemble those of other *Magnetospirillum* species. It was selected as model organism in our laboratory because it can be cultivated more readily than most other MTB, which has facilitated its physiological and biochemical analysis.

Because of the large amounts of iron required for magnetite biomineralization, MTB are likely to use very efficient systems for the assimilation of iron. In *M. gryphiswaldense*, iron is incorporated in the ferric form 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 [35, 36]. Saturation of iron uptake and maximum magnetite formation were detected at extracellular iron concentrations of 15–20 μM Fe, which is consistent with the observation that MTB are able to accumulate iron and form magnetosomes in environments with a relatively low abundance of iron [34]. Besides the availability of iron, microaerobic conditions are required for magnetite formation. Cells of *M. gryphiswaldense* are non-magnetic when grown under aerobic conditions, but start to produce Fe_3O_4 when the

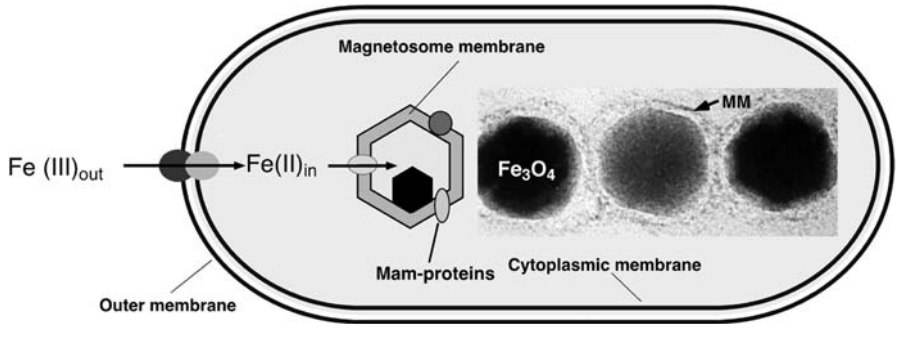

Fig. 3 Proposed model for magnetite biomineralization in *Magnetospirillum* species. Fe(III) is actively taken up by the cell, possibly via a reductive step. Iron is then thought to be reoxidized. In the last step, one-third of the Fe(III) ions are reduced, and magnetite is produced within the magnetosome vesicle. The magnetosome membrane contains specific Mam proteins, which are thought to have crucial functions in the accumulation of iron, nucleation of minerals, and redox and pH control

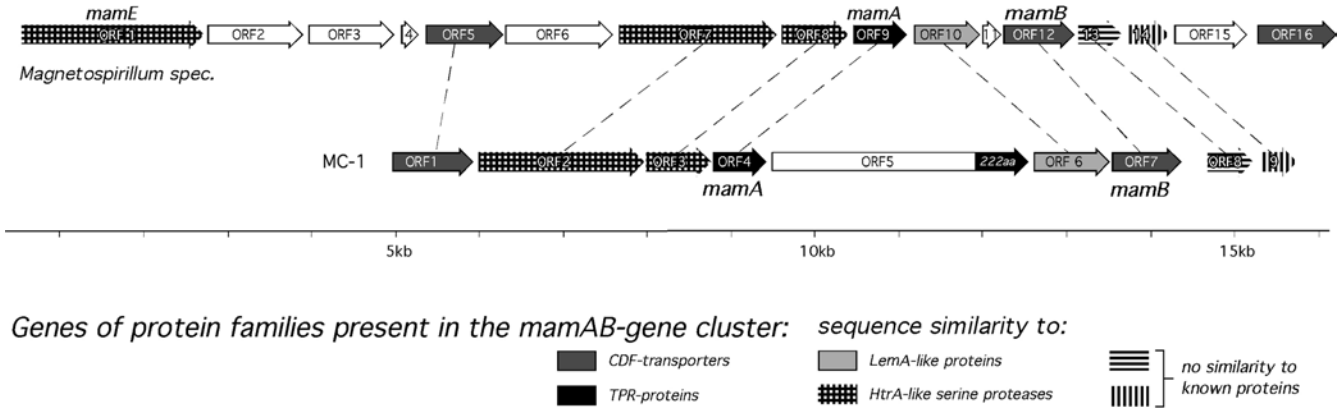

Fig. 4 Molecular organization of the *mamAB* gene cluster in *Magnetospirillum gryphiswaldense*, *M. magnetotacticum*, and the magnetic coccus strain MC-1. *Filled arrows* ORFs that belong to families of homologous genes shared by the *mamAB* clusters of all magnetotactic bacteria investigated. *Dashed lines* connect equivalent genes (closest homologs)

oxygen concentration is shifted below 2–7 μM. It was found that bulk iron uptake during growth is tightly coupled to the microaerobic induction of biomineralization [37, 40]. Cells of *Magnetospirillum* species are able to accumulate more than 2% iron on a dry weight basis during magnetosome formation [8].

The magnetosome membrane is a unique structure in MTB

In magnetospirilla, and probably other MTB, magnetite crystals are enveloped by the magnetosome membrane (MM). Compartmentalization by the formation of MM vesicles allows biochemical control of mineral formation. Biomineralization of magnetite crystals requires a precise regulation of iron supersaturation, and both the redox potential and the pH. The MM is thought to be the crucial component in the control of magnetosome formation [34].

In order to study the biochemical composition of the MM, magnetosomes were isolated from *M. gryphiswaldense* cells by a method combining ultracentrifugation and magnetic separation [33]. This procedure yielded high amounts of intact particles, which were apparently free of any contaminating material as indicated by electron microscopy. As in other *Magnetospirillum* species, analysis of the SDS-extracted MM from *M. gryphiswaldense* revealed that the MM is associated with a number of specific proteins. Electrophoresis detected a minimum of 13 polypeptide bands present in various quantities. N-terminal amino acid sequences were determined from several MM-specific polypeptides and used for cloning of their respective genes. At least five genes (*mamA, B, C, D, E*) encoding magnetosome proteins in *M. gryphiswaldense* could be identified [16]. Neither the identified genes nor the genes from their putative operons display significant sequence similarity to the *magA* and *mpsA* genes of *Magnetospirillum* sp. strain AMB-1, which were found to encode MM-associated proteins in this organism [23, 26]. These findings suggest that the genetic determination of magnetosome formation is complex and involves a number of genes located at several different genomic sites in addition to the identified *mam* gene clusters.

Molecular organization of the *mam*-gene cluster in *M. gryphiswaldense* and other MTB

Recently, the almost complete genome sequences of two magnetotactic α-Proteobacteria, *M. magnetotacticum* strain MS-1 and a magnetic coccus strain MC-1, have become available [http://www.jgi.doe.gov/tempweb/JGI_microbial/html/index.html], which now allows magnetosome formation to be studied at the genomic level. Comparative analysis of the *mam* gene sequences from *M. gryphiswaldense* and the almost complete genomic assemblies of *M. magnetotacticum* and strain MC-1 have allowed homologous genes in the latter organisms to be identified [16]. The sequences and arrangements of most of the *mam* genes, as well as the genetic organization of the flanking regions, were found to be surprisingly well-conserved in all magnetotactic strains investigated. Most of the so-far identified *mam* genes were found to be clustered and could be assigned to at least two different genomic regions. For example, *mamA* and *mamB* are part of a major gene cluster in *Magnetospirillum* strains comprising more than 16 kb and at least 16 genes that are arranged in a collinear fashion (Fig. 4). A very similar structure, which is indicative of an operon-like organization, is found in the corresponding chromosomal region of strain MC-1. Since in bacteria functionally related genes are often located close to each other, it can be concluded that the neighboring genes are likely to be related to magnetosome formation as well.

Because of the lack of biochemical data for most of the identified MM proteins as well as the unavailability of genetic tools for functional analysis, no conclusive evidence for their function in vivo is currently available.

However, it can be anticipated that proteins that are specifically associated with the MM have a crucial role in the control of biomineralization of magnetite crystals. It has been speculated that MM proteins have specific functions in the transport and accumulation of iron, nucleation of crystallization, and redox and pH control. Interestingly, one of the principal MM-proteins, MamB, exhibits extensive sequence homology to members of the ubiquitous cation-diffusion facilitator (CDF) family, the members of which are exclusively involved in the transport of several different heavy metals [30]. In addition to *mamB*, one and two more homologous CDF-genes are present in the *mamAB* clusters of strain MC-1 and *Magnetospirillum* species, respectively. The significant similarity of MamB homologues to proteins specifically involved in metal transport and the specific localization in the MM lead to the hypothesis that MamB-like proteins are involved in transport of iron into the magnetosome vesicles [33]. Several Mam proteins, including MamC and MamD, display no detectable similarity to any proteins or known functional motifs from databases. This implies that several biochemical functions involved in magnetosome formation are unique; however, it is thus impossible to deduce putative functions based on sequence homology. In other examples, MM proteins display homology to well-known protein families, but their specific role in biomineralization is not apparent. For example, the amino acid sequences of several specific constituents of the MM, such as MamE, resemble those of HtrA-like serine proteases [29]. Members of this family are widely distributed in nature and known to function as heat-shock induced proteases in the degradation of misfolded proteins in *E. coli* and other bacteria. In addition to the presence of a catalytic protease domain, PDZ domains [5, 31] characteristic for HtrA-like proteins were identified in the amino acid sequence of MamE. It has been speculated that these domains fulfill a function in the positioning of ion channels, receptors, or signaling molecules in the correct spatial arrangement in the MM [16]. Another characteristic group of proteins, which are shared by the MM of all MTB tested so far, are polypeptides bearing tetratricopeptide repeat (TPR) motifs, which have been identified in a large number of proteins with diverse functions across the biological kingdom and are known to mediate protein-protein interactions [20]. Accordingly, the function of TPR-proteins in the MM, which are represented by MamA and others, was speculated to be the interaction with proteins from the cytoplasm and may involve the formation of multi-protein complexes [16, 27, 28].

These examples clearly demonstrate that further biochemical and genetic analysis is indispensable and requires an improved set of tools order to elucidate the specific role of these proteins in bacterial magnetite biomineralization. In addition to the functional dissection of biomineralization in a well-studied model organism such as *M. gryphiswaldense*, the vast diversity of magnetosome formation in MTB needs to be further addressed by the investigation of uncultivated MTB from natural environments.

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