Genomics, Genetics, and Cell Biology of Magnetosome Formation

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Abstract
Magnetosomes are specialized organelles for magnetic navigation that comprise membrane-enveloped, nano-sized crystals of a magnetic iron mineral; they are formed by a diverse group of magnetotactic bacteria (MTB). The synthesis of magnetosomes involves strict genetic control over intracellular differentiation, biomineralization, and their assembly into highly ordered chains. Physicochemical control over biomineralization is achieved by compartmentalization within vesicles of the magnetosome membrane, which is a phospholipid bilayer associated with a specific set of proteins that have known or suspected functions in vesicle formation, iron transport, control of crystallization, and arrangement of magnetite particles. Magnetosome formation is genetically complex, and relevant genes are predominantly located in several operons within a conserved genomic magnetosome island that has been likely transferred horizontally and subsequently adapted between diverse MTB during evolution. This review summarizes the recent progress in our understanding of magnetobacterial cell biology, genomics, and the genetic control of magnetosome formation and magnetotaxis.
Magnetotactic bacteria (MTB): phylogenetically and physiologically diverse group of aquatic microorganisms that orient and swim along magnetic field lines by the permanent magnetic dipole moment imparted by magnetosomes (Figure 1a). Because the magnetic alignment of the cell reduces a three-dimensional search problem to a one-dimensional search problem, magnetotaxis is thought to make the cellular chemotactic response more efficient. However, the signal transduction mechanisms involved in magnetotaxis are poorly understood, and the recent observation of MTB with reversed or variable magnetic polarity suggests that the mechanism of magnetotaxis might be more complex than originally assumed and that the magnetosomes might fulfill different or additional functions (20, 23, 33, 77).

The synthesis of bacterial magnetosomes involves genetic control over the biomineralization of perfectly shaped and sized magnetic crystals and their assembly into highly ordered chains to serve most efficiently as a magnetic field sensor. Magnetosome formation is genetically complex, and a rather large set of candidate genes predominantly located within a genomic magnetosome island (MAI) has been identified so far (70).

Understanding the formation of magnetosomes is of interest to diverse disciplines such as microbiology, geobiology, and biotechnology (70, 72, 85). In addition, MTB recently emerged as a model for studying prokaryotic organelle formation and cell biology (39).

Although mud bacteria, whose motility is magnetically directed, were first documented by Salvatore Bellini as early as 1963 (10), it was only their rediscovery and the description of magnetotaxis by Richard Blakemore in the 1970s (11) that stimulated a wealth of diverse research activities. Since Blakemore’s seminal review of early discoveries, which was published in this journal in 1982 (12), the growing knowledge on MTB and magnetosome formation has been frequently summarized and recently has been the subject of several comprehensive review articles (7, 20, 39) and a monograph (69). This review summarizes the enormous progress recently made on the understanding while they are passively oriented along geomagnetic field lines by the permanent magnetic dipole moment imparted by magnetosomes (Figure 1a). Because the magnetic alignment of the cell reduces a three-dimensional search problem to a one-dimensional search problem, magnetotaxis is thought to make the cellular chemotactic response more efficient. However, the signal transduction mechanisms involved in magnetotaxis are poorly understood, and the recent observation of MTB with reversed or variable magnetic polarity suggests that the mechanism of magnetotaxis might be more complex than originally assumed and that the magnetosomes might fulfill different or additional functions (20, 23, 33, 77).

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of the unique cell biology of MTB as well as the genetic control of magnetosome formation and magnetotaxis, which has become possible by the availability of genomic information as well as novel genetic and ultrastructural tools for analysis and manipulation of MTB.

**STRUCTURE AND COMPOSITION OF MAGNETOSOMES**

**Biomineralization of the Inorganic Core**

In most MTB the mineral core of magnetosome consists of the magnetic iron oxide magnetite (Fe₃O₄). Several uncultivated MTB from marine environments synthesize magnetosomes consisting of the magnetic iron sulfide greigite (Fe₃S₄), the biomineralization of which, however, is poorly understood owing to the inability to cultivate greigite producer in the laboratory. The size and morphology of magnetite crystals are subject to species-specific genetic control, and a considerable diversity of magnetosome morphologies is found in different MTB, which are mostly unknown from magnetite particles formed by chemical synthesis (20) (Figure 1b).

The biomineralization of magnetite crystals requires the accumulation of large amounts (>4% per dry weight) of iron, which can be taken up in either its ferric or ferrous form from micromolar extracellular concentrations (18, 71). The intracellular pathway for uptake and sequestration then has to be strictly controlled because of the potentially harmful effect of free intracellular iron levels. Genomic analysis and preliminary experimental data suggest that common constituents of the iron metabolism, such as uptake systems for ferrous and ferric iron, iron storage, as well as iron-regulatory elements, and possibly siderophores, are present in MTB, although their significance for magnetite biomineralization is poorly understood (9, 16, 48, 91). There are indications that uptake and intracellular processing of iron for magnetite synthesis proceeds through a distinct pathway involving membrane-associated precursors that include a ferrous high-spin and a ferritin-like compound, but no mineral precursor other than magnetite (18). According to this study, a process has to be assumed by which nucleation of magnetite crystals predominantly occurs at the cytoplasmic membrane (CM) level, and subsequent crystal growth may proceed spatially separated from the CM after detachment of magnetosome vesicles. However, the proposed mechanism has not yet been fully characterized at the genetic and biochemical levels. Accumulation of supersaturating quantities of iron into the magnetosomes is assumed to proceed via distinct routes involving specific transport proteins. In *Magnetospirillum gryphiswaldense* the magnetosome-associated MamB and MamM proteins, which are members of the cation diffusion facilitator (CDF) family of metal transporters, are involved in magnetosomal iron transport (36).

**Magnetotaxis**: ability of certain motile, aquatic microorganisms to orient in a magnetic field

**Magnetosome island (MAI)**: large (80–150 kb) instable genomic region harboring many magnetosome genes that is found in all analyzed MTB and displays conserved structural and compositional characteristics, presumably transferred horizontally between different species.
This is achieved by compartmentalization of crystallization by the magnetosome membrane (MM), which forms a distinct vesicular compartment that serves as a nanoreactor in which conditions for magnetite precipitation can be strictly controlled. Despite the strict biological control exerted on magnetosome formation, magnetite crystallization is affected by environmental parameters. Specifically, the morphology of magnetite crystals is also determined by the rates of Fe uptake, and fast-growing crystallites lack the structural perfection of those formed slowly (19). Synthesis of magnetite crystals further depends on microoxic or anoxic conditions, whereas higher oxygen concentrations suppress magnetite biomineralization or result in the formation of smaller and aberrantly shaped crystals (32).

**Biochemical Composition of the MM**

Similar to eukaryotic organelles, magnetosome crystals are individually enveloped by a lipid bilayer membrane, the MM (27, 39, 67). The MM is formed prior to magnetite formation and originates as an invagination of the CM (39, 40), but it may become pinched off later in the cell cycle (18, 61). The MM has been studied at the structural and biochemical levels so far only in strains of *Magnetospirillum*, but it can be assumed that similar structures are also present in all other MTB. By electron microscopy the MM is visible as vesicular structures intimately attached to the CM (Figure 2) (40). Komeili et al. (41) have suggested that magnetosome vesicles require activation, possibly by the MM-associated tetratricopeptide repeat (TPR) MamA protein.

Biochemical analysis of isolated magnetosome revealed that they contain phospholipids and are associated with specific proteins (27, 29, 30, 66, 81). In *M. gryphiswaldense* the phospholipid composition of the MM resembles that of the CM and includes phosphatidylethanolamine, phosphatidylglycerol, ornithinamid lipid, and an unidentified amino lipid (29). Its protein composition, however, is rather distinct from that of other subcellular compartments. The MM of *M. gryphiswaldense* is associated with a specific set of more than 20 proteins present in various amounts (29, 30, 58). Similar biochemical studies and genomic analyses of other strains suggested that many magnetosome proteins are conserved among MTB (48, 52, 81, 82). MM proteins that have been named either Mam (magnetosome membrane), Mms (magnetic particle membrane specific), Mtx (magnetotaxis), or Mme (magnetosome membrane) belong to characteristic protein families, including TPR proteins (MamA), CDF transporters (MamB and MamM), HtrA-like serine proteases (MamE, MamP, and MamO), actin-like proteins (MamK), generic transporters (MamH).
and MamN), and MTB-specific proteins with no homology to other proteins in nonmagnetic organisms (MamG, F, D, C, J, W, X, and Y, Mms6, MmeA, and MtxA) (29, 30, 35, 58). As shown by 1D and 2D electrophoresis, the protein composition of the MM is distinct, and it has been demonstrated by GFP fusions and immunodetection that several proteins are specifically targeted to the MM. The molecular mechanisms controlling the sorting of MM proteins to the magnetosome compartment are currently unknown, and no common sequence motifs or targeting signals have been identified so far (39, 68). Whereas many MM proteins display the characteristics of typical membrane proteins and are tightly bound to the magnetosomes crystals, others appear to be rather hydrophilic and seem loosely attached so that they can be selectively solubilized by mild detergents (68).

Recruitment of magnetosome proteins could be by direct binding to the surface of magnetite crystals or by specific protein-protein interactions. The latter could be mediated for instance by the PDZ and TPR domains found in several MM proteins, which generally mediate protein-protein interactions, act as scaffolding proteins, and typically coordinate the assembly of proteins into multisubunit complexes at particular subcellular locations (7, 35). Although the role of many MM proteins has remained obscure, several proteins were already assigned functions in magnetosomal iron transport, magnetosomal iron crystalization, and assembly of magnetosome chains.

### GENETICS AND GENOMICS OF MAGNETOSOME FORMATION

#### General Genome Characteristics

Understanding the genetic basis of magnetosome formation has enormously benefited from the development of tools for the genetic manipulation as well as the genome analysis of several MTB. As of January 2009 two complete and two nearly complete genome sequences from Alphaproteobacteria-MTB are available to the public, which include the freshwater *M. gryphiswaldense* (58), *M. magneticum* (48), and *M. magnetotacticum*, as well as the marine magnetic coccus strain MC-1 (63) (for general genome characteristics see Table 1). In addition, there is substantial genome information available, including large contigs harboring the MAI for the marine vibrio strain MV-1 (33) and two uncultivated MTB that have been collected from freshwater habitats by

<table>
<thead>
<tr>
<th>Table 1</th>
<th>General genome features of the four magnetotactic bacteria with publicly available genomic sequencea,b</th>
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<tbody>
<tr>
<td><strong>Feature</strong></td>
<td><strong>MSR-1</strong></td>
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</tr>
<tr>
<td>No. of chemotaxis genes</td>
<td>ND</td>
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aModified after Reference 58.
bReference 64.

ND, not determined; ORFs, open reading frames; NA, not annotated in the original publication.
a metagenomic approach (34). Genome analysis of the deltaproteobacterium *Desulfovibrio magneticus* RS-1 was recently completed as well (NCBI: NC_012796). Genome comparisons and phylogenetic analysis of magnetospirilla and the magnetic coccus MC-1 have indicated that the magnetotactic phenotype is only weakly reflected by the overall gene content of the genome. For instance, Richter et al. (58) identified a magnetobacterial core genome shared by all four sequenced strains of 891 genes, and even the three closely related magnetospirilla have only 1932 of their nearly 5000 (4268–4925) genes in common. However, in addition to the magnetosome genes discussed below, all MTB seem to share a notable number of common characteristics: (a) genomes sizes between 4.2 and 4.9 Mb, (b) G+C content between 54.8 and 65.1%, and (c) unusually high numbers of putative chemotaxis transducers found in bacterial genomes (65 in *M. magnetotacticum* and *M. magnetotacticum*, 5 in *Escherichia coli*) (1, 25) as well as 65 signal transduction related genes in MC-1 (64). Another characteristic is that the highest number of hemerythrin genes (32 in *M. magneticum*) among prokaryotic genomes (compare with 5 in *Rhodospirillum rubrum*) are implicated in oxygen sensing and signal transduction and might play a central role in magneto-aerotaxis (23, 24). Despite their different habitats, all analyzed MTB have genes associated with an autotrophic lifestyle including those encoding putative enzymes for carbon fixation. While magnetospirilla seem to fix CO$_2$ via the Calvin-Benson-Bassham cycle (6, 48), MC-1 lacks RuBisCO genes but fixes CO$_2$ via the reverse tricarboxylic acid cycle (6, 64, 90). *Magnetospirillum* species and MV-1 are capable of anaerobic growth with nitrate or N$_2$O as terminal electron acceptor, respectively, while the magnetic coccus MC-1 depends on oxygen respiration (5, 8).

Among MTB the magnetic coccus MC-1 exhibits the most conspicuous genome, and its phylogenetic position was controversially discussed because of its mosaic-like genomic composition (17, 37). In addition, its genome contains 14 predicted prophages, which is the highest number found in a prokaryotic genome so far (13).

### Identification of Magnetosome Genes

Because of the limited amount of MTB species available in pure culture, genetic analysis has focused so far on the alphaproteobacterial freshwater strains *M. gryphiswaldense* and *M. magneticum* as model systems, which has been facilitated by the availability of genome data. Requirements for genetic manipulations, such as clonal selection on agar plates, suitable selection markers, gene transfer via conjugation or electroporation, site-directed and random mutagenesis, and the expression of GFP fusions, as well as the establishment of expression systems, are fulfilled only by these two MTB (41, 42, 44, 46, 51, 73–75).

The trait of magnetotaxis is genetically complex and requires a range of diverse gene functions, which are still not known completely. However, by using different approaches, the genetic basis of magnetosome formation was partially unveiled, and three main strategies identified a set of candidate genes with known or suspected functions in magnetosome formation, which are discussed below.

#### Reverse genetics by proteomic analysis

On the basis of the assumption that magnetosome-associated proteins might have specific roles in their biogenesis, several studies attempted to identify the corresponding genes in several MTB. For example, the first of these studies identified the *mam22* gene (equivalent to *mamA*) in *M. magnetotacticum* encoding a conserved TPR protein (52, 53). In *M. gryphiswaldense*, proteomic analysis and reverse genetics so far led to the identification of 23 genes encoding specific bona fide magnetosome-associated proteins (29, 30, 58). The proteins were assigned to various protein families and named MamA, B, C, D, E, F, G, J, M, N, O, Q, R, S, T, W, Y, and X, MmsF, Mms6; as well as MM22, MmeA, or MtxA. With the exception of *mmeA*, *mtxA*, *mms16*, and *MM22*, all identified genes were found colocalized within a
single genomic region that was recognized later as an MAI.

In a similar study in *M. magneticum*, 78 proteins copurified with isolated magnetosome particles. However, only 14 of them (Mms16, Mms6, and MamC, F, E, J, M, A, R, B, D, K, S, and O) overlapped with the magnetosome subproteome of *M. gryphiswaldense* (29, 81). Because a large proportion of the identified proteins are highly abundant cellular proteins involved in general housekeeping metabolism, most proteins identified by Tanaka et al. (81) probably represent contaminations from other cellular compartments that became adsorbed to the magnetosome particles during cell disruption. For example, the phasin Mms16 (ApdaA), which was originally suggested to be involved in the formation of MM vesicles in *M. magneticum* because of its detection in the MM fraction (50), is not associated with magnetosomes in vivo, and its function seems entirely unrelated to magnetosome formation but instead is associated with the formation of polyhydroxybutyrate granules (31, 73). In summary, although the proteomic approach is powerful for the identification of genes encoding abundant magnetosome-associated proteins, it is prone to false detection of contaminations and bears the risk of missing less abundant proteins, or those that are not or only temporarily localized at the MM but nevertheless have relevant functions for magnetosome formation.

**Forward genetics by mutant analysis.** Spontaneous mutants were observed at high frequency (up to $10^{-2}$) in *M. gryphiswaldense* during prolonged cold storage and stationary growth, or during exposure to oxidative stress. Mutants were recognized on plates by their pale appearance, compared with the dark brown color of wild-type colonies (63, 86), and displayed various phenotypes including smaller, fewer, or no magnetosomes. All nonmagnetic mutants harbored large and sometimes multiple deletions that mapped within a single genomic region corresponding to the MAI. Excision was likely caused by homologous recombination between the numerous direct repeats present in this area (33, 86). In *M. magneticum*, the spontaneous loss of the MAI, or of large parts of it, was also observed and resulted in nonmagnetic or weakly magnetic phenotypes (25, 49). The tendency of frequent, spontaneous loss of the magnetic phenotype and in particular of high genetic instability of the genomic MAI under certain conditions requires extreme caution because it might obscure the results of genetic analysis, as we discuss below.

Although transposon mutagenesis has the potential for an unbiased identification of all genes involved in magnetosome formation, the polar character of transposon insertions precludes higher-resolution analysis because of the operonal organization of most magnetosome genes. Nevertheless, several studies with partially controversial results were described. For example, a nonmagnetic, complementable transposon mutant (NM4) from *M. gryphiswaldense* had an insertion within a gene encoding a CheIII-like protein. The gene mapped within the neighborhood of *mamW* within the MAI (45). A mini-Tn5 mutagenesis in *M. magneticum* was correlated with the isolation of 62 nonmagnetic clones (46, 87). Curiously, neither of the previously identified *mam* and *mms* genes, nor other genes located within these operons, were identified in the studies mentioned above, which is in striking contrast to an independent study in which all transposon mutants obtained in the same organism resulted from insertions in the *mamAB* cluster (41). In addition, as none of the transposon insertants was trans-complemented so far, the conclusions of the aforementioned studies must be treated with caution, and many of the supposed mutants are likely to represent second-site mutations (46, 87, 88). In fact, Nash (49) demonstrated in a comprehensive mutagenesis study in the same organism that, by screening 5809 transposon insertants, 173 of 192 with various nonmagnetic or weakly magnetic phenotypes had lost one or several genes from the *mamAB* operon by spontaneous deletion. Fourteen of the remaining 19 had transposon insertion sites inside the *mamAB* cluster in six different genes, whereas only 5 of
them had insertions in previously unsuspected genes outside the MAI clusters, although it cannot be excluded that these carry second-site mutations outside the screened mamAB cluster.

### Comparative genome analyses

Cross-comparison of the available MTB genomes was motivated by the assumption that bacteria that share the common feature of magnetotaxis can be expected to have a distinct set of genes in common (58). By comparing the genomes of *M. gryphiswaldense*, *M. magneticum*, *M. magnetotacticum*, and the magnetic coccus MC-1 against each other and genome databases, 152 genes that are shared exclusively between various magnetospirilla were identified. Twenty-eight genes were group specific and thus exclusively found in all four analyzed genomes of MTB, but not in any nonmagnetic organism (MTB specific), or displayed only remote similarity to nonmagnetobacterial genes (MTB related). Eighteen of these genes are located within the MAI of *M. gryphiswaldense* (mamD, T, F, S, C, I, X, H, K, O, P, Q, A, B, M, E and mamH-like as well as mmsF), and 10 are located outside.

In conclusion, the three strategies resulted in the identification of a partially redundant set of candidate genes. For example, both proteomic analysis of the MM and bioinformatic prediction revealed an extensive overlap in the identified candidate genes. For some of them, including mamA, J, K, G, F, D, C, M, B, their function related to magnetosome formation was already experimentally confirmed by targeted deletion (36, 40, 41, 60, 61). On the other hand, the comparative approach also led to the identification of 14 previously unsuspected genes. If all genes that are colocated together with the predicted or verified candidate genes in several large operons within and outside the MAI were taken in account, the tentative number of genes potentially involved in magnetotaxis might be as high as 50. However, most genes identified by reverse genetics and bioinformatics are confined to the MAI, which is in agreement with the fact that all the reliable mutants with a magnetosome phenotype map to this region. In addition, it has been argued that the number of candidate genes identified outside the MAI in fact encode functions involved in signal transduction and motility rather than biomineralization and chain assembly (58). A proportion of the predicted genes seem redundant, as they provide accessory functions, or are present in multiple copies. Thus, the actual number of genes required for magnetosome formation might be as low as 15–25, and this number might shrink further if only genes essential for magnetite biomineralization, not chain assembly, are considered.

In summary, the most probable estimation of the minimum specific gene set currently seems to be between 20 and 50, a number that is sufficiently small for experimental analysis. That means that less than 1% of the genome is likely to be dedicated to magnetotaxis. Thus, the number of predicted genes approaches that implicated in other complex metabolic traits, such as, for example, bacterial photosynthesis (57).

### Molecular Organization of Magnetosome Genes

#### Characteristics of the genomic MAI

A reverse genetic approach in *M. gryphiswaldense* (30) led to the first indication that magnetosome genes are not scattered throughout the chromosome but are apparently confined to a single genomic region that was later recognized as an MAI (30), in analogy to other genomic islands described previously (15). Additional studies provided evidence that homologous MAI are present in the genomes of *M. magneticum* (25), MV-1 (33), MC-1 (64), and two uncultivated MTB (34). The MAI region displays the following characteristics: (a) It harbors most identified magnetosome (*mam/mms*) genes, (b) it contains a high proportion of transposase genes (up to 20% of the coding region), and (c) it is genetically unstable owing to frequent homologous recombination between numerous direct and inverted repeats (except for MC-1). In addition, a large fraction of hypothetical genes are present, as well as hemerythrins or other genes putatively involved in signal transduction.
Diversity and organization of magnetosome genes within the MAI. The operon organization of magnetosome genes is conserved among different *Magnetospirillum* strains and also, to a lesser extent, strains MC-1, MV-1, and two metagenomic clones (Fos001 and Fos002). This finding provides evidence that many magnetosome genes are universal among cultivated and uncultivated MTB (Figure 3). Encoded proteins display substantial sequence divergence, which is in the range of 30–45% identity for most magnetosome proteins. Besides some conspicuous organizational patterns (for instance, *mamK*, *M*, *O*, *P*, *A*, *Q*, and *B* are always arranged within a single *mamAB*-like operon, and *mamO*, *P*, *A* are always syntenic), several genes universally present in the MAI are shuffled between different magnetosome gene clusters in different MTB. The sequence and organization of magnetosome genes are less conserved in strains MC-1 and MV-1 than in magnetospirilla (Figure 3). In the MAI of MV-1, magnetosome genes are organized into five putative operons, along with a number of yet unknown hypothetical and conserved hypothetical genes. For example, the largest gene cluster comprises 17 genes, 14 of which (*maml*, *E*, *K*, *M*, *N*, *O*, *P*, *A*, *Q*, *R*, *B*, *S*, *T*, and *Y*) are orthologous to magnetosome genes found in all *Magnetospirillum* species and that are, with the exception of *mamN*, *R*, and *Y*, also present in MC-1.

Despite the general conservation, there are also some notable differences between various MTB. For instance, several magnetosome genes are present as duplicates in the MAI of some analyzed MTB. For example, homologs of *mamE*, *O*, *Q*, *R*, and *B* are present within an additional operon in *M. magnetotacticum* and *M. magneticum* but are missing in *M. gryphiswaldense*. Whereas only one *mreB*-like gene (*mamK*) is present within the *mamAB*-like cluster of MC-1 and the magnetospirilla, a second *mamK* gene is located within an additional operon in MV-1 (*mamDFHK* cluster), and two divergent copies of *mamK* are present in the metagenomic clone Fos001 as well. On the other hand, the presence of the *mamL* gene, which has a function in magnetosome chain assembly in *M. gryphiswaldense*, seems to be confined to the magnetospirilla, but it is absent from the genome of other MTB.

In summary, a set of genes including *mamH*, *I*, *E*, *K*, *M*, *O*, *P*, *A*, *Q*, *B*, *S*, *T*, *C*, *D*, *Z*, and *X*, as well as *mms6* and *mms1`, is shared by the MAI of all five cultivated MTB, while only *mamE*, *K*, *M*, *O*, *P*, *A*, *Q*, *B*, and *S* are present in the limited available genome sequence of magnetospirilla. That only a fraction of magnetosome genes is conserved with respect to sequence homology and MAI localization suggests that the minimal set of genes required for magnetosome biomineralization might be smaller than previously suggested (58) and that some genetic redundancy is present in the magnetospirilla. Alternatively, other functions essential for magnetosome formation might be performed by unrelated genes by nonorthologous displacement in other MTB. In general, the considerable genetic variability found in the MAI may account for the observed diversity in magnetosome morphologies and arrangements found in many MTB other than magnetospirilla.

Functions of Individual Genes and Proteins

Although a large set of candidate genes has been identified, only a few examples of genes and proteins have been characterized in detail with respect to their function.

**Mms6 affects magnetite crystallization in vitro.** The small Mms6 protein, which is a tightly bound constituent of the MM, may play a role in the magnetite crystallization process (47). Mms6 bears a Leu-Gly-rich motive that is also conserved in the magnetosome proteins MamG and MamD (29) and that is reminiscent of self-aggregating proteins of other biomineralization systems (3, 68). The overproduced Mms6 protein from *M. magneticum* exhibited...
iron-binding activity and had a striking effect on the morphology of growing magnetite crystals in vitro by catalyzing the formation of uniform 30-nm-sized, single-domain particles in solution (3, 56). However, the significance of Mms6 for magnetosomal magnetite synthesis in vivo remains to be shown.

MamGFDC control the size of magnetosomes. Mature magnetite crystals produced by MTB typically are within the stable single magnetic domain size range (between 30 and 120 nm) (7). Smaller, superparamagnetic particles would not efficiently contribute to the cellular magnetic moment, whereas larger crystals tend to reduce their total magnetic moment by developing multiple magnetic domains. Thus, biological control of crystal sizes within a narrow range is crucial for their efficient use in magnetic navigation.

The four small, hydrophobic magnetosome proteins MamG, MamF, MamD, and MamC are specifically involved in the size control of magnetite crystals in *M. gryphiswaldense* (Figure 4a) (60). Except for *mamG*, which is present only in *Magnetospirillum* strains, the *mamD*, *mamF*, and *mamC* genes are part of the MTB-specific set of 28 signature genes described above (58). The MamGFDC proteins account for approximately 35% of all MM proteins and are tightly bound to the MM (29), and their intracellular localization seems to be confined to the magnetosomes (44). The most abundant MM protein, MamC, has been utilized as an MM anchor in translational fusions for the magnetosome-specific display of heterologous hybrid proteins (44, 92). The second most abundant MM protein, MamF, tends to form stable oligomers even in the presence of sodium dodecyl sulfate (29). The MamD and MamG proteins share a conspicuous motif containing a Leu-Gly repeat also found in the Mms6 protein (Figure 4a). MamGFDC are not essential for magnetosome formation, as mutants deficient in either *mamC* or the entire *mamGFDC* operon still synthesize magnetite crystals and form intracellular MM vesicles (60). However, crystals formed by these mutants were significantly smaller and less regular with respect to morphology and chain-like organization (60) (Figure 4a). Apparently, growth of mutant crystals was not only spatially constrained by the size of MM vesicles, but the absence of MamGFDC likely also inhibited the growth of magnetite crystals directly. Formation of wild-type-sized magnetite crystals could be gradually restored by trans-complementation with any combination of one, two, or three genes of the *mamGFDC* operon, whereas the expression of all four genes resulted in large crystals, even exceeding wild-type size. These experiments have demonstrated that the MamGFDC proteins have partially redundant functions and, in a cumulative manner, control the growth of magnetite crystals (60).

MamJ and MamK: cytoskeletal elements are involved in the assembly of magnetosome chains. For maximum sensitivity of the magnetic orientation, magnetosomes are arranged in single or multiple linear chains in which the cellular magnetic dipole is the sum of the permanent magnetic dipole moments of the individual single-domain magnetosome particles (22). However, a string of magnetic dipoles has an inherent tendency of collapsing to lower its magnetostatic energy without mechanical stabilization (38). Dipolar attractions between particles are not sufficient for maintaining straight chains, as isolated
Figure 4
Characteristics and domain organization of the (a) MamGFDC and (b) MamJ proteins, and phenotypes of their respective deletion mutants. The MamJ protein contains several domains with highly biased amino acid composition. The central acidic repeat (CAR) domain has a variable length (Reference 62) and contains two identical stretches arranged as tandem repeats that are rich in acidic amino acid residues. Color pictures show cryoelectron tomograms of wild-type cells and a mamJ deletion mutant. Electron micrographs and diagrams by A. Scheffel (black-and-white pictures), cryoelectron tomograms (color pictures) by M. Gruska and J. Plitzko. Schematics and micrographs modified from References 60 (panel a) and 61 (panel b). MM, magnetosome membrane.

magnetosomes tend to form structures such as flux-closure rings and folded chains that represent lower magnetostatic energy states for in-plane dipoles (54). The assembly and maintenance of well-ordered chains in vivo are highly controlled at the genetic and structural levels. Apparently, constituents of the MM mediate coherence within the chain (61, 82), as isolated magnetosomes are interconnected by adherent organic material that appears to form junctions between individual particles. Recently, two complementary cryoelectron tomography studies demonstrated a network of filaments, 3–4 nm in diameter, that traverses cells of M. gryphiswaldense and M. magneticum closely adjacent to the CM. Magnetosomes were closely arranged along this cytoskeletal structure, which has been tentatively called a
magnetosome filament (MF) and is presumably formed by the MamK protein (21) (Figure 4b). MamK has homology to the cytoskeletal actin-like MreB protein, which is involved in a number of essential cellular processes in bacteria, such as cell shape determination, establishment of cell polarity, and chromosome segregation (26, 28). Because of this intriguing similarity it was speculated that MamK might have a role in aligning and stabilizing the magnetosome chain (68). In fact, the MF was absent in a mutant of M. magnetotacticum from which the mamk gene was deleted (40). The magnetosome chains were less regular and dispersed throughout the cell, which led to the conclusion that the MF is involved in the stabilization and anchoring of the magnetosome chain within the cell (39, 40). MamK of M. magnetotacticum formed straight filaments, structurally and functionally distinct from the known MreB and ParM filaments, rather than helical structures (55). The formation of MamK straight filaments in M. gryphiswaldense was independent of the presence of other magnetosome genes, as revealed by a GFP-based localization study in a deletion mutant lacking large parts of the MAI (62). Recombinant MamK of M. magnetotacticum expressed in E. coli in vitro polymerized into long straight filamentous bundles, the formation of which was dependent on the presence of a nonhydrolyzable ATP analogue (83). In MTB other than magnetospirilla and MC-1, multiple divergent mamK genes were speculated to have slightly distinct functions and might also be correlated to the more complex intracellular magnetosome architectures, such as the organization of multiple chains as found in several uncultivated MTB (34).

In addition to MamK, the acidic MamJ protein identified in the MM of M. gryphiswaldense (29) is involved in the control of magnetosome chain assembly. The mamJ gene immediately precedes mamK within the mamAB operon, which is transcribed from a single promoter (63, 65). The most conspicuous sequence feature of the MamJ protein is the central acidic repetitive (CAR) domain (Figure 4b), which, however, does not seem absolutely essential for its function in chain assembly (62). A deletion mutant of mamJ no longer produces straight magnetosome chains, but magnetite crystals are arranged in compact clusters (61, 62) (Figure 4b), whereas empty vesicles and immature crystals are scattered throughout the cytoplasm and detached from the MFs. Apparently, in cells lacking MamJ, mature magnetosome crystals are free to agglomerate once they are in close proximity to each other (39, 61). Localization studies with GFP suggest that MamJ interacts with the MF, and the direct interaction between MamK and MamJ was experimentally confirmed by two-hybrid experiments (62). One obvious model derived from these results is that MamJ connects magnetosomes to the cytoskeletal MF formed by MamK, which stabilizes the magnetosome chain and prevents it from collapsing (62). However, so far it is unknown if additional proteins are involved in chain formation, and several conflicting observations in two different Magnetospirillum species seem to indicate that MamJ and MamK proteins could perform different or additional functions (39, 70).

This additional function could be, for instance, the sorting and assembly of newly biomineralized crystals into the nascent chains (21, 80). In cells of M. gryphiswaldense that were grown at steadily high iron concentrations, immature crystals were preferentially found at the ends of the chain, whereas empty vesicles were dispersed along the entire length of the cell in iron-starved cells devoid of magnetite crystals (61). In contrast, immature crystallites were formed simultaneously at multiple sites along the entire length of the cell after induction of magnetite synthesis by the addition of iron to iron-starved, resting cells (18, 61). These growing crystals, subsequently concentrated at midcell, first assembling into imperfect, loosely spaced chains, gradually developed into straight, tightly spaced chains of mature particles. It is not yet known how this dynamic cellular localization is controlled or what causes new magnetosomes to form at the ends of the inherited chain (21). MamK, similar to some related MreB-like proteins, might function as a molecular motor (28, 84), thereby establishing
Horizontal gene transfer: incorporation of genetic material by an organism from another organism without being the offspring of that organism.

The chain by transporting newly formed magnetosomes with growing crystals to the ends of the nascent preexisting magnetosome chains. This notion is supported by the observation that the assembly of MamK filaments in E. coli is a highly dynamic and kinetically asymmetrical process (55), and polymerization and depolymerization processes might generate forces sufficient for the transport and relocation of magnetosomes. Furthermore, the position and polarity of the magnetosome chain might relate to other cellular structures relevant to magnetotaxis, such as the flagella motor (80), and it was suggested that the inherent molecular polarity of MamK is translated into a mechanism for controlling global cell polarity (55).

This also relates to the problem of how magnetosomes are correctly positioned at mid-cell, and how they are properly segregated to daughter cells during cell division (21). Loss of MamJ resulted in an uneven segregation of magnetosome particles during cell division (62), and there are further indications for a controlled mechanism of magnetosome positioning. It was suggested that spatial information for localization and distribution of the magnetosome chains could be provided by interaction with other positional determinants controlling cell division in bacteria, such as the tubulin-like FtsZ protein involved in divisome formation (26, 28, 84). In addition to the generic ftsZ gene present elsewhere in the genome, an ftsZ-like gene is colocalized with other putative magnetosome genes within the mamXY operon of M. gryphiswaldense, which led to speculations whether tubulin-like cytoskeletal structures other than the actin-like MF are involved in magnetosome assembly (58, 70). In one of the two mamK-like genes from an uncultivated MTB, an N-terminal FtsZ-like domain is linked to a C-terminal MamK-like actin domain protein. The fusion of a tubulin- and an actin-like domain within a single chimeric polypeptide lends further support to the hypothesis that both proteins may interact and that, in addition to MamK, FtsZ-like cytoskeletal elements might be involved in magnetosome assembly (34, 58, 70).

Evolution of Magnetotaxis

Magnetotaxis and magnetosome biomineralization are widespread among Proteobacteria and the different species produce crystals of different morphology (2, 7, 14). As illustrated in Figure 5, most of the known MTB belong to the Alphaproteobacteria, while Desulfovibrio magneticus and the many-celled magnetotactic prokaryote belong to the Deltaproteobacteria (14, 59, 89). The “Candidatus Magnetobacterium bavaricum” branches close to the Nitrospira phylum (79), and there is some evidence that uncultivated rod-shaped MTB produce greigite crystals in the Gammaproteobacteria (78).

Alphaproteobacteria–MTB do not form a coherent phylogenetic group, but based on 16S rRNA gene analysis some MTB-containing branches of the alphaproteobacterial phylogenetic tree are interspersed with nonmagnetotactic representatives (2). For example, nonmagnetotactic spirilla were repeatedly isolated that seem phylogenetically closely related to magnetotactic Magnetospirillum species (76). As shown in Figure 5, Magnetospirillum species and their nonmagnetotactic relatives branch closely together even below the genus level (33), whereas the closest relative outside the genus Magnetospirillum is the photosynthetic Phaeospirillum molischianum, and strains MV-1 and MC-1 are even more distantly related to the magnetospirilla.

Two possible evolutionary scenarios have been hypothesized to explain the phylogenetic situation of MTB. In the first scenario, the magnetotactic trait has evolved independently several times and was also lost in several lineages (14). The second scenario implies horizontal transfer of genes required to raise the magnetotactic phenotype among different phyla and species (33, 63, 86). The strict congruence of 16S rRNA gene and magnetosome gene phylogenies of all shared mamAB genes, as well as the occurrence of magnetosome genes outside the supposedly transferred MAI, seems to argue against a recent event of horizontal gene transfer in magnetospirilla (33, 58).
Figure 5
Putative evolution of magnetotaxis. The branching order of the schematic tree is according to Reference 2. Branching length is not to scale. Putative events of HGT are indicated based on sequence data; hypothetical HGT events are indicated with question marks. Representative morphotypes of displayed branches are shown. According to the model, the genomic MAI has been horizontally transferred among different phyla as indicated. The origin of magnetosome genes remains unknown. Abbreviations: HGT, horizontal gene transfer; MAI, magnetosome island; MMP, magnetotactic prokaryote; MTB, magnetotactic bacteria.

However, this finding might be explained by a period of coevolution of the transferred MAI and the genome of *Magnetospirillum* species after an ancient event of transfer. It is possible that magnetosome genes were acquired by the ancestors of recent magnetospirilla, which may have subsequently separated into different species such as *M. magneticum*, *M. gryphiswaldense*, and *M. magnetotacticum* (33).

During further evolution, the ancestral MAI then underwent extensive rearrangements, such as duplication, deletion, insertion, and phage integration, leading to distinctive compositional features. In the absence of selective pressure for magnetotaxis, nonmagnetotactic representatives branching within the MTB, such as *A. polymorphum*, may then have originated from secondary loss of the MAI, for example, by large deletions caused by homologous recombination between direct repeats within the MAI, a process that occurs frequently during lab cultivation of various magnetospirilla (25, 33, 86). In addition, this model suggests the independent horizontal transfer of magnetosome genes from an unknown ancestor to strains MC-1 and MV-1 occurred a longer time ago. The close phylogenetic affinity of a photosynthetic bacterium (*P. molischianum*) to the magnetospirilla suggests that their ancestor might have been a
phototrophic organism, with whom MTB also share the ability to form intracellular membranes. This hypothesis is further supported by the presence of genes in *M. magnetotacticum*, which might represent remnants of a previous photosynthetic lifestyle (9). For example, a gene encoding a putative photosynthetic reaction center M subunit displays similarity to homologs in *Methylobacterium chloromethanicum* and *Rhodospirillum centenum* (97% and 76% identity, respectively). Taken together, these results suggest that a model of MTB evolution involving horizontal gene transfer is much more likely than independent evolution of this complex trait.

**DIRECTIONS FOR FUTURE RESEARCH**

Despite the considerable progress in our understanding of the molecular mechanisms governing magnetotaxis and biomineralization, a number of remaining questions need to be solved by future approaches. Although the largest part of candidate genes involved in magnetosome formation has likely been identified, elucidation of their individual functions by genetic and biochemical analysis will remain a challenge for the next few years. On the other hand, only a few magnetotactic species, mostly from the Alphaproteobacteria, have been analyzed with respect to the genetic basis of magnetosome formation. It will be of interest to discover whether the same genetic mechanisms do apply in more remotely related MTB from the Delta-, Gamma-, or Nitrospira Proteobacteria phyla. However, only a small minority of MTB from natural populations have been isolated in pure culture, whereas the great diversity of naturally occurring species has been largely unexplored, including, for example, giant or multicellular forms of MTB, as well as those producing crystals of different chemical composition (i.e., greigite). However, unlike other uncultivated bacteria, MTB can be collected from environmental samples to virtual homogeneity by utilizing their magnetically directed motility. It has been already demonstrated that this can be used for selective cloning of large genomic fragments harboring the MAI from uncultivated MTB (34).

Not only will metagenomic analysis provide a census of genetic diversity in magnetosome formation, but by use of single-cell approaches this might also be extended to the analysis of complete genomes from single or a few individual cells, even from underrepresented species of natural populations. The increasing genome information will also bring us closer to the identification of the minimal set of genes required for magnetosome formation. This might in turn facilitate the heterologous expression of single genes, entire operons, or ultimately, the genetic reconstitution of the complete pathway from uncultivated MTB in heterologous hosts. In the future this could provide access to the biomineralization of the spectacular host of crystal morphologies and arrangements, which are expected to display unprecedented magnetic and crystalline characteristics. A complete understanding of magnetosome formation at the molecular level will also be useful for the engineering and functionalization of magnetosomes for their use as magnetic nanomaterials with tailored magnetic and crystalline properties that could be utilized in various biotechnological applications (43).

**SUMMARY POINTS**

1. Magnetosomes are specialized organelles for magnetic navigation comprising membrane-enveloped, nano-sized crystals of a magnetic iron mineral.

2. Physicochemical control over biomineralization is achieved by its compartmentalization within vesicles of the MM, that is a phospholipid bilayer originating from the CM by invagination.
3. The MM is associated with a specific set of Mam, Mms, Mtx, and Mme proteins that belong to characteristic families including TPR proteins, CDF transporters, HtrA-like serine proteases, actin-like proteins, generic transporters, and MTB-specific proteins with no homology to other proteins.

4. The magnetotactic phenotype is only weakly reflected by the overall gene content of magnetobacterial genomes, and MTB analyzed thus far share a magnetotactic core set of 891 genes and have a set of 28 conserved signature genes in common that is specifically implicated in magnetotaxis.

5. By various approaches, including forward and reverse genetics, as well as bioinformatic prediction, a partially redundant set of 15–50 candidate genes implicated in magnetosome formation was identified.

6. Most magnetosome genes are located in several operons within a conserved genomic MAI, which shares common structural and compositional characteristics between various MTB. The MAI also displays differences with respect to gene content and organization that may account for the diversity in magnetosome morphologies and arrangements in many MTB.

7. Examples with known in vivo functions are the MamGFDC proteins, which regulate the size of magnetite crystals, and the MamK and MamJ proteins, which control the assembly of magnetosome chains along dedicated cytoskeletal structures, presumably formed by the actin-like MamK protein.

8. Magnetotaxis is widespread within different lineages of eubacteria and most likely evolved by horizontal transfer of the MAI to various phylogenetic groups.

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LITERATURE CITED


is genetically unstable.

which comprises most the genomic MAI,

clusters.

magnetosome in agglomeration of chains and collapsed magnetosome MamJ results in magnetosome protein the mutagenesis of the 61. Demonstrates that the size of magnetite crystals.

collectively control the abundance of MamGFDC genes that is specifically identified with the magnetotactic phenotype.

60. Demonstrates by deletion mutagenesis and complementation analysis that the abundant MamGFDC magnetosomes have partially redundant functions and collectively control the size of magnetite crystals.

61. Demonstrates that the mutagenesis of the magnetosome protein MamJ results in collapsed magnetosome chains and agglomeration of magnetosome in clusters.


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