REVIEWS

From invagination to navigation: The story of magnetosome-associated proteins in magnetotactic bacteria

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Abstract: Magnetotactic bacteria (MTB) are a group of Gram-negative microorganisms that are able to sense and change their orientation in accordance with the geomagnetic field. This unique capability is due to the presence of a special suborganelle called the magnetosome, composed of either a magnetite or gregite crystal surrounded by a lipid membrane. MTB were first detected in 1975 and since then numerous efforts have been made to clarify the special mechanism of magnetosome formation at the molecular level. Magnetosome formation can be divided into several steps, beginning with vesicle invagination from the cell membrane, through protein sorting, followed by the combined steps of iron transportation, biomineralization, and the alignment of magnetosomes into a chain. The magnetosome-chain enables the sensing of the magnetic field, and thus, allows the MTB to navigate. It is known that magnetosome formation is tightly controlled by a distinctive set of magnetosome-associated proteins that are encoded mainly in a genomically conserved region within MTB called the magnetosome island (MAI). Most of these proteins were shown to have an impact on the magnetism of MTB. Here, we describe the process in which the magnetosome is formed with an emphasis on the different proteins that participate in each stage of the magnetosome formation scheme.

Keywords: magnetotactic bacteria; magnetosome; biomineralization; magnetic nanoparticles; protein function

Introduction

Magnetotactic bacteria (MTB) are a group of Gram-negative microorganisms that can align along external magnetic fields.[1] MTB were first described in Italian by Salvatore Bellini in 1963[2,3] but remained untranslated into English; in 1975, they were independently

Abbreviations: AMB-1, *Magnetospirillum magneticum* strain AMB-1; BAR, Bin/Amphiphysin/Rvs; CDF, cation diffusion facilitator; CM, cytoplasmic membrane; CTD, C-terminal domain; MAD, magnetosome-associated *Deltaproteobacteria*; MAI, magnetosome island; Mam, magnetosome-associated membrane; MCP, methyl-accepting chemotaxis proteins; MM, magnetosome membrane; Mms, magnetic particle membrane-specific; MSR-1, *Magnetospirillum gryphiswaldense* MSR-1; MTB, magnetotactic bacteria; NTD, N-terminal domain; TM, transmembrane; TMD, transmembrane domain; WT, wild-type.

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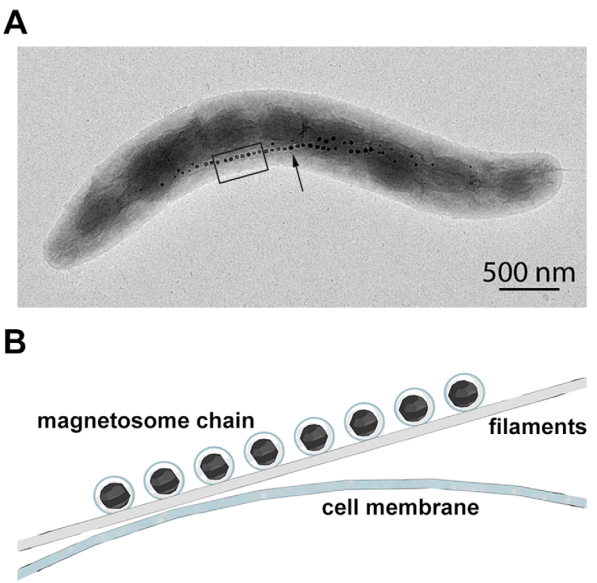

Figure 1. Magnetotactic bacterium. (A) Transmission electron microscope (TEM) image of *Magnetospirillum gryphiswaldense* MSR-1, contributed by Dr. René Uebe and Dr. Dirk Schüler. The black arrow points toward the magnetosome chain. (B) Magnified illustration of the black box in A: magnetosomes are made of magnetic particles surrounded by a lipid membrane—which invaginate from the cell membrane—and organized as a chain on filaments.

discovered by Richard Blakemore in marine sediments[4] and the worldwide MTB study was initiated.[5] The ability of MTB to orient themselves along magnetic fields is achieved by a chain-like organization of subcellular organelles, called magnetosomes, that are composed of a magnetic particle surrounded by a bilayer lipid membrane[6,7] (Fig. 1). Magnetosomes are able to biomineralize single crystals of magnetite or gregite[8,9] in strain-dependent sizes and morphologies, wherein each strain these properties are conserved.[10–12] The mineral crystal size is ~30–120 nm, which fits the size of a single-magnetic domain.[10] The common theory in the MTB community suggests that magnetosome membranes (MMs) invaginate from the cytoplasmic membrane (CM) to form vesicles.[5,13] creating the optimal conditions for crystal nucleation and growth.[10,13,14] Magnetosomes' alignment into a fixed linear chain or multiple chains requires cytoskeletal actin-like filamentous structures (Fig. 1B), and generates a permanent magnetic dipole moment. This enables the rotation of the entire cell to be aligned with the geomagnetic field lines, allowing the bacterium to move along these lines using their flagella. This behavior—magnetotaxis—increases their efficiency in finding suitable environmental conditions, usually the oxic-anoxic zone in aquatic enviroments.[4,5,7,15,16] The early model of magnetotaxis was based on the assumption that all MTB have a permanent polar preference to their swimming direction. In this model, north-seeking bacteria swimming northward in the Northern Hemisphere and south-seeking bacteria swimming southward in the Southern Hemisphere would migrate downward toward the sediments along the inclined geomagnetic field lines.[4,5,16,17] Later on, this model was shown not to be valid but only under specific conditions and cannot explain the taxis-behaviors of some strains.[18] A new model suggested that magnetotaxis together with aerotaxis enable the MTB to reach the appropriate environment, a behavior that was called "magneto-aerotaxis."[18,19] Two different mechanisms were proposed: (1) a polar magneto-aerotaxis mechanism, in which the bacterium moves persistently in a specific direction (parallel or antiparallel to the magnetic field), depends on the oxic conditions, which results in an efficient aerotactic response in the vertical oxygen gradients, and (2) an axial magneto-aerotaxis, in which the bacterium does not have a preference for the swimming direction and swims with frequent, spontaneous reversals of swimming directions (with no distinction between north-seeking and south-seeking bacteria).[5,13,16,18] Recently, six different magneto-aerotactic behaviors were observed in different strains.[20] Despite the above, the navigation mechanism of MTB does not depend only on oxygen concentration but is thought to be more complicated and to involve other mechanisms such as phototaxis[21,22] and chemotaxis.[5,13,23,24]

From their rediscovery in 1975, MTB were greatly studied in many research groups around the world. Genetic studies showed that MTB are highly divergent: they are affiliated mainly with the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* from the *Proteobacteria* phylum, as well as with the *Nitrospirae*[25,26] and *Omnitrophica*[27,28] phyla. The most characterized strains today include the cultivated *Alphaproteobacteria* species *Magnetospirillum magneticum* strain AMB-1 (AMB-1), *Magnetospirillum gryphiswaldense* MSR-1 (MSR-1), *Magnetospirillum magnetotacticum* strain MS-1 and *Magnetococcus marinus* strain MC-1.[29] There are also cultivated, studied strains affiliated to *Deltaproteobacteria* and *Gammaproteobacteri*a classes, such as *Desulfovibrio magneticus* strain RS-1[30] and BW-2,[31] respectively.

The study of different strains showed that the formation of a functional magnetosome is a highly controlled process.[32–35] Most of the genes responsible for this process are located in the magnetosome island (MAI), a genomic segment (of 130 kb in MSR-1) that is conserved among different species.[32,33,35–37] This region contains a few operons; the most conserved and essential operon is *mamAB*, which can be found in all identified MTB, while other operons, such as *mamGFDC*, *mamXY*, and *mms6* in MSR-1, are specific to *Alphaproteobacteria*.[25,35,38–41] To simplify the information discussed in this review, we will focus on a set of identified genes within the MSR-1 strain that are classified as MTB-related and -specific genes, meaning that they share only slight or no

Table I. *Key features of all discussed Mam and Mms proteins*

Protein	# Amino acids[a]	Encoding operon[a]	Suggested role	Main related articles
MamA	217	*mamAB*	Protein sorting	14, 46, 47
MamB	297	*mamAB*	Membrane invagination, iron transport	48
MamC	125	*mamGFDC*	Crystal size and shape control	44, 49–52
MamD	314	*mamGFDC*	Crystal size and shape control	44, 50, 51
MamE	772	*mamAB*	Protein sorting, redox control	34, 53, 54
MamF	111	*mamGFDC*	Crystal size control	42, 44, 49, 55
MamG	84	*mamGFDC*	Crystal size and shape control	44, 49–51
MamH	427	*mamAB*	Iron transport	56
MamI	76	*mamAB*	Membrane invagination	34
MamJ	466	*mamAB*	Magnetosome alignment	57–61
MamK	359	*mamAB*	Magnetosome alignment	62–66
MamL	122	*mamAB*	Membrane invagination	34
MamM	318	*mamAB*	Iron transport	48, 67
MamN	437	*mamAB*	pH control	–
MamO	632	*mamAB*	Crystal nucleation	53, 54, 68
MamP	269	*mamAB*	Redox control	69–72
MamQ	271	*mamAB*	Membrane invagination	–
MamR	72	*mamAB*	Crystal size and number control	–
MamS	180	*mamAB*	Crystal size and shape control	–
MamT	174	*mamAB*	Redox control	70, 72
MamX	268	*mamXY*	Redox control	45, 56
MamY	370	*mamXY*	Membrane invagination	73, 74
MamZ	660	*mamXY*	Iron transport, redox control	56
FtsZm	323	*mamXY*	Crystal size and shape control, denitrification	75, 76
Mms6	136	*mms6*	Crystal size and shape control	77–79
MmsF	124	*mms6*	Crystal size and shape control	55, 80

[a] Data are from Schleifer *et al*.,[81] Richter *et al*.,[32] Grünberg *et al*.,[35] and Wang *et al*.[82]

similarities to the genes of other nonmagnetic organisms, respectively. These genes encode for most of the magnetosome-associated membrane (Mam) proteins and magnetic particle membrane-specific (Mms) proteins.[5,32] Examples of magnetosome-related genes from other strains are the magnetosome-associated *Deltaproteobacteria* (*mad*) group of genes that can be found in *Deltaproteobacteria* strains and in *Nitrospirae* and *Omnitrophica* phyla strains.[25,28,40] Mam and Mms proteins can be classified approximately into several groups according to their roles during magnetosome formation processes, such as membrane invagination, protein sorting, magnetosome alignment into chains, biomineralization, and the control of mineral crystal shape and size.[34,42–45] Here, by describing the known data regarding Mam and Mms proteins and focusing on their role (Table I),[14,32,34,35,42,44–82] we present the process of magnetosome formation.

Membrane invagination

Magnetosome invagination is the first step in magnetosome formation,[5,13,34] in which a few proteins are suspected to take part (Fig. 2A). Based on genetic dissection studies, *mamB, I, L* and *Q* deletion resulted in the lack of magnetosome vesicles in AMB-1[34] and apart from *mamI*, also in no magnetic response in MSR-1.[42] This suggests that *mamB*, *I*, *L*, and *Q* are important for magnetosome invagination, although they are not sufficient by themselves in the Δ*mamAB* operon to restore the biogenesis of magnetosomes.[34] MamB is suspected to have a dual role in MTB, in magnetosome invagination as well as in iron transportation,[48] hence, it will be discussed in detail later in the biomineralization section. MamY was also suggested to have a role in membrane invagination,[73] indicating that a set of at least five proteins control this process.

MamI and MamL are small, integral membrane proteins conserved within different strains and are unique to MTB.[13,32,43] MamI was shown to be associated with the MM in AMB-1, whereas MamL was shown to be associated mainly with the CM and less with the MM, suggesting a transient MM association of MamL.[34] Deletion of *mamI* in MSR-1 resulted in only a few, much smaller, nonmagnetic magnetosomes, in contrast to AMB-1, which lacked magnetosome vesicles, suggesting it has a role also in early magnetite nucleation, perhaps by controlling the conditions for proper nucleation and growth.[42] Both MamI and L are predicted to contain two transmembrane (TM) helices. The MamI connecting loop between the integral membrane helices is not predicted to bind magnetite, which further supports the proposed role of MamI in participating in MM bending.[83] MamL has a basic C-terminal tail that can bind the phospholipid heads on the inner MM, which can help in the membrane-bending process.[84]

MamQ is a magnetosome-integrated membrane protein conserved within different MTB phyla.[28,32,34,83]

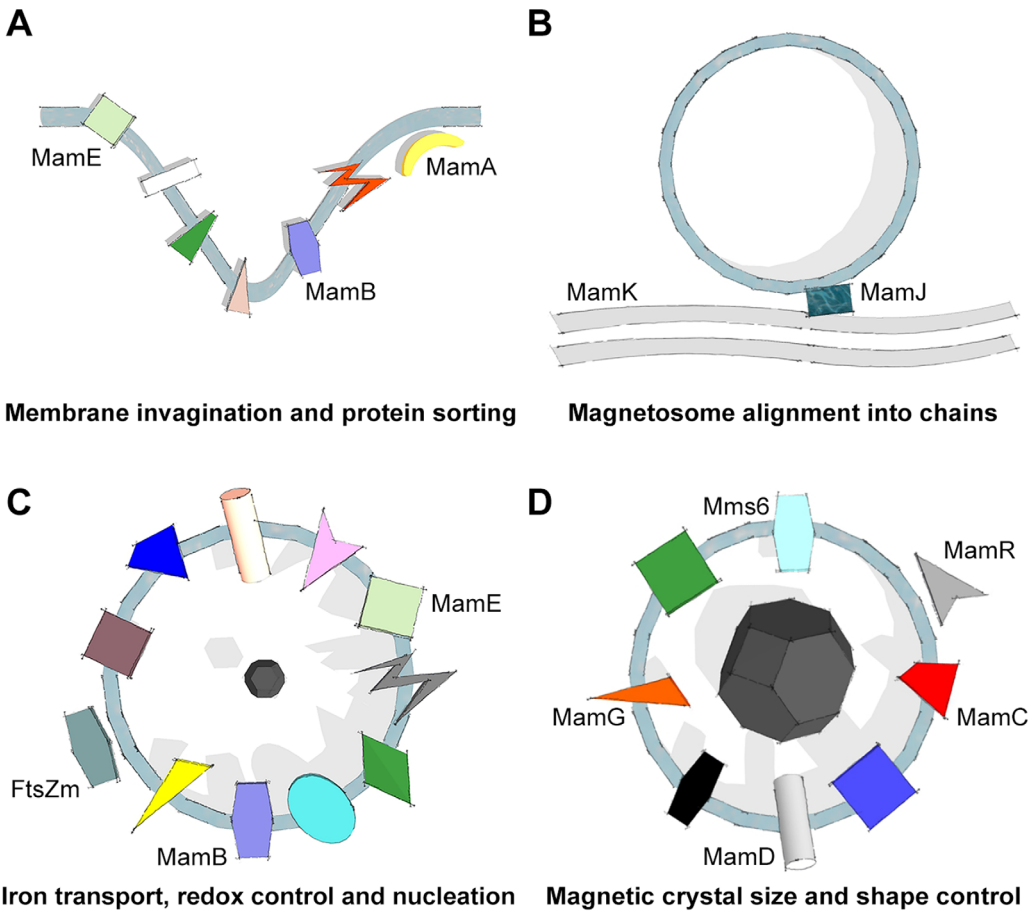

Figure 2. Schematic model of magnetosome formation. Proteins (each is presented in a different shape or color) can be roughly divided into different stages of magnetosome formation (TM proteins cross the membrane; proteins' sizes, shapes, colors and locations are meaningless, unless specified): (A) MamB, I, L, Q, and Y were suggested to take part in magnetosome invagination, and MamA and E in protein sorting. (B) MamK and J participate in magnetosome alignment into chains. (C) MamB, E, H, M, N, O, P, T, X, Z, and FtsZm are involved in processes such as iron transport, nucleation and chemical environment control. (D) MamC, D, G, F, R, S, Mms6 and MmsF all influence the magnetic particle size and morphology. MamC, D, G and Mms6 locations correspond to the presumed locations in the magnetosome.

MamQ secondary structure prediction displays an integral helix followed by a C-terminal domain (CTD), presumably located in the magnetosome lumen.[83] MamQ is homologous to the LemA protein family that have no known function[34,84] and shares weak similarity to BAR (Bin/Amphiphysin/Rvs) proteins.[42,84] The BAR domain is a coiled-coil, membrane-bound domain that takes part in membrane deformation.[42,73,83–86] Since MamQ presumably contains a coiled-coil domain similar to BAR proteins, it may have a role in membrane bending during magnetosome invagination.[42,84]

MamY is an MTB-specific protein.[32] In AMB-1, MamY was only present in magnetosomes containing small immature crystals (SM),[73] whereas it was not identified in regular-sized magnetite magnetosomes or in the CM.[73,74] When *mamY* was deleted, magnetosome vesicles were bigger but had a larger population of SM compared to wild type (WT).[73] MamY is able to bind liposomes, to form tubules and to deform the membrane of both liposomes' and magnetosomes' lipid extract.[73] MamY is predicted to contain two integral membrane helices followed by a large cytosolic CTD.[83] MamY is similar to MCPs (methyl-accepting chemotaxis proteins), BAR[73,83] and to talin, a cytoskeletal protein that links actin to the membrane.[83] All of these together suggest that MamY participates in: (a) constriction of the cell membrane that leads to the invagination of the magnetosome membrane, (b) deformation and size control of the magnetosome membrane, and (c) magnetite nucleation or growth.[73,83]

Protein sorting

Many of the proteins discussed in this review are uniquely found or enriched in the magnetosome membrane (containing at least one transmembrane domain [TMD]) or on the MM cytoplasmic side.[6,13,35,49,87,88] Some of these proteins, such as MamA and MamC, show dynamic localization.[14,49] Hence, an exclusive mechanism is needed to ensure that these proteins will be located in the MM or at the MM surface at the proper stage. This

mechanism is not yet determined, but few proteins are thus far suspected to have a role in the protein sorting process (Fig. 2A).

MamA is one of the most conserved and abundant magnetosome-associated proteins.[13,46,87] In AMB-1, MamA deletion does not abolish magnetosome formation, but not all magnetosomes are populated with magnetite.[14] MamA has a dynamic localization during cell-growth, which is independent of magnetite formation.[14] MamA was shown to form in vitro globular homo-oligomers with a central pore cavity.[46] Moreover, MamA forms high-molecular weight complexes that surround and cover the entire cytosolic side of the magnetosome membrane.[47] Determined MamA structures from several species showed great structural similarity and interphyla conservation, despite the variances in their sequences.[46,89,90] It contains five identified, and a total of putatively six, tetra-tricopeptide repeat (TPR) motifs.[89,91,92] Proteins with repetitive TPR motifs are known to take part in protein–protein interactions.[93,94] MamA has three proposed protein–protein interaction sites,[46] two of which are assumed to participate in the oligomerization of MamA, whereas the third can bind other magnetosome-associated proteins and by this may enable the association of MamA complexes to the magnetosome surface.[46] In vivo and in vitro studies showed that MamA can bind different proteins,[47,57] which supports the assumption that the MamA homo-oligomer surface forms a multiprotein interaction site.[46]

MamE is predicted to be an integral membrane protein with one TM region. MamE shares high identity with different *Magnetospirillum* strains[53,83] and it acts at two functionally distinct magnetosome formation steps, the protein sorting and the crystal biomineralization initiation (which will be discussed in the biomineralization section).[53,54] MamE contains a putative trypsin-like serine protease domain, two putative PDZ domains belonging to the HtrA/DegP serine protease protein family and two putative CXXCH heme-binding motifs.[53,54] The putative heme-binding motifs are expected to take part in the iron redox chain, during magnetic particle biomineralization. Deletion of *mamE* in AMB-1 lead to mislocalization of several proteins,[34] indicating that the localization of magnetosome proteins can be accomplished through physical interaction of MamE with one or more magnetosome-associated proteins at the MM. Furthermore, it was shown that magnetosome protein localization does not require MamE's protease acticity.[54,95]

Magnetosome alignment into chains

Each magnetosome contains a crystal that is within the size of a single-magnetic domain, which is not sufficient to sense magnetic fields. The passive alignment to a geomagnetic field is achieved by the alignment of magnetosomes into a linear chain, which creates a larger magnetic dipole moment than a single crystal.[15] The current literature indicates that chain assembly formation is mediated mainly via two proteins, MamK and MamJ (Fig. 2B).

MamK is an MTB-related protein that is homologous to the bacterial filaments-forming actin-like protein MreB.[32,62,63] MamK forms long, linear filaments from one cell pole to the other, along the cell's inner curvature and aligned with the magnetosomes.[62,96,97] In dividing cells, MamK was shown to have a role in positioning the magnetic dipole in each of the daughter cells.[96] MamK polymerization into the filamentous chains is a dynamic process and is kinetically asymmetrical.[58,96] Thus, MamK is found not only next to the magnetosome chains but it is also dispersed throughout the cell.[98] MamK contains an ATP-binding site with ATPase activity, which is assumed to be related to the dissociation of filament aggregates and not directly to polymerization.[58,63–65,96,97] MamK's surface is largely hydrophobic or neutral, suggesting that the salts in the cell cytoplasm limit and control filament assembly.[63] MamK monomers in solution assemble into two-stranded helical filament structures, from unstaggered, parallel strands.[64]

In AMB-1 Δ*mamK* cells, a MamK-like protein creates filaments and magnetosomes are aligned into a chain, despite the lack of MamK[65] (though not supported by another study[62]). MamK-like is a protein encoded outside of the MAI region in AMB-1 that is very similar to MamK,[65] but MamK was found to have a more dominant function in magnetosome chain formation.[99] In MSR-1 Δ*mamK* cells, magnetosome chains were detected, but were smaller, misplaced, less organized and with less ability to self-orient.[66] MamK was suggested to serve as a track that magnetosomes can move on[96,98] and to have a role in the assembly and localization of mature magnetosomes into a chain in the midcell for some strains,[58,62,66,96] which was further supported by in silico analysis of magnetosome formation, assembly, and localization.[100]

AMB-1 MamK was shown to interact with a few MCPs,[57,101] suggesting that magnetotaxis via MamK in AMB-1 may relate in some way to the mechanism of chemotaxis.[57] Also, flagella-motor-associated proteins were shown to interact with MamK, suggesting that the magnetosome torque produced by the magnetosome chain can impact on flagella rotation via the interaction with flagella motor proteins.[57]

MamJ is an MTB-specific protein cotranscribed with *mamK*.[32,35,59] MamJ is located in a linear structure that stretches between two ends of the cell, which extends beyond the magnetosome chain and is situated in the midcell.[59] Deletion of *mamJ* in MSR-1 and of *mamJ* together with its homologue *limJ* in AMB-1 impaired magnetosome chain

organization, suggesting MamJ has a role in this process.[58,60] MamJ's location is affected by the presence of other magnetosome proteins.[59] MamJ was shown to physically interact with MamK in both MSR-1 and AMB-1,[57,60,61] and it is assumed to be involved in the assembly of the magnetically attracted magnetosomes into chains by connecting them to the cytoskeletal structure formed by MamK.[59] Supporting this, MamK was found to be associated with the MM of AMB-1,[74] possibly via the interactions with MamJ.[60] Moreover, in MSR-1, *mamJ* deletion does not affect the localization of MamK[66] but MamK is needed for the proper localization of MamJ.[59,66] While MamK filaments are stable and not deformed under strong magnetic fields, strong magnetic fields can disturb MamJ function and/or the MamJ–MamK interaction, resulting in magnetosome alignment in the direction of the strong magnetic field.[102] Additionally, MamJ and MamE were shown to interact, suggesting that this interaction anchors MamJ–MamK to the MM.[57] Despite all of the above, MamJ is not conserved within all MTB, therefore, it is most likely that some other mechanisms are also involved in magnetosome alignment.[98]

Biomineralization

The MM, which is present before magnetic-crystal formation, serves as a secluded compartment in the cell that provides a suitable environment for efficient crystal formation and growth into a proper mature crystal.[5,13,14] This requires iron transfer into the magnetosome, adjustment of the chemical environment for crystal nucleation and maturation control.[13,34] Here, we describe the proteins participating in the biomineralization of magnetic nanocrystals (Fig. 2C). The proteins that are responsible for species-specific size and shape control will be discussed in the next section.

MamM is an integral membrane protein that shares 47% sequence similarity to MamB.[35] MamM was shown to be localized to the MM and to be involved in magnetite nucleation and crystal growth.[48] It was recently proposed that MamM acts as an iron transporter, since it is homologous to the cation diffusion facilitator (CDF) protein family and its deletion or single point mutations have been shown to abolish magnetite biomineralization or cause alterations in magnetite crystals' sizes and morphologies.[34,48,67,103] Deletion of *mamM* was shown to affect localization of other MM proteins, such as MamC-GFP,[104] in an elusive mechanism.[34,48] Recent structural and functional studies of MamM confirmed that its overall topology fits to the CDF protein family.[67] MamM is suspected to share the TMD conserved fold, the putative metal-binding sites and shares the CTD fold with other CDFs.[67,105] CDF proteins transport divalent transition-metal-cations by exploiting the proton motive force.[106] Biophysical analyses of MamM demonstrated that a conformational change takes place upon binding of divalent cations to the CTD, which triggers the CTD conformation toward a compact fold that is believed to allow the activation of a two-step ion transport mechanism through the TMD.[67] This transport is essential not only for iron accumulation in the magnetosome lumen, but also for maintaining the high pH needed for magnetite/gregite biomineralization.

MamB is an integral membrane protein that is classified as a CDF protein.[83,107] Accordingly, MamB is expected to assemble as a dimer and to contain metal-ion-binding sites at its TMD and CTD. Additionally, deletion of *mamB* led to the lack of the intracellular MM,[48] suggesting that MamB participates in two different magnetosome formation stages: first in membrane invagination and second in magnetosome iron accumulation.[48] Of note, MamM is required for the stability of MamB in MSR-1,[48] consequently suggesting that MSR-1 MamM and MamB interact with each other and might form heterodimers. MamB and MamM cannot functionally compensate for each other,[48] supporting the hypothesis that these two proteins have different roles in the MTB.

MamO is a large integral membrane protein with eight predicted TM α-helices that share high-sequence identity with different *Magnetospirillum* strains.[53,83] MamO contains two domains: a domain of unknown function (DUF81)[54] and a trypsin-like serine protease domain.[83] The MamO DUF81 domain may function as an anion transporter or as a magnetosome localization determinant via protein–protein interaction.[53,108–110] Deletion of *mamO* leads to empty magnetosomes with low magnetism and intracellular iron content.[68] Similarly, insertion and deletion mutations within *mamO* were shown to be sufficient to abolish crystal biomineralization,[53] whereas several point-mutations at the trypsin-like domain did not affect crystal formation.[53,54] Altogether, these results suggest that MamO may take part in crystal nucleation.

Iron oxidation-reduction proteins have a key role in the magnetosome biomineralization process. Since magnetite and gregite nanocrystals' formations require the oxidation of Fe^{2+} to Fe^{3+}, it was suggested that some proteins take part in iron redox control.[54,56] The CXXCH motif, a typical c-type cytochrome motif that is known to bind haem,[111] is found in MamE, P, T, and X,[54,56] and can act in the reduction/oxidation of iron.[69] The CXXCH-containing domain in these proteins, the "magnetochrome," seems to be specific to MTB, suggesting that it is a new, functional, unique class of cytochromes.[69] Since magnetochrome-containing proteins are the only redox proteins associated with the magnetosome, the possibility arises in which the

presence of magnetochrome-containing proteins could be related to the evolution of MTB and may be associated with magnetite/gregite crystal shape.[112]

Further to MamE's previously discussed role in protein sorting,[53,54] MamE plays a role in the biomineralization process. *mamE* deletion leads to empty magnetosome vesicles and to the loss of magnetite synthesis,[34] whereas a mutation within MamE's heme-binding site did not abolish biomineralization of mature magnetite-crystals. These results indicate that MamE may act as a molecular switch to initiate crystal biomineralization. Biomineralization can be mediated via interaction with MamO or other proteins,[53,110] to create a network of redox activity in the center of the process.[54,56] Supporting this hypothesis, AMB-1 MamE's magnetochrome domain was identified as a cytochrome c-like domain.[70] Since some serine proteases were shown to be capable of precipitating metal oxides, and since MamO and MamE contain this domain, a possibility arose in which MamO and MamE could play a direct role in the formation of iron-oxide crystals.[113]

Additionally, MamE may have a role in the maturation of small 20 nm crystals. Once a magnetite crystal reaches the 20-nm transition point, degradation of biomineralization inhibitors or proteolytical activation by MamE is essential for further crystal growth.[54]

MamP is an integral membrane protein with one predicted TM helix, two c-type cytochrome motifs and a single PDZ domain.[69,70,83] The structure for the soluble portion of MamP from magnetotactic ovoidal bacterium MO-1 was determined, enabling the description of the magnetochrome domain structure.[69] This structure confirms that the magnetochrome domain defines a single heme-binding domain belonging to a new family of c-type cytochrome; it folds as one of the smallest heme-binding units known thus far.[69] In vitro studies found that MamP can oxidize Fe_2SO_4 at alkaline pH efficiently, similarly to Fe^{2+} oxidation by the multihaem cytochrome c MtoA protein.[69,114] The optimal pH of MamP iron oxidase activity coincides with that found for the redox potential[70] and for in vitro ferrihydrite and magnetite synthesis.[69,115] The combination of these data clearly demonstrates the identity of the magnetochrome domain as a cytochrome c-like domain.[70] Deletion of *mamP* resulted in smaller and irregular-shaped crystals (suspected to be haematite) than the WT,[34,42] suggesting that MamP could play a role in controlling crystal size and number.[34,71] MamP was also proposed to optimize the stoichiometry of Fe^{2+} and Fe^{3+} so that the magnetite nanoparticle can be grown without defects,[72] therefore, MamP was suggested to mediate the production of ferrihydrite or magnetite.[69,116] Beyond these roles, MamP may take part in the MTB cell-cycle, since it was shown that its cell content is temporally regulated throughout the growth cycle and increased during the exponential growth phase.[71]

MamT is an integral membrane protein with a predicted N-terminal TM helix.[83] Furthermore, secondary structure prediction shows a double-magnetochrome motif assumed to be located in the magnetosome lumen.[70,83] *mamT* deletion resulted in smaller particles compared to the WT[34,42]. These phenotypes and the presence of magnetochrome motifs suggest that MamT has a role in magnetite crystal growth[34,72] and the electron redox chain.[70,72]

MamX structure prediction is indicative for a TM helix at the protein's N-terminus, a DNA-binding domain at its CTD and two magnetochrome domains.[45,56,117,118] Deletion of either full-length *mamX* or the substitution of its paired CXXCH motifs impaired magnetite biomineralization.[56] Additionally, irregular small particles with no magnetic response and low-iron content were observed in Δ*mamX* cells.[45] MamX, thus, is likely involved in crystal maturation and shape control[45] and in redox control needed for the synthesis of the mixed-valence iron oxide Fe_3O_4 under oxidant-limiting conditions.

MamH and MamZ (MamH-like[32]) share high identity to the major facilitator superfamily (MFS) domain,[43,56] suggesting that these two proteins are involved in magnetosomal iron transport.[48,56] The MamH structural model supports this hypothesis—the model displays a negative cavity that can bind positive ions and transfer them through the magnetosome membrane.[56,83] Deletion of *mamH* leads to a decrease in magnetic response, suggesting it is also involved in magnetite biomineralization.[34]

MamZ contains a conserved ferric reductase TM component of the YedZ-type that is assumed to bind heme B, therefore, MamZ might be involved in electron shuttling and redox reactions.[119] In addition to the putative ferric reductase domain, MamZ contains an MFS transporter domain and represents the only known example in which this domain is fused to a ferric reductase domain.[56,120,121] Based on this unique domain combination, MamZ was hypothesized to be an iron transporter[120,121] or to mediate ferric iron transport.[56] Deletion of only the ferric reductase domain abolished the protein function.[56]

Double deletion of *mamZ* and *mamH* had larger effects than each individual deletion. Therefore, it was assumed that both proteins have partially redundant functions, and that the presence of at least one protein is necessary for the synthesis of regular magnetite crystals.[56] However, it can be assumed that their functions are distinct from the functions of MamM and MamB.[48,56]

MamN is a TM protein that is expected to share homology with Na^+/H^+ antiporters and to form a

dimer, suggesting it has a role in increasing the pH within the magnetosome, a required condition for in vitro magnetite synthesis.[13,83] Deletion of *mamN* in AMB-1 resulted in empty magnetosomes, which is also reflected in the absence of a magnetic response,[34] whereas in MSR-1 it resulted in half-sized crystals.[42] These findings suggest that MamN may be involved in pH regulation, by exporting the protons that are released during the magnetite precipitation.[42]

FtsZ are structural, bacterial tubulin-like cell-division proteins that assembles into ring-like structures in a GTP-dependent manner in the dividing cell septum.[122,123] In at least three *Magnetospirillum* species, there is a second copy of a conserved *ftsZ*-like gene located in the *mamXY* operon[32,75] that encodes FtsZm, a truncated C-terminal FtsZ protein.[75,76,83] FtsZm's structural model consists of a GTP binding site in the N-terminal domain (NTD) and a CTD, that is essential for FtsZ polymerization and interactions with other proteins.[76,83] In agreement with the model, FtsZm was shown to have both ATPase and GTPase activity.[75,76] Due to the gene's location in the MAI, the FtsZ-like protein is suspected to be involved in magnetosome chain-assembly with other actin-like proteins or to have a role in the asymmetrical cell division in *Magnetospirillum* species.[41,76] In MSR-1, FtsZm and FtsZ do not create filaments but instead a large number of spots at midcell.[76] In the absence of FtsZm, magnetosome chains contained mature crystals in the chain center and flake crystals at the ends of the chain.[76] Deletion of *ftsZm* had no significant impact on cell growth, which suggests that it does not have the cell-division function of other bacterial FtsZ proteins.[75,76] Conversely, overexpression of FtsZm interfered in cell division similarly to other FtsZ proteins.[76] Studies showed that *ftsZm* deletion caused changes in magnetite size and morphology only when cells grew in the presence of ammonia instead of nitrate.[75,76] This suggests FtsZm has a role in denitrification, redox control, and iron uptake.[76]

MamS is an MTB-specific protein[32,35] that shares similarity to the putative serine-protease domain of MamE and MamX in MSR-1.[42] In AMB-1, and likewise in MSR-1, the crystals of Δ*mamS* cells were small, mainly amorphous and with weak magnetic response, creating small clusters within the chain with irregular spacing.[34,42] This suggests that MamS has a function in the regulation of magnetosome size and morphology.[34]

MamR is an MTB-specific protein that is predicted to be localized to the cytoplasmic side of the MM.[32,35] Deletion of *mamR*, together with its duplicated gene in AMB-1, resulted in shorter magnetosome chains, smaller particles, and a weak magnetic response, which suggest that MamR plays a role in the control of the numbers and sizes of magnetosomes.[34] MSR-1 Δ*mamR* cells presented similar magnetic responses and magnetosome numbers to WT cells. In contrast, other phenotypes, such as magnetosome size and chain formation modifications, were similar to those of AMB-1 Δ*mamR* cells, suggesting the same function for MamR as in AMB-1.[42]

Crystals' size and morphology

The last part that will be discussed here is the formation of functional magnetosomes. There is a large set of proteins that have a role in controlling the magnetite size and morphology, but are not essential for biomineralization (Fig. 2D). For example, in *Alphaproteobacteria* strains, there are at least six MTB-specific proteins encoded by the *mms6* and *mamGFDC* operons that have this role.[32,34,35,42–44,50,80] Deletion of both operons separately[42–44] resulted in smaller magnetite crystals than WT, while their simultaneous deletion resulted in stronger phenotypes compared to each deletion alone.[34,43] In MSR-1 Δ*mamGFDC* cells, magnetite crystals were 75% the size of WT crystals. Only complementation of three out of four of the proteins in any combination produced crystals of the size of the WT, suggesting these four proteins have a cumulative action in the regulation of crystal size.[44] In AMB-1, MamC, D and G (named Mms13, 7, and 5, respectively, in this strain) and Mms6 were shown to be tightly bound to magnetite.[51] These genes are absent in all strains studied so far that synthesize bullet-shaped magnetite, therefore, it is suggested that MamC, D, G, and Mms6 might have a role in the size control of octahedral-shaped crystals.[50] Moreover, these proteins are assumed to colocalize in the MM and to interact with the magnetite surface.[50,77] Mms6, MamD, and G all contain a hydrophobic leucine–glycine repeat region that is also found in other biomineralization proteins.[35,51,83,124]

MamC (Mms13) is the most abundant protein found uniquely in the MM.[35,49,51,87,125,126] Deletion of *mamC* in MSR-1 and AMB-1 had only a small impact on magnetite size.[44,50,127] MamC is predicted to contain two integral TM helices that are connected by an acidic alpha-helical loop in the magnetosome lumen[52,83] that is suspected to be the magnetite binding site.[128] MamC is proposed to influence magnetite formation via a specific mechanism: the high acidic region in the loop between the TM helices binds iron, which increases the local iron concentration and creates a favorable environment for magnetite nucleation.[52] In vitro coprecipitation of magnetite with MamC resulted in magnetite particles that possessed missing corners, suggesting that the protein is bound to specific magnetite faces, preventing the crystals from growing in these directions.[52]

MamD (Mms7) is an abundant protein in the MM.[32,33,44,87] AMB-1 MamD is composed of a

hydrophobic NTD and hydrophilic CTD.[51,87,124] The NTD integrates the protein into the magnetosome membrane and the CTD—which is located in the magnetosome lumen and contains acidic amino acids—is suspect to interact with the magnetite surface.[51] In AMB-1 Δ*mamD* cells, crystals had decreased size in the minor axis, and the crystal face was different from those in WT cells, which further supports MamD's role in controlling crystal morphology.[50]

MamG (Mms5) is an abundant protein, homologous to MamD in MSR-1 and Mms6 and MamD in AMB-1.[33,35,51] AMB-1 *mamG* deletion leads to the formation of smaller, spherical crystals, suggesting that MamG has a role in crystal growth via interaction with a specific crystal face.[50] MamG is exclusively located in the magnetosome membrane along the chain.[49] Secondary structure prediction suggest that MamG contain two integral TM helices and a charged, unstructured C-terminal that faces the cytosol,[83] which suggests that MamG interacts with the magnetite crystal via a connecting loop between the two TM helices.

MamF is the second most abundant MM protein.[32,35] In MSR-1, MamF forms stable oligomers even in the presence of sodium dodecyl phosphate (SDS).[35] MamF is encoded by the *mamGFDC* operon and is similar to MmsF (61% identity).[32,42] In MSR-1, *mamF* deletion caused no change in crystal size or number, but its codeletion with *mmsF* increased the effect of *mmsF* deletion phenotypes, that is, a decrease in magnetosome numbers and sizes. This suggests a role for MamF in magnetosome size and number control.[42] MamF is located in the MM exclusively[49] and is predicted to contain three TM helices.[44,55,83] The loop between the first two helices is rich in charged residues and is suspected to be located in the magnetosome lumen, hence it may interact with the magnetite crystal.[83]

MmsF is a protein encoded by the *mms6* operon, in which its encoding gene deletion in MSR-1 and AMB-1 caused similar phenotypes of smaller crystals and lower magnetic responses.[42,80] MmsF participates in controlling the size and shape of magnetite, and was suggested to have a role in the control of crystal maturation after the nucleation stage.[80] MmsF in AMB-1 is predicted to be composed of three TM helices with a cytoplasmic N-terminal and a C-terminal in the magnetosome lumen. The C-terminal and the loop between helices one and two, which are rich in acidic amino acids, are assumed to bind the magnetite crystal.[55,80] In vitro purification of MmsF without detergents led to oligomers or aggregates in the cell lysate soluble fraction.[55] MmsF in solution was found to create artificial, doughnut-shaped assemblies, named "proteinosomes," that are probably high-ordered aggregates. These proteinosomes coprecipitate iron in vitro into magnetic particles similar to those of AMB-1. MmsF has an aspartate residue in the loop between helices one and two that is suspected to be a magnetite binder and has the same motif as Mms6 that is known to bind magnetite.[55]

Mms6 is a protein suspected to undergo proteolytic cleavage from its pro-protein to create a shorter, C-terminal, functional protein.[35,51] Not only the full protein but also the Mms6 C-terminal peptide was shown to be tightly bound to magnetite and affect its size and shape in vitro.[78] Mms6 NTD is suspected to have a random coil structure with a TM helix, while the C-terminal is acidic and is suggested to form an alpha helix with a negative surface.[51,79,83] The predicted helical CTD is suspected to bind the magnetite particles.[51,79,83] Full-Mms6 and C-terminal-Mms6 were shown to bind iron ions[51,79,129] and to create homogenous-sized and regular-shaped magnetite particles in vitro.[51,79] These all occurred only if the C-terminal was in its native form that contained the acidic amino acids,[78,129] thus suggesting that the CTD is responsible for iron binding. A negative surface charge composed of the CTD acidic amino acids enables iron nucleation at the Mms6 surface, and in turn specific magnetite face interaction with Mms6 controls the magnetite size and shape.[79,129–132] Deletion of *mms6* from AMB-1 and MSR-1 resulted in smaller crystals and in AMB-1 also in different morphology compared to the WTs,[42,50,77,80] supporting the proposed role of Mms6 in magnetite maturation and orientation during magnetite crystal growth.[77]

From the different morphology phenotypes that were obtained in MamC, D, G, and Mms6 deletion studies in AMB-1, the proteins' locations were suggested. In a linear chain of n magnetosomes, MamC and G are mainly found at the location inside magnetosome k proximal to magnetosome $k+1$. In contrast, MamD and Mms6 are still within the magnetosome but are presumed to be located within the vesicle such that they face away from the magnetosome chain.[50]

The proteins discussed in this section are not conserved within all MTB branches.[25] The *mad* group of 30 genes, which can be found in *Deltaproteobacteria* strains,[40,133] *Nitrospirae* phylum strains[134,135] and in *Omnitrophica* phylum strain,[28] can be divided into three groups: magnetite biomineralization-related genes, gregite magnetosome formation-related genes and a third, unclassified group that can be found in all the *Deltaproteobacteria* strains that were studied.[40] Most of the Mad proteins share no homology to any other proteins, and some were suggested to have a putative role based on their homology to other proteins, such as in iron uptake and in magnetosomes alignment into chains. Since these genes are unique to gregite and bullet-shaped magnetite crystal-

synthesizing strains, they might have a role in size and morphology control specifically for these crystal types, which is analogous to the *mamGFDC* and *mms* genes in *Alphaproteobacteria* that are not found in those species.[40]

Concluding remarks

Here, we presented briefly the current known data about the main proteins involved in magnetosome formation. Yet, more data is needed to clarify this unique process. The revealing of magnetosome formation mechanisms in general, together with specific studies of relevant proteins, has an impact on many scientific fields that were not discussed here, such as nanotechnology, medicine and ecology. This makes magnetosome studies a "hot topic" that interests many groups around the world who continuously study different aspects of magnetosome-related processes.

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