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MINI-REVIEW

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Molecular analysis of a subcellular compartment: the magnetosome membrane in *Magnetospirillum gryphiswaldense*

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Abstract The ability of magnetotactic bacteria (MTB) to orient and migrate along magnetic field lines is based on magnetosomes, which are membrane-enclosed intracellular crystals of a magnetic iron mineral. Magnetosome biomineralization is achieved by a process involving control over the accumulation of iron and deposition of the magnetic particle, which has a specific morphology, within a vesicle provided by the magnetosome membrane. In *Magnetospirillum gryphiswaldense*, the magnetosome membrane has a distinct biochemical composition and comprises a complex and specific subset of magnetosome membrane proteins (MMPs). Classes of MMPs include those with presumed function in magnetosome-directed uptake and binding of iron, nucleation of crystal growth, and the assembly of magnetosome membrane multiprotein complexes. Other MMPs comprise protein families of so far unknown function, which apparently are conserved between all other MTB. The *mam* and *mms* genes encode most of the MMPs and are clustered within several operons, which are part of a large, unstable genomic region constituting a putative magnetosome island. Current research is directed towards the biochemical and genetic analysis of MMP functions in magnetite biomineralization as well as their expression and localization during growth.

Keywords Magnetite biomineralization · Magnetosome membrane · Magnetotactic bacteria · "*Magnetospirillum gryphiswaldense*"

Abbreviations *MM* Magnetosome membrane · *MMP* Magnetosome membrane protein · *MTB* Magnetotactic bacteria

Introduction

Many prokaryotes are able to build intracytoplasmic "inclusions" or compartments, which in several cases display a remarkable degree of complexity and intracellular differentiation. One of the most intriguing examples are the magnetosomes of magnetotactic bacteria (MTB), which provide a navigational device for magnetotaxis by interaction of the bacteria with the Earth's magnetic field (Frankel et al. 1997). Magnetosomes are defined as intracellular, magnetic-single-domain crystals of a magnetic iron mineral that are enveloped by a membrane (Balkwill et al. 1980). Magnetosomes have been found in numerous species of aquatic prokaryotes affiliated within the α, δ and *Nitrospira* lineages of Proteobacteria. Most of them, however, have not been isolated in pure culture (Amann et al. 2000; Schüler et al. 1999). The superior crystalline and magnetic characteristics of bacterial magnetosomes make them potentially useful in a number of biotechnological applications (Safarik and Safarikova 2002; Schüler and Frankel 1999), and their characteristics have recently been considered for use as biosignatures to identify presumptive Martian magnetofossils (Thomas-Keprta et al. 2002). The biomineralization of magnetosome particles is achieved by a complex mechanism involving the uptake, accumulation, and precipitation of iron.

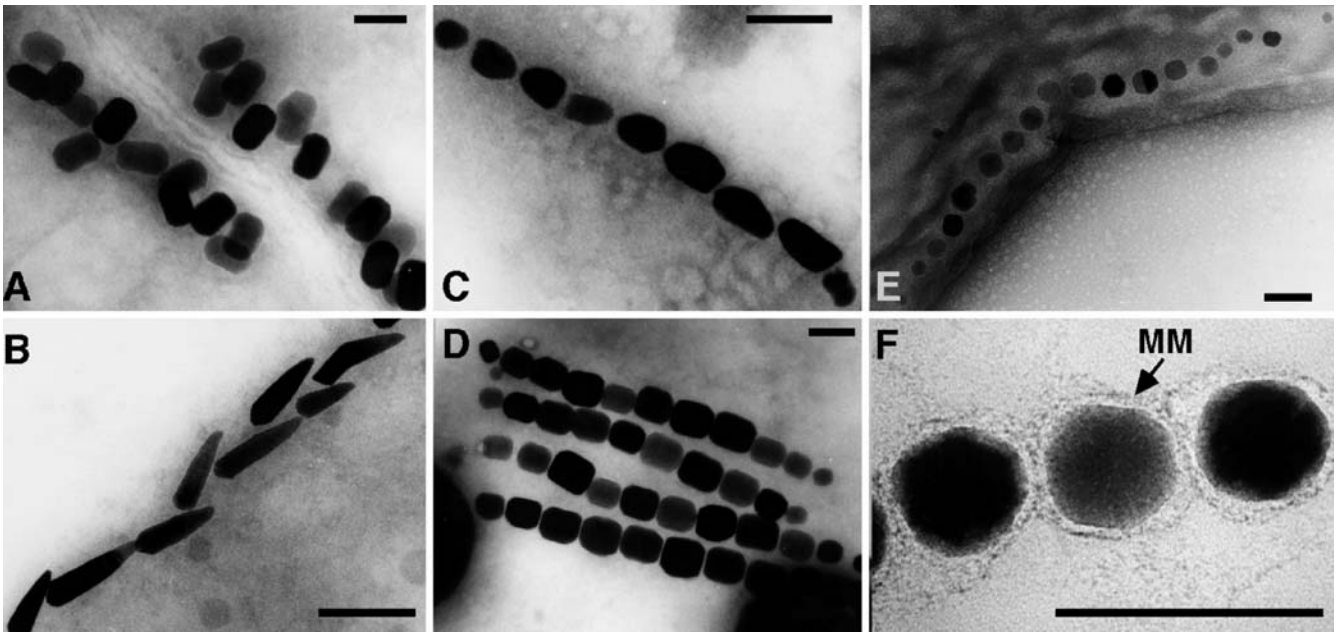

Fig. 1 A–D Electron micrographs of magnetosomes from various uncultivated magnetotactic bacteria. **E** Magnetosome chain within a cell of *Magnetospirillum gryphiswaldense*. **F** Isolated magnetosomes from *M. gryphiswaldense* showing the intact magnetosome membrane (*MM*). *Bar* 100 nm

Despite a considerable interdisciplinary interest in magnetosome biomineralization, this process was, until recently, poorly understood at the molecular and biochemical level, largely due to the lack of appropriate laboratory models. The α-proteobacterium *Magnetospirillum gryphiswaldense* (Schleifer et al. 1991) has recently emerged as a model for the investigation of magnetosome formation, as it is genetically tractable (Schultheiss and Schüler 2003) and can be readily grown by microaerobic mass cultivation (Heyen and Schüler 2003). Moreover, sequence analysis of its genome is under way. Therefore, much of the following review will focus on recent insights obtained from this organism and related *Magnetospirillum* species, which, seem to have relevance for the understanding of magnetosome biomineralization in other MTB as well.

Magnetite biomineralization

Most MTB synthesize intracytoplasmic crystals of the ferrimagnetic iron mineral magnetite (Fe_3O_4). Unlike magnetite produced in inorganic systems, the magnetosome particles are characterized by nearly perfect crystallinity and narrow size distributions that are within the magnetic-single-domain range (35–120 nm) (Moskowitz 1995). While the size and appearance of magnetic crystals are species-specific and uniform within a single cell, there is a considerable diversity of magnetosome morphologies found in different MTB (Fig. 1).

The assimilation of iron for magnetite synthesis occurs very efficiently from relatively low environmental concentrations. In *M. gryphiswaldense*, ferric iron is incorporated by a high-affinity uptake system that is saturated at extracellular iron concentrations of 15–20 μM Fe (Schüler and Bäuerlein 1996). Interestingly, the growth of a mutant that had lost the ability to form magnetosomes was more sensitive to elevated concentrations of iron (Schübbe et al. 2003). This might be indicative of a contribution of magnetite formation to iron homeostasis and detoxification of potentially harmful high intracellular levels, functions similar to those of the iron-storage proteins ferritin and bacterioferritin (Andrews et al. 2003).

The number of magnetite particles per cell is variable depending on the growth conditions. Besides the availability of micromolar amounts of iron, microoxic conditions are required for magnetite formation. Cells of *M. gryphiswaldense* are non-magnetic when grown under oxic conditions, but start to produce Fe_3O_4 when the oxygen concentration is shifted below a threshold value of 20 mbar (Schüler and Bäuerlein 1998; Heyen and Schüler 2003). There is a clear correlation between the increase in magnetosome content and a decreasing extracellular oxygen concentration, with maximum magnetite synthesis occurring at 0.25 mbar oxygen. Under those conditions, bacteria can synthesize up to 60 magnetosome particles per cell, which corresponds to an accumulation of iron up to 4% of the total dry cellular weight (Schüler and Bäuerlein 1998; Heyen and Schüler 2003).

The magnetosome membrane is a unique intracellular structure

Individual magnetite crystals are enveloped by a trilaminate structure, the magnetosome membrane (MM) (Balkwill et al. 1980). Magnetosome particles are mostly arranged in chain-like structures adjacent or in close proximity to the cytoplasmic membrane. However, connections be-

tween the MM and the cytoplasmic membrane have not been observed so far and the MM does not appear to be continuous with the cell membrane. Empty and partially filled vesicles have been seen in iron-starved cells of *Magnetospirillum magnetotacticum* and *M. gryphiswaldense* (Gorby et al. 1988; Schüler and Bäuerlein 1997). Thus, the MM likely pre-exists as an "empty" MM vesicle prior to the synthesis of the mineral phase.

Magnetite formation requires the presence of mixed-valence iron complexes in solution. Biomineralization of this material, therefore, depends on precise regulation of iron supersaturation and both the redox potential and the pH. Compartmentalization by the formation of MM vesicles enables the process of mineral formation to be controlled by biochemical means.

Biochemical composition of the magnetosome membrane

Magnetosomes can be readily isolated and purified from disrupted cells by means of magnetic separation and ultracentrifugation, and the membrane can be solubilized by hot SDS treatment or organic solvents (Grünberg et al. 2001; Schüler 2000). Isolated magnetosomes have a strong tendency to form chains, even after treatment with mild detergents, indicating that an interparticle connection mediated by MM components is involved in the organization of chains. Initial attempts to analyze the biochemical composition of the MM in *M. magnetotacticum* revealed the presence of phospholipids associated with isolated magnetosome particles as well as numerous proteins, some of which appeared to be unique to the MM (Gorby et al. 1988).

A number of common fatty acids were identified in isolated magnetosomes of *M. gryphiswaldense* (Grünberg et al. 2003). Phosphatidylethanolamine and phosphatidylglycerol are the most abundant polar lipids, whereas ornithine amide lipid and an unidentified aminolipid are less abundant in the MM compared to the fraction of lipids from the outer and cytoplasmic membranes (Bäuerlein 2000; Grünberg et al. 2003). Analysis of the extracted membrane revealed that the magnetosome is associated with a highly specific and rather complex subset of proteins present in various quantities. The amount of MM-bound polypeptides was estimated to represent approximately 0.1% of the total cellular protein (Grünberg et al. 2001). By various one- and two dimensional electrophoresis methods in combination with N-terminal and mass spectrometric sequencing techniques, between 15 and 20 major polypeptides have been identified in the MM, several of them representing post-translational modifications of the same gene product. The different resistances of magnetosome proteins to proteases and detergents indicate that some proteins are very tightly bound to the magnetosome crystals and/or embedded within the membrane. Others seem to be loosely attached and can be selectively solubilized by mild detergents (Grünberg et al. 2003). Several of the proteins contain covalently bound c-type heme as revealed by peroxidase staining. Glycoproteins, which are common constituents of other biomineralizing systems, have not been detected.

The magnetosome subproteome

Proteomic analysis together with reverse genetics, and most recently, the availability of substantial genomic data have led to identification of the genes for all major MM-associated proteins. A number of minor constituents bound to isolated magnetosomes were occasionally observed. Because they were mostly present in small amounts and represent highly abundant cellular proteins, they are probably contaminations from other subcellular compartments, although it cannot be entirely excluded that some them, for instance several detected ATPase subunits, might be associated in vivo with magnetosome function. The magnetosome subproteome of *M. gryphiswaldense* comprises at least 18 different bona-fide magnetosome membrane proteins (MMPs); their characteristics are shown in Table 1. Based on sequence analysis, most MMPs can be assigned to a number of protein families that seem to be present in all MTB and which are discussed below.

TPR proteins

MamA, which has been also identified in the MM of other *Magnetospirillum* species (Okuda et al. 1996), is an abundant protein of the MM and contains four to five copies of the tetratricopeptide repeat (TPR) motif. These motifs have been identified in a growing number of proteins with diverse functions, and they are known to mediate protein-protein interactions (Blatch and Lassle 1999). It therefore has been speculated that MamA is a receptor in the MM that interacts with cytoplasmic proteins or is involved in the assembly of multiprotein complexes within the MM (Okuda et al. 1996; Okuda and Fukumori 2001).

CDF proteins

Both MamB and MamM were identified as members of the cation diffusion facilitator (CDF) family of metal transporters, which consists of proteins that function as efflux pumps of toxic divalent cations, such as zinc, cadmium, cobalt, and other heavy-metal ions. More specifically, MamB and MamM have greatest similarity to the CDF3 subfamily, which was postulated to comprise putative iron transporters (Nies 2003). It has been speculated that MamB and MamM are involved in the magnetosome-directed uptake of iron (Grünberg et al. 2001), and preliminary evidence obtained from mutant analysis seems to support this assumption (Schüler et al., unpublished data).

HtrA-like serine proteases

MamE and MamO display sequence similarity to HtrA-like serine proteases, although they share only relatively

Table 1 Characteristics of magnetosome membrane proteins (MMPs) that have been identified in *Magnetospirillum gryphiswaldense*. *CDF* Cation diffusion facilitator, PDZ, *PHB* polyhydroxybutyric-acid

Protein	Length (amino acids)	Deduced mol. mass (kDa)/pI	Blast homologue[a]	Characteristics	Putative function
MamA	217	24.01/5.64	MM2348 *Methanosarcina mazei*	TPR motifs (relative abundance >10%)	Protein-protein interaction
MamB	297	31.96/5.25	YdfM *Bacillus subtilis*	Cation transporter (CDF)	Iron transport
MamC	125	12.40/4.88	–	Relative abundance[b] >16%	Unknown
MamD	314	30.20/9.68	–	Leu/Gly-rich motif	Unknown
MamE	772	78.00/8.69	MLL5022 *Mesorhizobium loti*	PDZ domains protease domain	Serine protease
MamF	111	12.30/9.57	–	Relative abundance >15%	Unknown
MamG	84	7.70/9.28	–	Leu/Gly-rich motif	Unknown
MamJ	466	48.51/3.80	–	Asp/Glu-rich repeats	Unknown
MamM	318	34.50/5.82	BH 1238 *Bacillus halodurans*	Cation transporter (CDF)	Unknown
MamN	437	46.14/6.70	TM0934 *Thermotoga maritima*	Membrane protein	Inorganic ion transport
MamO	632	65.40/6.51	CC1282 *Caulobacter crescentus*	PDZ domains protease domain	Serine protease
MamQ	272	30.00/8.48	LemA *T. maritima*	Membrane protein	Unknown
MamR	72	8.10/8.48	–	Hydrophilic protein	Unknown
MamS	180	18.71/7.02	–	Membrane protein	Unknown
MamT	174	18.88/10.05	–	Heme binding	Unknown
Mms6	136	14.26/9.79	–	Leu/Gly-rich motif	Iron binding
Mms16	145	16.35/5.49	Apd *Rhodospirillum rubrum*	Weakly similar to phasins	Activator of PHB depolymerase
MM22	196	20.00/7.14	*Enterococcus faecalis* V583	Membrane protein	Unknown

[a]Only hits with an *e*-value <0.01 are considered. Hits to other magnetotactic bacteria are excluded. With the exception of MM22, all MMPs have close homologues in the genome of *Magnetospirillum magnetotacticum*

[b]Relative abundances are with respect to the total amount of MM-associated protein

weak (31%) sequence similarity to each other. *mamP*, encoding a further putative serine protease, is colocated with *mamE* and *mamO* within the same operon (Fig. 2), but MamP has not been identified in the MM. HtrA-like proteins share a conserved trypsin-like protease domain and one or two PDZ domains. They act as molecular chaperones and heat-shock induced proteases, which degrade misfolded proteins in the periplasm (Clausen et al. 2002). It has been suggested that MamE and MamO are involved in magnetosome formation, perhaps by the processing, maturation, and targeting of MMPs during MM assembly (Grünberg et al. 2001).

MTB-specific protein families

Most of the identified MMPs including, for example, the most abundant MM-associated proteins MamC and MamF, have no known homologues in organisms other than MTB and thus represent members of unique, MTB-specific protein families. One feature common to several of these proteins is the presence of repetitive motifs. Examples are found in MamD, Mms6, and MamG, which share conspicuous hydrophobic sequence motifs that are rich in stretches of repeated leucine and glycine residues. These motifs display an intriguing similarity to LG-rich repetitive sequences found in silk-like (fibroin) proteins (Zurovec and Sehnal 2002), mollusk shell framework proteins (Sudo et al. 1997), as well as elastins and cartilage proteins (Bochicchio et al. 2001), which are known to have a remarkable tendency for self-aggregation and several of which are involved in other biomineralization processes. Interestingly, the small Mms6 protein was described recently in *Magnetospirillum* strain AMB-1 as a tightly bound constituent of the MM that exhibited iron-binding activity and had an effect on the morphology of growing magnetite crystals in vitro (Arakaki et al. 2003).

An additional sequence pattern with potential relevance for magnetite biomineralization is found in MamJ. This protein, which displays extensive self-similarity, is particularly rich (18.7%) in repeats of the acidic amino acid residues glutamate and aspartate. A number of additional conspicuous proteins with highly repetitive and/or acidic sequence motifs can be deduced from the genome assemblies of *M. gryphiswaldense* and other MTB. Clusters of acidic groups are commonly found in biomineralizing systems, such as in mollusk shells (Bäuerlein 2003; Gotliv et al. 2003). Acidic groups have a strong affinity for metal ions and are involved in the initiation of crystal nucleation by binding of metal ligands. MamJ and other

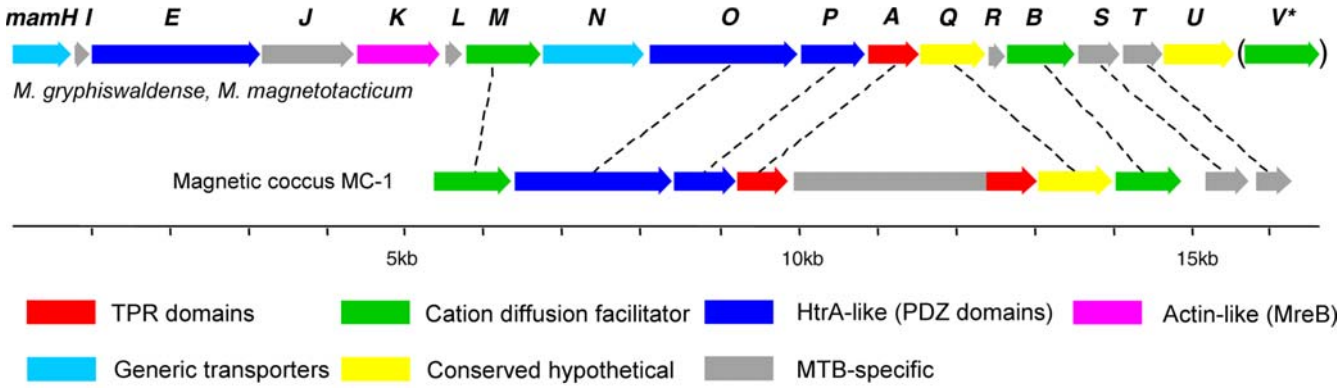

Fig. 2 Molecular organization of the *mamAB* gene cluster in *M. gryphiswaldense*, *M. magnetotacticum*, and the magnetic coccus strain MC-1. *Colors* indicate similarity of the deduced proteins to functionally characterized protein families, conserved hypothetical proteins in non-magnetic organisms, or lack of similarity to known proteins (MTB-specific). The *dashed lines* connect equivalent genes (closest orthologues). **mamV* is present in the genome assembly of *M. magnetotacticum*, but absent from the homologous region in *M. gryphiswaldense*

acidic or repetitive proteins are therefore candidates for a function in magnetite biomineralization by providing local supersaturation of iron.

Other proteins that were previously reported to be associated with isolated magnetosomes in the closely related *Magnetospirillum* strain AMB-1 are MpsA and MagA (Matsunaga et al. 2000; Nakamura et al. 1995). Although equivalent genes are present in the genome of *M. gryphiswaldense*, they are not part of the putative magnetosome island, and an extensive analysis did not reveal their presence in the MM. Thus, it must be considered that neither MpsA and MagA are universal constituents of the MM. Alternatively, their co-purification with the magnetosomes may have been accidental, resulting from unspecific binding during magnetosome preparation.

This may be also true for Mms16, which has been detected in isolated magnetosomes of *M. gryphiswaldense* and *Magnetospirillum* strain AMB-1. Because of its observed GTPase activity in vitro, Mms16 was suggested to be involved in the formation of MM vesicles (Okamura et al. 2001). However, it turns out that Mms16 has striking similarity to Apd, an activator of polyhydroxybutyric-acid depolymerization in *Rhodospirillum rubrum*, and the protein is able to substitute Apd function in vitro (Handrick et al. 2003). Hence, its function in *Magnetospirillum* species seems to be independent of magnetosome formation, and its in vivo association with the MM requires further clarification.

How are MMPs targeted to the membrane during magnetosome assembly?

With the notion of the considerable complexity of the MM, several questions arise. How is such a macromolecular structure assembled, and how are the MMPs expressed and targeted to their proper subcellular location? Preliminary results indicate that the expression of MMPs is not strictly coupled to the formation of complete magnetosomes (Schüler et al., unpublished data). Proteomic analysis revealed indications for the post-translational cleavage of several of the MMPs. However, so far, no sequence motifs or sorting signals universal to MMPs have been identified. While many MMPs display the characteristics of typical membrane proteins, others appear to be rather hydrophilic with a predicted cytoplasmic localization. This means that binding of MMPs cannot be only by hydrophobic interactions, but for some MMPs may involve other types of interactions, such as protein-protein interactions or direct interaction with the mineral surface of magnetite crystals. Interestingly, several MMPs contain PDZ and TPR domains, which are known to mediate protein-protein interactions, act as scaffolding proteins, and typically coordinate the assembly of proteins into multisubunit complexes at particular subcellular locations (Blatch and Lassle 1999; Chung et al. 2002; Nourry et al. 2003; Sheng and Sala 2001). It could be envisioned that the organization around a PDZ- or TPR-based scaffold may allow the stable localization of interacting proteins during magnetosome assembly. Additional experiments, such as in situ localization studies, are of importance to follow the expression and targeting of MMPs and magnetosome assembly during growth. With the availability of in vivo genetic technology, gene fusions of MMPs coupled to, e.g., green fluorescence protein (GFP) have already proven to be an extremely powerful tool to address these questions (Schüler et al., unpublished data).

Genes encoding MMPs are organized within a putative magnetosome island

With the exception of *mm22* and *mms16,* most of the genes encoding MMPs are encoded within a single genomic region. This region, the magnetosome island, was functionally linked to magnetosome synthesis in a nonmagnetic mutant strain harboring a large chromosomal deletion (Schübbe et al. 2003). Magnetosome genes are colocated in three different operons, which are linked within less than 35 kb in the genome *of M. gryphiswaldense*.

The *mamAB* cluster extends over 16.5 kb and comprises 17 consecutive, colinear genes that were assigned

mamH–mamU (mam for magnetosome membrane). As can be inferred from the available genome data of different MTB (http://www.jgi.doe.gov/tempweb/JGI_microbial/html/index.html), the gene order and amino acid sequences of the predicted Mam proteins are highly conserved, even in the remotely related magnetic coccus strain MC-1 (Fig. 2). The putative 2.7-kb *mms6* operon (*mms* for magnetic particle membrane-specific protein; Okamura et al. 2001) consists of four genes and is located 15 kb upstream from the left border of the *mamAB* cluster, while the *mamGFDC* operon is located 9.2 kb upstream of the *mamAB* cluster and extends over 2.1 kb. Remarkably, the regions within, adjacent, and between these clusters contain many ORFs that have close homologues in the genomes of *M. magnetotacticum* MS-1 and strain MC-1 but yield no database hits to nonmagnetic organisms. Hence, they can be considered MTB-specific and are likely to be involved in magnetosome formation. Other deduced gene products are predicted to have related functions based on sequence similarity to functionally characterized proteins. For example, *mamK* of the *mamAB* cluster displays extensive similarity to a gene (*mreB)* encoding an actin-like cytoskeletal protein (Jones et al. 2001). So far, it is not clear how the magnetosome chain is positioned within the cell. However, it seems unlikely that the individual particles are free to rotate within the cytoplasm; instead, some sort of mechanical anchoring to the cell envelope must exist. It is therefore tempting to speculate that MamK is associated with the formation of a cytoskeletal "superstructure" involved in the organization and segregation of magnetosome chains.

A further intriguing feature of this region is the presence of numerous genes encoding mobile DNA elements, such as insertion sequence elements and integrases. In total, these genes represent more than 14% of the coding sequence. The presence of this high number of mobile elements may account for the observed genetic instability of this region under conditions of stationary growth (Schübbe et al. 2003). In summary, all these features are strongly reminiscent of those described for genome islands in other bacteria (Finan 2002; Hacker and Kaper 2000). Genome islands usually comprise large genomic regions, which, for instance, are present as pathogenicity islands (PAIs) in the genomes of pathogenic strains but absent from the genomes of nonpathogenic members of the same or related species. They often encode "accessory" gene functions, tend to be genetically unstable, and can transfer horizontally. Thus, it seems plausible that most of the gene functions required for magnetite synthesis are organized within a large genomic magnetosome island that may have been distributed by lateral gene transfer.

Concluding remarks

The molecular and genetic basis of magnetosome formation remained elusive for many years following Richard Blakemore's discovery of MTB (Blakemore 1975), but our understanding is finally beginning to improve. Nonetheless, the emerging picture is that the genetic and biochemical control of bacterial magnetosome biomineralization is complex and there are many questions that remain to be answered. For instance, there is an urgent need for functional characterization of the identified magnetosome constituents by biochemical studies and mutational analysis. This will be of particular interest with respect to the interaction of biomineralization proteins in the transport, binding, and nucleation of iron and their involvement in the physico-chemical control of crystal growth. This necessarily will also include in vitro approaches to reconstitute magnetite biomineralization based on purified individual components. A further intriguing question is how a complex structure like the bacterial MM is organized and assembled during the cell cycle, and how a highly specific subset of proteins is targeted into the MM. Finally, another fascinating problem to be solved is how the structure and composition of the MM at the molecular level controls the great diversity in size, structure, and shape of magnetite crystals found in many, so far mostly uncultivated MTB (Fig. 1 A–D). An in-depth understanding of the biomineralization mechanism could have immediate biotechnological relevance with respect to the tailoring of magnetic nanoparticles with desired structural and magnetic characteristics using biomimetic approaches.

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References

Amann R, Rossello-Mora R, Schüler D (2000) Phylogeny and *in situ* identification of magnetotactic bacteria. In *Biomineralization*, pp. 47–60. Edited by E Baeuerlein. Weinheim: Wiley-VCH

Andrews SC, Robinson AK, Rodriguez-Quinones F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27: 215–37

Arakaki A, Webb J, Matsunaga T (2003) A novel protein tightly bound to bacterial magnetic particles in *Magnetospirillum magneticum* strain AMB-1. J Biol Chem 278:8745–50

Balkwill D, Maratea D, BlakemoreR (1980) Ultrastructure of a magnetotactic spirillum. J Bacteriol 141:1399–1408

Bäuerlein E (2000) Single magnetic crystals of magnetite (Fe_3O_4) synthesized in intracytoplasmic vesicles of *Magnetospirillum gryphiswaldense*. In: Baeuerlein E (ed) Biomineralization. Wiley-VCH, Weinheim, pp 61–80

Bäuerlein E (2003) Biomineralization of unicellular organisms: an unusual membrane biochemistry for the production of inorganic nano- and microstructures. Angew Chem Int Ed Engl 42:614–41

Blakemore R (1975) Magnetotactic bacteria. Science 190:377–9

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21:932–9

Bochicchio B, Pepe A, Tamburro AM (2001) On (GGLGY) synthetic repeating sequences of lamprin and analogous sequences. Matrix Biol 20:243–50

Chung JJ, Shikano S, Hanyu Y, Li M (2002) Functional diversity of protein C-termini: more than zipcoding? Trends Cell Biol 12:146–50

Clausen T, Southan C, Ehrmann M (2002) The HtrA family of proteases: implications for protein composition and cell fate. Mol Cell 10:443–55

Finan T (2002) Evolving insights: symbiosis islands and horizontal gene transfer. J Bacteriol 184:2855–2856

Frankel RB, Bazylinski DA, Johnson MS, Taylor BL (1997) Magneto-aerotaxis in marine coccoid bacteria. Biophys J 73:994–1000

Gorby YA, Beveridge TJ, Blakemore RP (1988) Characterization of the bacterial magnetosome membrane. J Bacteriol 170:834–841

Gotliv BA, Addadi L, Weiner S (2003) Mollusk shell acidic proteins: in search of individual functions. Chembiochem 4:522–9

Grünberg K, Wawer C, Tebo BM, Schüler D (2001) A large gene cluster encoding several magnetosome proteins is conserved in different species of magnetotactic bacteria. Appl Environ Microbiol 67:4573–82

Grünberg K, Müller EC, Otto A, Reszka R, Linder D, Kube M, Reinhardt R, Schüler D (2003) Biochemical and proteomic analysis of the magnetosome membrane in *Magnetospirillum gryphiswaldense*. Appl Environ Microbiol (in press)

Hacker J, Kaper JB (2000) Pathogenicity islands and the evolution of microbes. Annu Rev Microbiol 54:641–79

Handrick R, Reinhardt S, Schultheiss D, Reichart T, Schüler D, Jendrossek D (2003) Unraveling the function of the *Rhodospirillum rubrum* activator of polyhydroxybutyrate (PHB) degradation: the activator is a PHB granule bound protein (phasin). (in press)

Heyen U, Schüler D (2003) Growth and magnetosome formation by microaerophilic *Magnetospirillum* strains in an oxygen-controlled fermentor. Appl Microbiol Biotechnol 61:536–544

Jones LJ, Carballido-Lopez R, Errington J (2001) Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. Cell 104:913–22

Matsunaga T, Tsujimura N, Okamura H, Takeyama H (2000) Cloning and characterization of a gene, *mpsA*, encoding a protein associated with intracellular magnetic particles from *Magnetospirillum* sp. strain AMB-1. Biochem Biophys Res Communications 268:932–937

Moskowitz BM (1995) Biomineralization of magnetic minerals. Rev Geophysics 33:123–128

Nakamura C, Kikuchi T, Burgess JG, Matsunaga T (1995) Iron-regulated expression and membrane localization of the MagA protein in *Magnetospirillum s*p. strain AMB-1. J Biochemistry 118:23–27

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27:313–39

Nourry C, Grant SG, Borg JP (2003) PDZ domain proteins: plug and play! Sci STKE 2003:RE7

Okamura Y, Takeyama H, Matsunaga T (2001) A magnetosome-specific GTPase from the magnetic bacterium *Magnetospirillum magneticum* AMB-1. J Biol Chem 276:48183–8

Okuda Y, Fukumori Y (2001) Expression and characterization of a magnetosome-associated protein, TPR-containing MAM22, in *Escherichia coli*. FEBS Lett 491:169–73

Okuda Y, Denda K, Fukumori Y (1996) Cloning and sequencing of a gene encoding a new member of the tetratricopeptide protein family from magnetosomes of *Magnetospirillum magnetotacticum*. Gene 171:99–102

Safarik I, Safarikova M (2002) Magnetic nanoparticles and biosciences. Monatsheft Chemie 133:737–759

Schleifer K, Schüler D, Spring S, Weizenegger M, Amann R, Ludwig W, Köhler M (1991) The genus *Magnetospirillum* gen. nov., description of *Magnetospirillum gryphiswaldense* sp. nov. and transfer of *Aquaspirillum magnetotacticum* to *Magnetospirillum magnetotacticum* comb. nov. System Appl Microbiol 14:379–385

Schübbe S, Kube M, Scheffel A, Wawer C, Heyen U, Meyerdierks A, Madkour M, Mayer F, Reinhardt R, Schüler D (2003) Characterization of a spontaneous nonmagnetic mutant of *Magnetospirillum gryphiswaldense* reveals a large deletion comprising a putative magnetosome island. J Bact 185: 5779–5790

Schüler D (2000) Characterization of the magnetosome membrane in *Magnetospirillum gryphiswaldense*. In *Biomineralization*, pp. 109–118. Edited by E Baeuerlein. Weinheim: Wiley-VCH

Schüler D, Baeuerlein E (1996) Iron-limited growth and kinetics of iron uptake in *Magnetospirillum gryphiswaldense*. Arch Microbiol 166:301–307

Schüler D, Baeuerlein E (1997) Iron transport and magnetite crystal formation of the magnetic bacterium *Magnetospirillum gryphiswaldense*. J Phys IV 7: 647–650

Schüler D, Baeuerlein E (1998) Dynamics of iron uptake and Fe_3O_4 biomineralization during aerobic and microaerobic growth of *Magnetospirillum gryphiswaldense*. J Bacteriol 180:159–162

Schüler D, Frankel RB (1999) Bacterial magnetosomes: Microbiology, biomineralization and biotechnological applications. Appl Microbiol Biotechnol 52: 464–473

Schüler D, Spring S, Bazylinski DA (1999) Improved technique for the isolation of magnetotactic spirilla from a freshwater sediment and their phylogenetic characterization. System Appl Microbiol 22:466–471

Schultheiss D, Schüler D (2003) Development of a genetic system for *Magnetospirillum gryphiswaldense*. Arch Microbiol 179:89–94

Sheng M, Sala C (2001) PDZ domains and the organization of supramolecular complexes. Annu Rev Neurosci 24: 1–29

Sudo S, Fujikawa T, Nagakura T, Ohkubo T, Sakaguchi K, Tanaka M, Nakashima K, Takahashi T (1997) Structures of mollusc shell framework proteins. Nature 387:563–4

Thomas-Keprta KL, Clemett SJ, Bazylinski DA, Kirschvink JL, McKay DS, Wentworth SJ, Vali H, Gibson EK Jr, Romanek CS (2002) Magnetofossils from ancient Mars: a robust biosignature in the martian meteorite ALH84001. Appl Env Microbiol 68: 3663–72

Zurovec M, Sehnal F (2002) Unique molecular architecture of silk fibroin in the waxmoth, *Galleria mellonella*. J Biol Chem 277:22639–47