REVIEW ARTICLE

Genetics and cell biology of magnetosome formation in magnetotactic bacteria

Dirk Schüler

Faculty of Biology, Microbiology, Ludwig Maximilians University, München, Germany

Correspondence: Dirk Schüler, Faculty of Biology, Microbiology, Ludwig Maximilians University, Maria-Ward-Str. 1a, D-80638 München, Germany. Tel.: +49 0 89 2180 6126; fax: +49 0 89 2180 6127; e-mail: dirk.schueler@lrz.uni-muenchen.de

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Abstract

The ability of magnetotactic bacteria (MTB) to orient in magnetic fields is based on the synthesis of magnetosomes, which are unique prokaryotic organelles comprising membrane-enveloped, nano-sized crystals of a magnetic iron mineral that are aligned in well-ordered intracellular chains. Magnetosome crystals have species-specific morphologies, sizes, and arrangements. The magnetosome membrane, which originates from the cytoplasmic membrane by invagination, represents a distinct subcellular compartment and has a unique biochemical composition. The roughly 20 magnetosome-specific proteins have functions in vesicle formation, magnetosomal iron transport, and the control of crystallization and intracellular arrangement of magnetite particles. The assembly of magnetosome chains is under genetic control and involves the action of an acidic protein that links magnetosomes to a novel cytoskeletal structure, presumably formed by a specific actin-like protein. A total of 28 conserved genes present in various magnetic bacteria were identified to be specifically associated with the magnetotactic phenotype, most of which are located in the genomic magnetosome island. The unique properties of magnetosomes attracted broad interdisciplinary interest, and MTB have recently emerged as a model to study prokaryotic organelle formation and evolution.

Introduction

Magnetosomes are specialized organelles synthesized by magnetotactic bacteria (MTB) for direction sensing along the geomagnetic field. The magnetosomes comprise membrane-enveloped, nano-sized crystals of a magnetic iron mineral, which are arranged in one or multiple chain-like structures that enable the cell to passively align along external magnetic fields, a behavior known as 'magnetotaxis' (Blakemore, 1975). Magnetotaxis, in combination with chemotaxis, aerotaxis, and perhaps phototaxis, is thought to direct the swimming of cells toward growth-favoring microoxic zones on the bottom of chemically stratified natural waters (Frankel *et al.*, 2006). Synthesis of the bacterial magnetosome chain is achieved by a remarkable degree of control over the biomineralization of perfectly shaped mineral crystals, which are then assembled into a highly ordered chain-like structure. Magnetosome formation is a fascinating example of how simple organisms can generate complex inorganic structures from genetic blueprint information encoded in the genome. Magnetosome crystals have species-specific morphologies, sizes, and arrangements. Their unique properties have attracted a broad interdisciplinary interest and might be exploited for a variety of applications in diverse disciplines from geobiology to biotechnology. For instance, the molecular mechanisms governing the formation of magnetosome chains are of great interest for the generation of bioinspired materials, such as functionalized magnetic nanoparticles and nanotubes (Banerjee *et al.*, 2005; Ceyhan *et al.*, 2006; Lang & Schüler, 2006; Lisy *et al.*, 2007; Matsunaga *et al.*, 2007; Wacker *et al.*, 2007), and magnetosomes have been even suggested as biomarkers to detect extraterrestrial life (Frankel & Buseck, 2000; Thomas-Keprta *et al.*, 2002; Arato *et al.*, 2005).

Since the first description of magneto-responsive prokaryotes as 'magnetotactic bacteria' (MTB) by Blakemore (1975), the knowledge on MTB and magnetotaxis has been summarized in a number of excellent review articles (e.g. Blakemore, 1982; Mann *et al.*, 1990b; Schüler & Frankel, 1999; Bazylinski & Frankel, 2004; Frankel & Bazylinski, 2004

and others). For example, for a comprehensive coverage of the subject the reader is referred to a recent monograph covering all aspects such as the microbiology, magnetotactic orientation, geobiology, phylogeny, mineralogy, and applications of MTB and magnetosomes (Schüler, 2006). The present review is intended to highlight the remarkable advances especially in the understanding of cell biology and molecular genetics of magnetosome synthesis that has been seen within the past decade.

Characteristics of MTB

All known MTB form multiple magnetosome particles and are motile by means of flagella, but they are diverse with respect to morphology, physiology, and phylogeny. Magnetosome formation has been found in several distinct phylogenetic lineages of Gram-negative bacteria (Amann *et al.*, 2006). Most known MTB are affiliated with the *Alphaproteobacteria*, but magnetosome-like inclusions and magnetic orientation have also been described for some representatives of the *Deltaproteobacteria* (DeLong *et al.*, 1993; Kawaguchi *et al.*, 1995; Posfai *et al.*, 2006; Simmons & Edwards, 2007), *Gammaproteobacteria* (Simmons *et al.*, 2004, 2006), and the *Nitrospira* phylum (Spring *et al.*, 1994; Flies *et al.*, 2005). Common morphotypes include coccoid cells as well as rods, vibrios, and spirilla (Blakemore, 1975, 1982; Vali *et al.*, 1987; Spring *et al.*, 1994; Schüler, 1999, 2000; Simmons *et al.*, 2004; Flies *et al.*, 2005). Some unusual morphologies were observed among as-yet uncultivated MTB, including the large magnetotactic rod '*Magnetobacterium bavaricum*' containing up to nearly 1000 magnetosome particles per cell (Vali *et al.*, 1987; Spring *et al.*, 1993, 1992), and a multicellular magnetotactic prokaryote (Farina *et al.*, 1990; Mann *et al.*, 1990b; Abreu *et al.*, 2007). Despite their high abundance and ubiquitous occurrence in many marine and freshwater habitats, most MTB are difficult to isolate and cultivate in the laboratory, which is probably due to their lifestyle that is adapted to complex chemical gradients typically encountered in stratified sediments. Only a limited number of strains have been isolated in pure culture (Schüler & Bazylinski, 2007). All known MTB have a solely respiratory form of metabolism, can fix nitrogen, and are mesophilic with respect to pH and temperature requirements (Bazylinski *et al.*, 2000; Bazylinski & Williams, 2006). Most magnetotactic isolates are *Alphaproteobacteria*, which grow either microaerobically using oxygen, or anaerobically using nitrate or nitrous oxide as electron acceptors, whereas the magnetite-forming Deltaproteobacterium *Desulfovibrio magneticus* grows anaerobically by reduction of fumarate or sulfate (Sakaguchi *et al.*, 2002). Many MTB metabolize short-chained organic acids, but several MTB have been recently shown to be capable of chemo-lithoautotrophic growth (Bazylinski *et al.*, 2004; Bazylinski & Williams, 2006; Williams *et al.*, 2006; Schüler & Bazylinski, 2007). Although cultivation of fastidious MTB has been considered notoriously difficult in the past, protocols for mass cultivation and genetic manipulation have been recently advised for several strains, which greatly facilitated their physiological and molecular analysis.

Magnetosomes

Magnetic crystals

The term 'magnetosomes' was coined for the membrane-enveloped magnetic crystals found in *Magnetospirillum* (*Aquaspirillum*) *magnetotacticum* by Richard Blakemore *et al.* (Balkwill *et al.*, 1980). It has been realized since then that bacterial magnetosomes represent true prokaryotic membrane-bound organelles, displaying a comparable degree of complexity as their eukaryotic counterparts (Komeili *et al.*, 2004). All MTB synthesize ferrimagnetic crystals of either magnetite (Fe_3O_4) or the iron sulfide greigite (Fe_3S_4) (Frankel *et al.*, 1983; Rodgers *et al.*, 1990; Bazylinski *et al.*, 1993, 1990; Moskowitz *et al.*, 1993; Lins & Farina, 2001; Posfai *et al.*, 2001, 1998a, b). As none of the magnetotactic iron sulfide producers has been isolated in pure culture, only MTB of the magnetite type will be further considered in this review.

The size, morphology, and chemical composition of magnetite crystals are subject to a species-specific genetic control. While crystals are uniform within a single species, their different MTB display a considerable diversity with respect to magnetosome morphologies, which are mostly unknown from magnetite particles formed by chemical synthesis (Bazylinski *et al.*, 1994; Devouard *et al.*, 1998; Thomas-Keprta *et al.*, 2001; Faivre *et al.*, 2004; Arato *et al.*, 2005) (Fig. 1).

Mature magnetite crystals produced by MTB typically fall within the stable single magnetic domain range between 30 and 120 nm (Stolz *et al.*, 1986; Moskowitz *et al.*, 1988; Bazylinski & Frankel, 2004), and thus are of the optimum size for magnetotaxis. While crystals of this size range are permanently magnetic, smaller sizes would not efficiently contribute to the cellular magnetic moment, as those crystals are superparamagnetic at ambient temperatures, which means they do not show persistent magnetization. On the contrary, in crystals larger than 120 nm, multiple magnetic domains of opposite magnetic orientation can be formed, which reduces the total magnetic remanence of the crystal.

The magnetosome membrane (MM)

Structure

Similar to eukaryotic organelles, magnetosomes consist of a lipid bilayer membrane, which represents a third

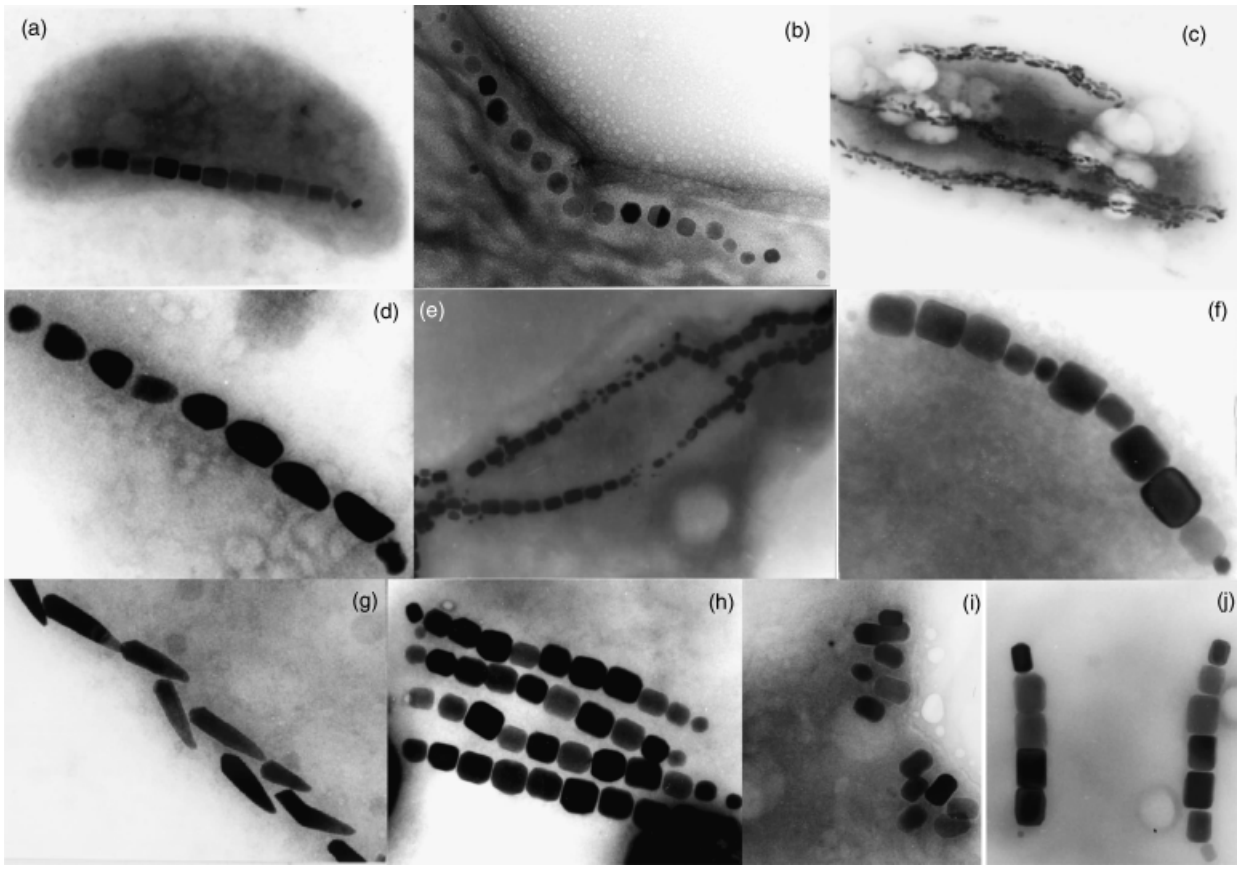

Fig. 1. Diversity of magnetosome crystals and arrangements in various MTB. Characteristic crystal habits found in various MTB are elongated prisms (a, e, f, h, i, j), cubo-octahedral (b), and bullet-shaped morphologies (c, d, g). Crystals can be arranged in single or multiple chains.

membranous compartment in addition to the outer membrane (OM) and cytoplasmic membrane (CM) (Gorby *et al*., 1988; Komeili, 2007). The MM has been studied at the structural and biochemical level only in strains of *Magnetospirillum*, but it can be assumed that similar structures are also present in other MTB. The MM can be most readily seen by electron microscopy of thin sections or cryo-electron tomographs as vesicular structures that are empty or partially filled by tiny immature crystallites of magnetite in iron-starved or 'premagnetic' cells (Gorby *et al*., 1988; Komeili *et al*., 2004; Scheffel *et al*., 2006) (Fig. 2). These structures are intimately attached to the CM, from which they seem to originate (Komeili *et al*., 2006). This has led to the assumption that MMs are closed vesicles that form before magnetite formation (Gorby *et al*., 1988; Komeili *et al*., 2004) and serve as a 'nanoreactor', in which conditions for magnetite synthesis can be strictly controlled. This notion has been partially challenged by the recent observation that some growing magnetite crystals in *Magnetospirillum magneticum* have been found to be associated with invaginations of the CM (Komeili *et al*., 2006) (Fig. 2c), which seemed to be extensions contiguous with the periplasmic space (Komeili, 2007). However, it is not clear if these invaginations remain attached with the CM as they assemble into a chain (Scheffel *et al*., 2006). On the other hand, the need of control of physicochemical conditions favoring magnetite synthesis, such as solution chemistry, redox, and pH control and supersaturation, is not consistent with a free chemical exchange between MM and periplasm.

Compartmentalization and sequestration might be achieved by a barrier, such as a transport protein, channeling iron directly from the periplasm into the magnetosome vesicles. The latter notion would be consistent with the results of a recent study on iron metabolism of *Magnetospirillum gryphiswaldense* by Mößbauer spectroscopy, which suggested that iron required to build magnetite crystals is processed directly to the MM without previous iron transport and accumulation via the cytoplasm. According to this scenario, nucleation of small crystallites predominantly occurs at the CM, and further growth proceeds after vesicles are detached from the CM (Faivre *et al*., 2007a).

Apparently, magnetite biomineralization is not limited by the number of MM vesicles, as studies in *M. gryphiswaldense*

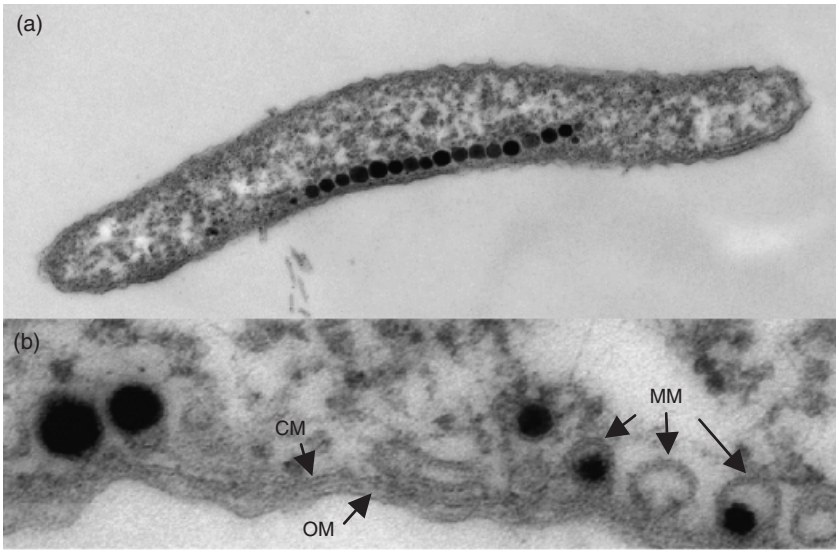

Fig. 2. Structure of the MM (CM, OM). (a) Transmission electron micrograph of a thin-sectioned cell of *Magnetospirillum gryphiswaldense* displaying the chain of membrane-enveloped magnetite crystals. (b) Section of an *M. gryphiswaldense* cell showing empty and partially filled MM vesicles (a and b by G. Wanner, LMU München). (c) Invaginated MMs of *M. magneticum*, containing magnetite crystals at different growth stages (micrographs courtesy of A. Komeili, UC Berkeley, and *Science* magazine).

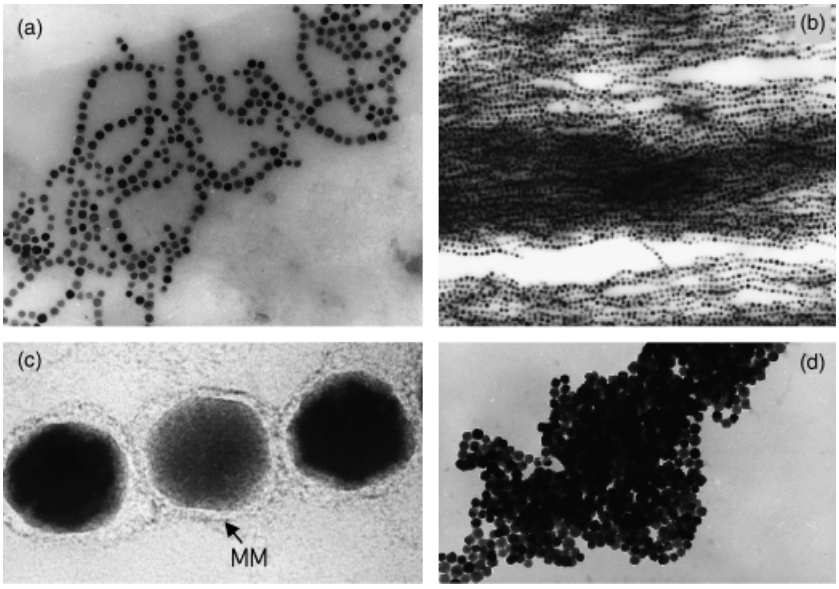

Fig. 3. Isolated magnetosomes from *Magnetospirillum gryphiswaldense*. The magnetite particles tend to form chains (a, b) and are enveloped by the intact MM (c), which can be removed by detergents (e.g. sodium dodecyl sulfate), resulting in the agglomeration of naked magnetite crystals as shown in (d).

suggest that the number of empty MM vesicles apparently exceeds the maximum number of mature crystals (Scheffel *et al.*, 2006). It has been postulated by Komeili *et al.* (2004) that magnetosome vesicles require 'activation' by an MM constituent, possibly the MM-associated tetratricopeptide repeat (TPR) MamA protein. However, the putative mechanism of this suspected activation has remained unclear.

Biochemical composition of the MM

Magnetosome crystals, which are enveloped by the intact MM, can be isolated from disrupted cells by sucrose cushion ultracentrifugation and magnetic separation (Schüler, 2000; Grünberg *et al.*, 2001, 2004) (Fig. 3). Isolated MMs of *M. gryphiswaldense* were found to contain a set of phospholipids resembling the lipid composition of the CM, although

the individual constituents are present in different amounts between the two compartments. In contrast, the protein pattern of solubilized MMs is very distinct compared with other subcellular compartments (Gorby *et al.*, 1988; Grünberg *et al.*, 2001, 2004; Tanaka *et al.*, 2006). Proteomic analysis revealed that the MM of *M. gryphiswaldense* is associated with a specific set of *c.* 20 proteins present in various amounts (Grünberg *et al.*, 2001, 2004; Schüler, 2004a). Similar studies in *M. magneticum* and *M. magnetotacticum* suggested that most magnetosome proteins are shared by different magnetospirilla (Tanaka *et al.*, 2006), and probably other MTB. To some extent, technical variations might account for the observed differences in MM protein profiles between different species, and some of the proteins that were occasionally found attached to isolated particles were then shown to be contaminants from other subcellular compartments (Grünberg *et al.*, 2004; Handrick *et al.*, 2004; Schüler, 2004a; Schultheiss *et al.*, 2005).

The identified magnetosome-specific proteins are thought to have functions in protein–protein interactions, magnetosomal iron transport, magnetite crystallization, and activation of magnetosomes (for a compilation of identified magnetosome proteins in *M. gryphiswaldense* and other MTB, see Richter *et al.*, 2007). Only a few magnetosome proteins were characterized experimentally with respect to their functions, as will be described further below.

Specific localization and targeting of magnetosome proteins

The complexity and specificity of the MM subproteome raises questions of how such a macromolecular structure is assembled, and how the magnetosome-associated proteins are expressed and targeted to their proper subcellular location? Preliminary results of green fluorescent protein-based localization experiments have indicated that their expression and targeting is not strictly coupled to the formation of intact magnetosomes (D. Schüler *et al.*, unpublished data). Proteomic analysis revealed indications for the posttranslational cleavage of several of the magnetosome proteins. However, so far no sequence motifs or sorting signals universal to all proteins have been identified. While most magnetosome proteins display the characteristics of typical membrane proteins, others appear to be rather hydrophilic with a predicted cytoplasmic localization, meaning that their MM association may involve other types of interactions, such as protein–protein interactions or direct interaction with the mineral surface of magnetite crystals. Interestingly, several magnetosome proteins contain PDZ and TPR domains, which in other organisms 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 (Grünberg *et al.*, 2004; Schüler, 2004b). It might be speculated that the organization around a PDZ- or TPR-based scaffold may thus facilitate the targeting of interacting proteins during magnetosome assembly, which, however, remains to be demonstrated experimentally.

Biomineralization of magnetite crystals

Uptake and metabolism of iron

General characteristics

The biomineralization of more than 100 magnetite (Fe_3O_4) crystals relies on the uptake and transport of large amounts of iron, which can reach an intracellular accumulation of more than 4% of the dry weight. Both ferric and ferrous iron can be taken up actively from micromolar concentrations (Schüler & Baeuerlein, 1996; Faivre *et al.*, 2007a), and the intracellular pathway for uptake and sequestration then has to be strictly controlled because of the potentially harmful effect of free intracellular iron levels (Imlay, 2003). It has been shown that iron uptake is regulated and coupled to magnetite synthesis in *M. gryphiswaldense* (Schüler & Baeuerlein, 1996, 1998). The general iron metabolism in MTB is poorly understood at the molecular level. However, 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 siderophores, are present in MTB, although their significance for magnetite biomineralization is not fully understood (Bertani *et al.*, 1997; Dubbels *et al.*, 2004; Suzuki *et al.*, 2006, 2007). It has been discussed that magnetite synthesis has to be integrated with the general and biochemical iron metabolism (Dubbels *et al.*, 2004; Suzuki *et al.*, 2006). On the other hand, there are some indications that uptake and intracellular processing of iron for magnetite synthesis proceeds through a distinct pathway, possibly through membrane-associated precursors involving a ferrous high-spin compound and a ferritin-like compound (Faivre *et al.*, 2007a), which has not been characterized yet at the genetic and biochemical level.

The periplasmic ChpA protein is involved in iron uptake for magnetite synthesis in strain MV-1

In the marine magnetotactic vibrio MV-1, a major copper-containing periplasmic protein (ChpA) was found to be involved in iron uptake. Spontaneous nonmagnetic mutants were isolated, which failed to express this protein due to two point mutations in the *chpA* gene. ChpA (copper-handling protein) of MV-1 forms a homodimer with an apparent subunit mass of about 19 kDa and is iron regulated (Dubbels *et al.*, 2004). Two potential 'copper-handling' motifs (MXM/

MX2M), as well as an MX3M motif, are present in its amino acid sequence, and the native protein bound copper in a 1 : 1 ratio. These features are characteristic of copper transport proteins, the best characterized of which is Ctr1p, an integral plasma membrane component of a copper-dependent, high-affinity iron transport system in the yeast *Saccharomyces cerevisiae*. It was hypothesized by Dubbels *et al.* (2004) that ChpA is part of a three-component iron uptake system, which resembles this copper-dependent high-affinity iron uptake system in *S. cerevisiae* including an iron permease and an Fe(II) oxidase. Given its cellular location and the fact that it binds copper, ChpA might have a similar copper transport function as the Ctr1 protein in *S. cerevisiae*, providing copper to an Fe(II) oxidase for the formation of an active oxidase–permease complex for the transport of iron(III) across the cell membrane. The *chpA* mutant continued to synthesize a hydroxamate type of siderophore but differed in the patterns of putative siderophore production (Dubbels *et al.*, 2004; Bazylinski & Williams, 2006). Whereas wild-type MV-1 produces the highest levels of siderophore between initial media iron concentrations of about 8–28 μM, siderophore production by the *chpA* mutant was maximum at iron concentrations of $< 8\,\mu M$ (Dubbels *et al.*, 2004; Bazylinski & Williams, 2006). Homologs of *chpA* are present not only in the genomes of many nonmagnetic bacteria but also in other magnetospirilla, suggesting that a similar pathyway might also be effective in other MTB.

Iron transport into the magnetosomes

Specific routes for iron uptake into the magnetosomes are likely to be used for accumulation of supersaturating quantities of iron into the magnetosomes. Previous genetic and biochemical studies suggested that the MagA protein may play a role in magnetosomal iron uptake in *M. magneticum* (Nakamura *et al.*, 1995). However, these experiments left some ambiguity because of the lack of genetic complementation and have not been verified in other MTB so far. In *M. gryphiswaldense*, proteomic analysis identified two abundant putative transport proteins MamB and MamM, which are encoded within the *mamAB* operon (Grünberg *et al.*, 2004). Both MamB and MamM are members of the cation diffusion facilitator (CDF) family of metal transporters, which comprises 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.*, 2004), and preliminary evidence obtained from mutant analysis supports this notion (Junge K *et al.*, unpublished data). Intriguingly, FieF, a CDF transporter identified in *Escherichia coli* with sequence similarity to the MamB and MamM proteins, was recently demonstrated besides zinc to also export iron over the cytoplasm membrane (Grass *et al.*, 2005; Lu & Fu, 2007), which lends further support to the notion that CDF3 proteins might be primarily involved in the transport of iron.

Nucleation and crystal growth of magnetite particles

The synthesis of magnetite crystals in *Magnetospirillum* strains depends on the prevalence of microoxic or anoxic conditions, whereas higher oxygen concentrations entirely suppress magnetite biomineralization, or result in the formation of smaller and aberrantly shaped crystals (Heyen & Schüler, 2003). The small Mms6 protein, bearing a Leu–Gly-rich motive also conserved in the unrelated magnetosome proteins MamG and MamD (Grünberg *et al.*, 2004), and which is reminiscent to self-aggregating proteins of other biomineralization systems (Arakaki *et al.*, 2003; Schüler, 2004a), may play a role in the magnetite crystallization process (Matsunaga & Okamura, 2003). Mms6 protein was described in *M. magneticum* as a tightly bound constituent of the MM that exhibited iron-binding activity and had a striking effect on the morphology of growing magnetite crystals *in vitro* by facilitating the formation of uniform 30-nm-sized, single domain particles in solution (Arakaki *et al.*, 2003; Prozorov *et al.*, 2007). However, the significance of the Mms6 for magnetosomal magnetite synthesis *in vivo* remains to be shown.

As protons are released in stoichiometric amounts during magnetite synthesis, the solution within the MM compartment has to be sufficiently buffered to ensure that the solubility product of magnetite is always exceeded (Cornell & Schwertmann, 2003; Faivre *et al.*, 2004). It has been speculated that specific magnetosome proteins such as MamN, which exhibits some similarity to H^+-translocating proteins, might mediate active H^+ efflux from the magnetosome compartment (Jogler & Schüler, 2007). Another magnetosome protein, MamT, contains two conserved putative cytochrome *c* heme-binding sites and thus might represent a redox-active protein, which could be responsible for redox mediation of iron within the MM compartment (Jogler & Schüler, 2007).

Control of crystal size: role of the abundant MamGFDC proteins

It is currently unknown how observed nonisometric morphologies such as prismatic and bullet-, or hook-shaped crystals are achieved at the biochemical and genetic level by various MTB, but it has been speculated that these crystal

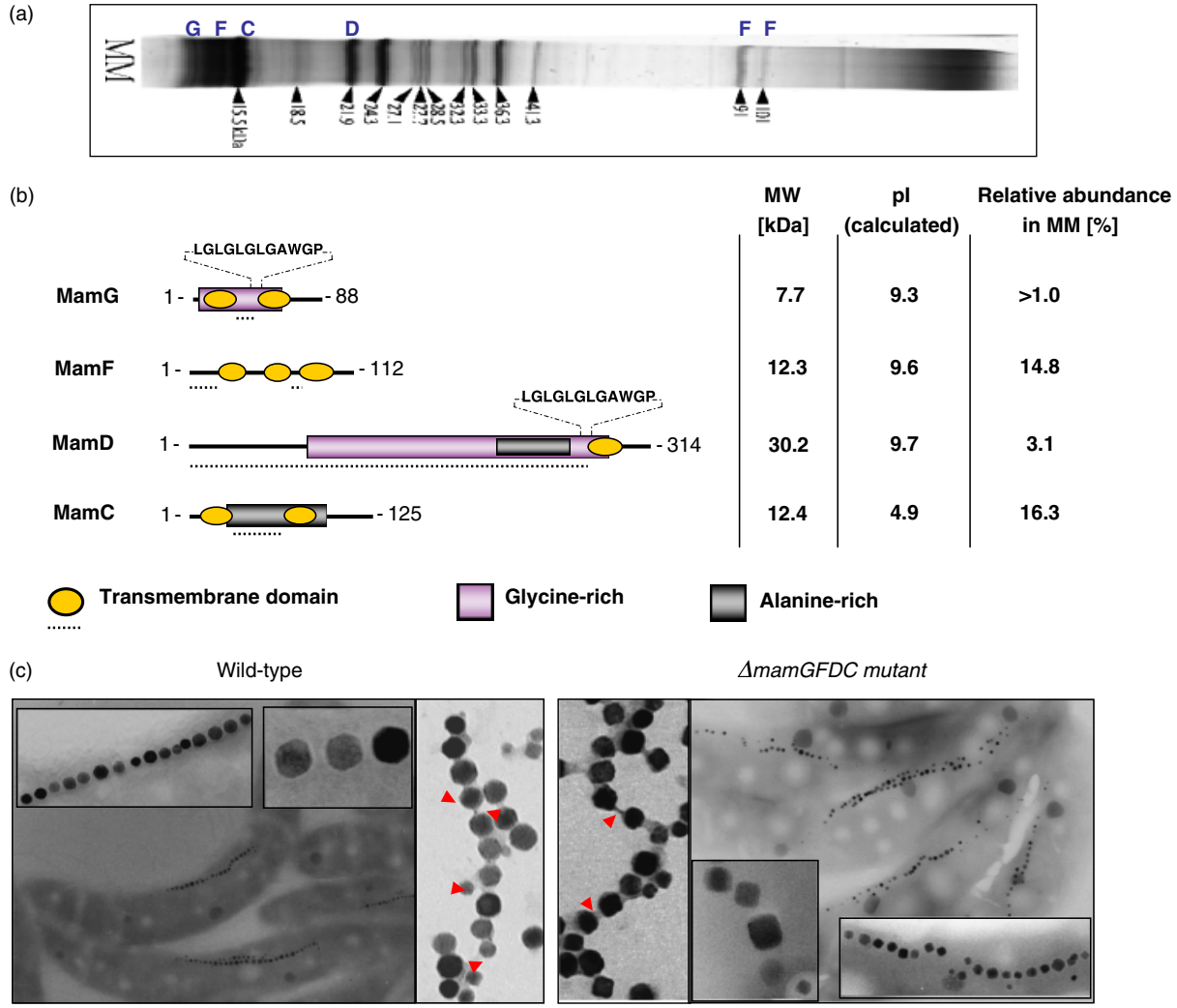

Fig. 4. Characteristics of the MamGFDC magnetosome proteins. (a) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profile of magnetosome proteins solubilized by SDS. Arrows indicate the molecular masses of the most abundant polypeptides. Positions of bands corresponding to the MamG, F, D, and C are indicated by characters. (b) Domain structure and selected protein properties. (c) Phenotype of a Δ*mamGFDC* deletion mutant compared with the wild type. Arrows indicate organic material that connects the isolated particles (b and c modified after Scheffel *et al.*, 2008).

habits might result from anisotropic growth due to chemical gradients or specific inhibition of growth sites by organic molecules (Mann *et al.*, 1990a). It has recently been demonstrated that immature and very fast growing magnetite crystals lack the biological control and structural perfection found in mature particles, and that environmental parameters, such as oxygen concentration, rate of growth, and iron uptake, may affect the habits of magnetosome crystals (Heyen & Schüler, 2003; Faivre *et al.*, 2007b).

The isolation of spontaneous *M. gryphiswaldense* mutants, which produce smaller and aberrantly shaped particles (Hoell *et al.*, 2004; Ullrich *et al.*, 2005), indicated that in addition to shapes of crystals their dimensions are under genetic control. Clearly, the growth of magnetite crystals has to be regulated to generate the species-specific shapes and sizes of particles. However, it is unknown how this regulation is achieved at the structural and genetic level. Recently, the four small magnetosome proteins MamG, MamF, MamD, and MamC were shown to control the size of magnetite crystals (Scheffel *et al.*, 2008).The MamGFDC proteins are encoded by a single operon in *M. gryphiswaldense* and other magnetospirilla and altogether account for approximately 35% of all magnetosome-associated polypeptides in the MM (Fig. 4a) (Schübbe *et al.*, 2003; Grünberg *et al.*, 2001, 2004; Ullrich *et al.*, 2005). With the exception of *mamG*, which is a *Magnetospirillum*-specific gene with no orthologs in other MTB, the *mamD*, *mamF*, and *mamC* genes are part of the MTB-specific set of 28 'signature' genes (Richter *et al.*, 2007). The 12.4 kDa MamC protein, which represents the most abundant magnetosome

polypeptide, was shown to be resistant against solubilization by weak detergents and is tightly associated with the MM (Grünberg *et al.*, 2004), probably owing to its two predicted transmembrane segments (Fig. 4b). A protein orthologous to MamC (Mam12) was found by immunogold staining to localize in the MM also in *M. magnetotacticum* (Taoka *et al.*, 2006). The second-most abundant MM protein identified in *M. gryphiswaldense* is the 12.3 kDa MamF protein, which contains three predicted transmembrane segments and tends to form stable oligomers even in the presence of SDS (Grünberg *et al.*, 2004). The hydrophobic proteins MamD (30.2 kDa) and MamG (7.7 kDa) are partially identical and share a conspicuous motif containing a Leu–Gly-deceptive repeat. Based on their high abundance in the MM, their exclusive occurrence and high conservation in other MTB, MamC and the further gene products of the *mamGFDC* operon were assumed to play a key role in magnetite crystal formation (Schüler, 2004b). However, mutants lacking either *mamC* or the entire *mamGFDC* operon continue to synthesize magnetite crystals, form intracellular MM vesicles, and align in magnetic fields. However, cells lacking *mamGFDC* produced crystals, that had only 75% of the wild-type size, and that were less regular with respect to morphology and chain-like organization (Fig. 4c) (Scheffel *et al.*, 2008). The inhibition of crystal formation could not be rescued by increased iron concentrations, and growth of mutant crystals apparently was not spatially constrained by the size of MM vesicles, as cells lacking *mamGFDC* formed vesicles with only slightly reduced size compared with the wild type. Formation of wild-type-sized magnetite crystals could be gradually restored by the *in trans* complementation with one, two, or three genes of the *mamGFDC* operon, respectively, regardless of their combination, whereas the expression of all four genes resulted in crystals exceeding the wild-type size. These observations suggest that the MamGFDC proteins have partially redundant functions, and in a cumulative manner control the growth of magnetite crystals by an as-yet unknown mechanism (Scheffel *et al.*, 2008). Intriguingly, by selective expression of the four different magnetosome proteins the sizes of particles could be controlled within a narrow range of a few nanometers. This might provide a useful strategy for the synthesis of size-adjusted magnetic nanoparticles with potential for a number of technological applications.

Assembly of magnetosome chains

Organization and structure of the magnetosome chain

The magnetic dipole moments of individual magnetite crystals are not large enough to align a bacterial cell in the geomagnetic field against thermal disorientation. Therefore, the cell has maximized its magnetic dipole by arranging the magnetosomes in chains, resulting in a single magnetic dipole that is the sum of the permanent magnetic dipole moments of the individual single-domain magnetosome particles (Frankel *et al.*, 2006). A single linear chain is built in *Magnetospirillum* and other MTB, which is arranged adjacent to the CM, apparently following the cell curvature in a helical manner, thus facilitating the alignment of magnetosome chains (Fig. 5a and b). In a deep-etching electron microscopy study, it has been reported by Martins *et al.* (2007) that filamentous structure seems to connect the magnetosome chain to the cell boundary. Even more complex intracellular architectures have been found in some uncultivated MTB, including two or multiple chains (Fig. 5c, e, h, j), which in some bacteria are organized in various twisted strands (Fig. 5c), implying a highly sophisticated process of assembly.

In cells of *M. gryphiswaldense* permanently grown at iron concentrations saturating for magnetite synthesis, the chain is predominantly located at midcell, and growing crystals are preferentially found at the ends of the chain (Figs 2a and 5a). In contrast, empty vesicles are found along the entire length of the cell in iron-starved cells. Time-course experiments, in which magnetite synthesis was induced in iron-starved resting cells, revealed that immature crystallites were formed simultaneously at multiple sites along the entire length (Fig. 5c) (Scheffel *et al.*, 2006). Later, growing crystals started to concentrate at midcell, first assembling into imperfect, loosely spaced chains, which gradually developed into straight, tightly spaced chains of mature particles. This suggests that magnetosome particles undergo a dynamic intracellular localization during maturation and chain assembly.

The cytoskeletal magnetosome filament (MF)

A string of magnetic dipoles has an immanent tendency of collapsing to lower its magnetostatic energy without some form of support (Kirschvink, 1982), which had been attributed to an elusive cellular structure that could properly anchor the chain to rotate the whole cell into alignment with magnetic field lines (Kirschvink, 1982; Gorby *et al.*, 1988; Schüler, 2000). Recently, the existence of such a structure was confirmed by the application of cryo-electron tomography in two complementary studies on the closely related *M. gryphiswaldense* and *M. magneticum*, respectively (Komeili *et al.*, 2006; Scheffel *et al.*, 2006). Cryo-electron tomography is an emerging technology that enables intact prokaryotic cells to be imaged in three dimensions in a near-native 'frozen-hydrated' state at a resolution sufficient to recognize very large macromolecular complexes *in situ* (Jensen & Briegel, 2007). These analyses revealed a network of filaments 3–4 nm in thickness, which are traversing the cell

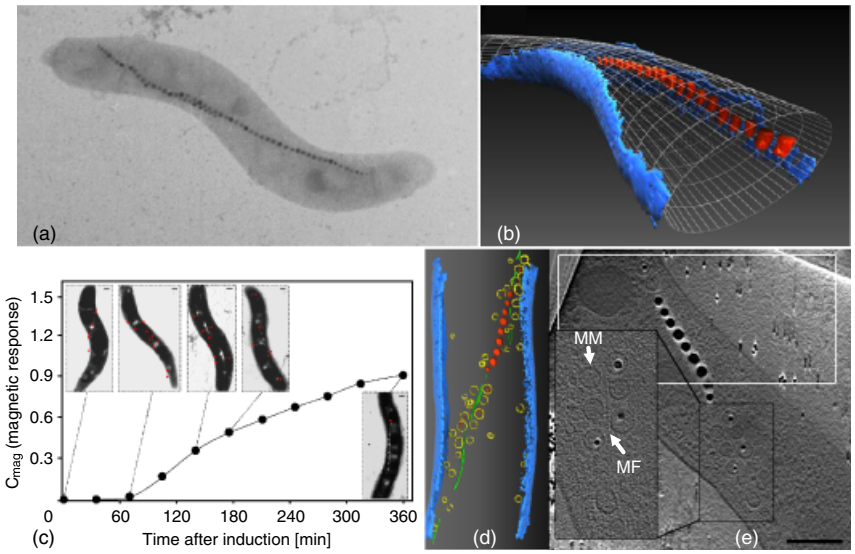

Fig. 5. Organization and assembly of the magnetosome chain and the cytoskeletal MF in *Magnetospirillum gryphiswaldense*. (a) Transmission electron micrograph (TEM) of a cell with a chain of magnetosomes. (b) Three-dimensional reconstruction by cryo-electron tomography of a magnetic cell showing the intracellular location of the magnetosome chain, which is intimately attached to the CM and follows the curvature of the cell (visualization by M. Gruska and J. Plitzko, MPI Martinsried). (c) Time course of magnetite formation in wild-type and Δ*mamJ* cells after induction: magnetite formation was induced in nongrowing iron-deprived cells by the addition of 50 μM ferric citrate and followed by magnetic response (C_{mag}) (Schüler *et al.*, 1995) and energy-filtered TEM. Localization of growing magnetite crystals (arrows) is indicated after different elapsed times. The micrographs illustrate that growing magnetosome particles undergo a dynamic localization during chain assembly. (d) Tomographic reconstruction of a magnetic cell showing the CM (blue), empty vesicles (yellow), growing and mature magnetite crystals (red), and the MF (green). (e) Empty MM vesicles and those containing growing, immature magnetite crystals are closely attached to the cytoskeletal MF.

closely adjacent to the CM (Fig. 5d and e). Most magnetosomes were found arranged intimately along this novel cytoskeletal structure referred to as 'MF' (Frankel & Bazylinski, 2006).

The actin-like MamK protein is likely to form the MF

The two proteins MamJ and MamK, which are encoded by two consecutive genes within a single operon, seem to play key roles in the assembly of magnetosome chains and the formation of the MF. MamK represents a further lineage within the bacterial actin-like proteins along with MreB and ParM, which are phylogenetically and functionally distinct. For example, the ubiquitous MreB is involved in cell shape determination, establishment of cell polarity, and chromosome segregation in bacteria (Gitai, 2007). All prokaryotic actin-like proteins including MamK comprise five characteristic conserved domains, including two phosphate and one adenosine-binding domains that are linked by two 'connect' domains (Bork *et al.*, 1992; van den Ent *et al.*, 2001; Schübbe, 2005).

Evidence that MamK, unlike MreB, is not essential for growth and cell shape in *M. gryphiswaldense* came from the isolation of nonmagnetic, but otherwise fully vital mutant (MSR-1B), in which *mamK* was codeleted with a number of other magnetosome genes (Schübbe *et al.*, 2003). Cells of *M. magneticum* deleted for *mamK* lost their chain-like magnetosome structure, but instead magnetosomes were dispersed throughout the cell (Komeili *et al.*, 2006). In addition, the cytoskeletal MF, whose dimensions are similar to MreB filaments formed *in vitro*, appeared absent from a Δ*mamK* cell, implying that the MF is potentially identical to MamK. MamK fused to enhanced green fluorescent protein (EGFP) displays a filament-like organization *in vivo*, appearing as a thin line within the cells (Komeili *et al.*, 2006; Scheffel & Schüler, 2007). The formation of filament-like structures seems to be an intrinsic property of MamK, because MamK of *M. magneticum* alone is sufficient to direct the synthesis of straight filaments in *E. coli*, which are structurally and functionally distinct from the known MreB and ParM filaments (Pradel *et al.*, 2006). Consistently, MamK localization does not depend on the presence of other magnetosome genes, because a MamK–EGFP fusion in *M. gryphiswaldense* displays a localization pattern in the Δ*mamJ* and MSR-1B deletion mutant indistinguishable from that in the wild type (Scheffel & Schüler, 2007). Using dual fluorescent labeling of MamK, it was shown that MamK in *E. coli* nucleates at multiple sites and dynamically assembles into mosaic filaments (Pradel *et al.*, 2006). In a different study, recombinant MamK of *M. magnetotacticum* expressed in *E. coli in vitro* polymerized into long straight

filamentous bundles, which were more than 100 μm in length and 100 nm in width (Taoka *et al.*, 2007). Filaments were observed in the presence of a nonhydrolyzable ATP analog, but not if ATP was added, suggesting that MamK filaments might depolymerize in the presence of ATP. The smallest bundles were twisted with a total diameter of 8–18 nm, and the twisted bundle was composed of fine helical protofilaments about 6 nm in width (Taoka *et al.*, 2007). These observations indicated that the nature of the MamK polymer is distinct from the other known bacterial actin-like proteins.

The acidic mamJ protein interacts with MamK and may attach MM vesicles to the MF

In addition to MamK, the acidic MamJ protein, which was identified in the MM of *M. gryphiswaldense* (Grünberg *et al.*, 2004), was recently implicated in control of the magnetosome chain assembly. Its most conspicuous sequence feature is the central acidic repetitive (CAR) domain (Scheffel & Schüler, 2007), which comprises a direct repetition of 88 amino acid (aa), followed by tandemly arranged copies of a highly acidic motif of 20 aa consisting of Pro and Glu residues (Fig. 6a). The CAR domain is followed by an Ala-rich domain, and a Gly-rich domain is positioned near the C-terminus. MamJ was originally assumed to be involved in the control of magnetite crystallization because of its resemblance to other biomineralizing proteins, i.e. the acidic pI and the presence of highly repetitive domains. However, a mutant strain, in which the *mamJ* gene was removed, surprisingly was not affected in the synthesis of magnetite crystals, but no longer produced straight magnetosome chains (Scheffel *et al.*,

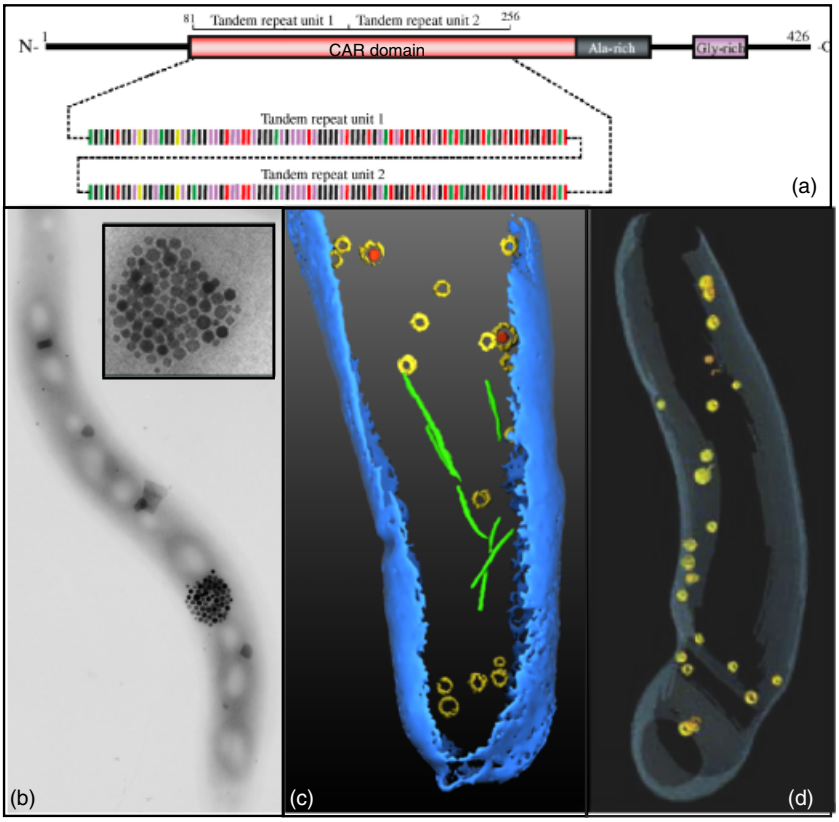

Fig. 6. (a) Domain structure of the MamJ protein of *Magnetospirillum gryphiswaldense*. The 426-aa protein with a mass of 44.3 kDa and an isoelectric point of 3.9 contains several domains with highly biased amino acid composition. The CAR domain contains two identical Glu-rich stretches (81–168 and 169–256) that are arranged as tandem repeats (acidic amino acid, red; basic amino acid, yellow; hydrophobic amino acid, black; hydrophilic amino acid except Glu and Asp, purple). Within the CAR domain, Glu and Asp residues are predominantly located between positions 224 and 294, accounting for 32.4% of all amino acid residues within that region. The CAR domain is followed by an Ala- and Gly-rich domain (modified after Scheffel *et al.*, 2006). (b) Phenotype of a Δ*mamJ* mutant of *M. gryphiswaldense* shown by transmission electron micrograph. Cells are deficient in the chain assembly, but instead mature magnetosome particles agglomerate in clusters (modified after Scheffel *et al.*, 2006). (c) Tomographic reconstruction of an iron-deprived Δ*mamJ* mutant cell of *M. gryphiswaldense*, which contains empty and partially filled MM vesicles (yellow), which are scattered throughout the cell and detached from the MF (green) (modified after Scheffel *et al.*, 2006). (d) For comparison, the phenotype of a Δ*mamK* mutant of *Magnetospirillum magneticum* is shown. Although the MFs appear to be absent, magnetosomes still maintain a dispersed, chain-like configuration (picture courtesy of A. Komeili and Science magazine).

2006). Instead, magnetite crystals were arranged in compact clusters (Fig. 6b).

The cytoskeletal network of MFs was still present in the Δ*mamJ* mutant; however, empty vesicles and immature crystals were scattered throughout the cytoplasm and detached from the filaments in Δ*mamJ* cells (Fig. 6c). In Δ*mamJ* cells empty vesicles were widely spaced and located along the entire length of cells, which suggested that agglomeration of vesicles occurs only after synthesis and maturation of magnetite crystals (Scheffel *et al.*, 2006).

MamJ protein displays an extensive intra- and sequence variability, which is probably caused by frequent recombination events between repeated domains. However, all tested *mamJ* polymorphs were functional in the restoration of wild-type-like magnetosome chains in Δ*mamJ*. Complementation experiments with truncated *mamJ* constructs revealed that the hypervariable CAR domain as well as the Ala-rich domain and the first N-terminal residues (1–24) are not essential for magnetosome chain restoration in Δ*mamJ*, whereas residues 25–80 and 335–392 are indispensable for MamJ function (Scheffel & Schüler, 2007). A functional MamJ–EGFP fusion was *in vivo* localized as a linear structure traversing longitudinally from pole-to-pole both in wild-type and in Δ*mamJ* cells. In contrast, cytosolic localization of the MamJ–EGFP fusion was observed if expressed in the nonmagnetic mutant strain MSR-1B, in which a number of magnetosome genes are absent by deletion, or in cells of *E. coli*. This suggested that MamJ interacts with a linear structure encoded within the MAI, but is not likely to form this structure itself. Direct interaction between the putative filament-forming MamK protein and MamJ was experimentally confirmed by two-hybrid experiments which revealed that MamJ interacts with MamK and itself through a C-terminal (295–368 aa) and a second N-terminal domain (23–81 aa) (Scheffel & Schüler, 2007).

Acidic proteins in other MTB

Whereas single or even multiple *mamK* orthologs are common to the *mamAB* operons in all MTB analyzed so far, the presence of *mamJ* genes seems to be confined to the genus *Magnetospirillum* (Richter *et al.*, 2007). This raised the question of whether the same functions might be performed in other MTB by nonorthologous proteins. A search for genes encoding acidic repetitive proteins in the genome of MC-1 revealed several conspicuous ORFs (A. Scheffel and D. Schüler, unpublished data), which share the acidic repetitive domain structures but are not homologous to MamJ. For example, a large ORF denoted as ORF5 Mmc1_2252 (Grünberg *et al.*, 2001) is present in the *mamAB* operon of MC-1, which encodes a large (1025 aa) putative multidomain protein comprising a C-terminal TPR domain resembling the magnetosomal MamA protein, and a central acidic domain that exhibits multiple (> 20) repeats of a slightly varied QEVESVQEVEP motif, which is somehow reminiscent of the CAR domain of the MamJ protein. A further large hypothetical histidine-rich protein (1250 aa, MW >), which contains a conspicuous AVPEAASHEPEAS motif present in multiple repeats, is encoded by Mmc1_2266 that is located in the vicinity of the *mamAB* operon in strain MC-1. It remains to be shown experimentally whether these large, highly acidic, and repetitive proteins are specifically involved in magnetosome formation or magnetite biomineralization.

Cell biology of magnetosome formation: known facts and open questions

One model derived from these data is that the MF is composed of the MamK protein and that MamJ mediates the interaction with this dedicated cytoskeletal structure (Fig. 7). This implies that the MamJ protein connects magnetosome vesicles to the putative cytoskeletal MF, which is intimately associated with the magnetosome chain, thereby preventing the magnetosome chain from collapsing. In contrast, in cells lacking MamJ, mature magnetosome crystals are free to agglomerate once they are in close proximity (Scheffel, 2007).

However, the notion described above is likely to be an oversimplification, as it does not take into account the role of additional proteins, and alternative scenarios cannot be precluded. There are several conflicting observations, that raise further questions. For instance, according to the proposed model, deletion of either *mamJ* or *mamK* would be expected to yield very similar phenotypes, which in reality, however, are strikingly different between $\Delta mamJ_{Mgryph}$ (aggregated magnetosomes) and $\Delta mamK_{Mmagneticum}$ (dispersed magnetosomes) (Fig. 6c and d). There were other structural differences observed between *M. gryphiswaldense* (pole-to-pole extension of the MF) and *M. magneticum* (shorter MF). It is currently not clear if these discrepancies are due to experimental differences, or cellular mechanisms that are different between these two strains. Moreover, although the absence of filaments in Δ*mamK* suggests that the cytoskeletal MF might indeed be formed by MamK, its identity has not yet been proven unambiguously. Instead, further proteins in addition to MamK might be involved in the assembly of the filament. One of the most intriguing questions in magnetosome assembly is what controls the dynamic localization of magnetosomes during chain assembly and what causes new magnetosomes to form at the ends of the inherited chain? There is precedence that bacterial MreB-like proteins may function as intracellular motors (Graumann & Defeu Soufo, 2004), and it was proposed that MamK might function in establishing the chain by

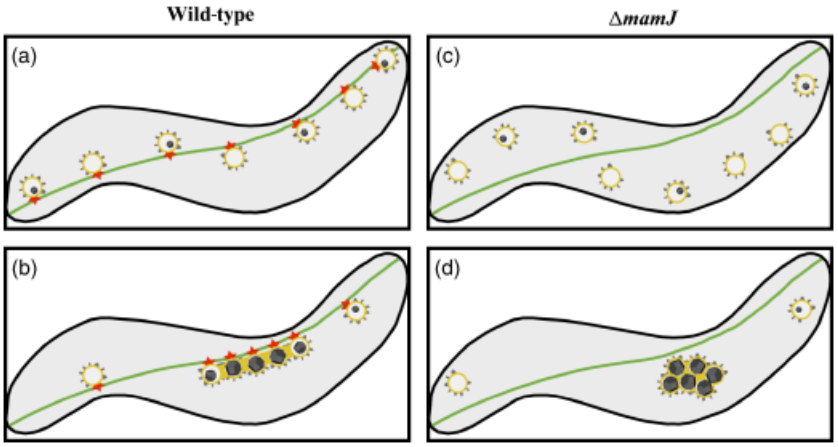

Fig. 7. Model for magnetosome chain assembly in *Magnetospirillum gryphiswaldense* MamJ (red) attaches the empty magnetosome vesicles (yellow) to the cytoskeletal filaments (green), resulting in a dispersed 'beads-on-a-string'-like alignment. Magnetite biomineralization is initiated at multiple sites distributed over the length of the cell (a). As magnetite crystals (black) continue to grow, their magnetic moments increasingly force the particles to interact. Positioning of mature magnetosomes at midcell is then directed along filaments (b). Once particles are in close contact, growing chains are further stabilized by interaction with the MM. In contrast, vesicles in the Δ*mamJ* mutant are detached from the filaments (c). This results in agglomeration of crystals as magnetic interactions between growing magnetite crystals increase (d) (modified after Scheffel *et al.*, 2006).

transporting newly formed magnetosomes with growing crystals to the ends of the nascent pre-existing magnetosome chains (Frankel & Bazylinski, 2006). Time-lapse experiments revealed that the assembly of the MamK filaments in *E. coli* is a highly dynamic and kinetically asymmetrical process (Pradel *et al.*, 2006), and polymerization and depolymerization processes might generate forces sufficient for the transport and relocation of magnetosomes. It has been further speculated that the position and polarity of the magnetosome chain might relate to other cellular structures relevant for magnetotaxis, such as the flagella motor (Shih & Rothfield, 2006; Stephens, 2006), and it was suggested that the inherent molecular polarity of MamK might be translated into a mechanism for directing global-cell polarity (Pradel *et al.*, 2006).

It is currently unknown what causes the growing magnetosome chain to be located at midcell, and how are magnetosomes properly divided and segregated to daughter cells. Loss of MamJ resulted in an uneven segregation of magnetosome particles during cell division (Scheffel & Schüler, 2007), and there are some further indications that suggest a controlled mechanism of magnetosome positioning and segregation. Positional information for localization of the magnetosome chains as well as its coordination of assembly and distribution with the cell cycle could be provided by interaction with other positional determinants, such as for example the divisome (Stephens, 2006). A gene with similarity to such additional putative cytoskeletal elements is represented by the *ftsZ*-like ORF present in the *mamXY* operon of *M. gryphiswaldense* (in addition to a further genuine *ftsZ* gene located elsewhere in the genome). FtsZ of other bacteria are universal cytoskeletal tubulin-like proteins, which assemble into the Z ring at the center of the bacterial cell during division, and are required for the association of all other division proteins (Margolin, 2005; Shih & Rothfield, 2006). The idea that further cytoskeletal proteins might be involved in the assembly and positioning of magnetosome chains is intriguing, but so far lacks any experimental evidence.

Genomics and genetics of magnetosome formation

Genome characteristics of MTB

As only a few species of MTB are available in pure culture, most of our current knowledge on genetics of magnetosome synthesis comes from analysis of *Magnetospirillum* species and closely related MTB within the *Alphaproteobacteria*. Systems for genetic manipulation have been reported so far only for *M. magneticum* (Matsunaga *et al.*, 1992; Komeili *et al.*, 2004) and *M. gryphiswaldense* (Schultheiss & Schüler, 2003; Schultheiss *et al.*, 2004, 2005). During the last years, genome information from several magnetite-producing, magnetotactic *Alphaproteobacteria* has become available. Draft genome assemblies were reported for *M. magnetotacticum* strain MS-1 (http://www.jgi.doe.gov) and *M. gryphiswaldense* strain MSR-1 (Richter *et al.*, 2007). Complete genome sequences of *M. magneticum* strain AMB-1 (Matsunaga *et al.*, 2005) and the marine magnetic coccus strains MC-1 provided by JGI (Richter *et al.*, 2007) became recently available. Genome sequencing of the magnetic sulfate-reducing bacterium *Desulfovibrio magneticus* strain RS-1 is underway (http://www.bio.nite.go.jp/ngac/e/project-e.html).

Genome sizes of MTB are between 4.3 (*M. gryphiswaldense*) and nearly 5 Mb (*M. magneticum*), corresponding to a number of 3716 (MC-1) and 4559 (*M. magneticum*) predicted ORFs. Their G+C content is between 54.8% (MC-1) and 62.8–65.1% (*Magnetospirillum* sp.). Most best BLAST hits to ORFs identified in *M. gryphisw*aldense were found in other *Alphaproteobacteria*, such as *Rhodospirillum rubrum*, the closest relative with a complete genome sequence available. The magnetotactic coccus strain MC-1 is only distantly related to *Magnetospirillum,* and the common magnetotactic phenotype apparently is only rather weakly reflected at genome level (Richter *et al*., 2007).

By comparative genome analysis of the three sequenced strains of *Magnetospirillum* strains and the magnetic coccus MC-1, a magnetobacterial core genome of only about 891 genes was found shared by all four MTB. In addition to a set of *c*. 152 genus-specific genes shared exclusively by the three *Magnetospirillum* strains, 28 genes were identified as group specific, i.e. they occur in all four analyzed MTB, but exhibit either no (MTB-specific genes) or only remote (MTB-related genes) similarity to any genes from nonmagnetotactic organisms. These group-specific genes include all *mam* and *mms* genes, which were implicated previously in magnetosome formation by proteomic and genetic approaches. The MTB-specific and MTB-related genes, which represent $< 1\%$ of the 4268 ORFs of the MSR-1 genome, display synteny and are likely to be specifically involved in magnetotaxis, although many of them are of as-yet unknown functions.

The genomic magnetosome island

Most magnetosome genes, which were first identified by a reverse genetic approach in *M. gryphiswaldense*, are located within a 130 kb genomic magnetosome island that exhibits the following characteristics: (1) it harbors the majority of genes encoding MM proteins, (2) it contains a high proportion of transposase genes ($> 20\%$ of the coding region), and (3) it contains many hypothetical genes (Fig. 8).

Its G+C content is slightly distinct from that of the rest of the genome and displays a more heterogeneous distribution. Three *tRNA* genes are present within this region, which is also bounded by a putative integrase gene. As all these features are strongly reminiscent of those described for other genome islands, such as 'pathogenicity' or 'environmental' islands in other bacteria (Dobrindt *et al*., 2004), the genomic cluster was described as genomic 'magnetosome island' (MAI) (Schübbe *et al*., 2003; Ullrich *et al*., 2005; Bazylinski & Schübbe, 2007). A genomic region with similar molecular structure, gene content, and operon organization was also identified in *M. magneticum* AMB-1 and other magnetospirilla (Fukuda *et al*., 2006; Jogler & Schüler, 2006). From the conserved 28 group-specific (MTB-specific and MTB-related) genes, which are exclusively present in all MTB genomes, 18 are located within the MAI of *M. gryphiswaldense* whereas 10 are located outside the MAI. This might suggest that the MAI encodes many, but not all, gene functions required for magnetotaxis. However, based on preliminary experimental analysis and prediction of gene functions it was speculated that genes required for the synthesis and biomineralization of magnetosomes are confined within the MAI, whereas the genomic organization of gene functions required for the 'taxis' part of magnetotaxis may be less conserved and display a wider genomic distribution (Richter *et al*., 2007).

Extensive sequence polymorphism within the MAI was observed between clones isolated from different subcultures of *M. gryphiswaldense* MSR-1 (Ullrich *et al*., 2005), indicating that this region undergoes frequent rearrangements during serial subcultivation in the laboratory. Spontaneous magnetosome mutants accumulate in bacterial cultures after prolonged cold storage or exposure to oxidative stress at a frequency of up to 10^{-2} (Ullrich *et al*., 2005). All analyzed nonmagnetic mutants were harboring different deletions within the MAI, probably caused by homologous recombination between identical copies of the numerous insertion elements and direct and inverted repeats contained in the MAI (Schübbe *et al*., 2003; Ullrich *et al*., 2005). Spontaneous and frequent loss of the ability to synthesize magnetosomes has also been reported for other MTB (Frankel *et al*., 1979; Dubbels *et al*., 2004). For example, Fukuda *et al*. (2006) suggested a direct repeat-dependent mechanism of integrase mediated excision of the entire MAI followed by transient circularization in *M. magneticum*. The existence of a mobile magnetosome island might also explain the widespread occurrence of magnetic phenotypes among various unrelated *Proteobacteria* and the phylum *Nitrospira* (Amann *et al*., 2006). It can be speculated that magnetosome genes

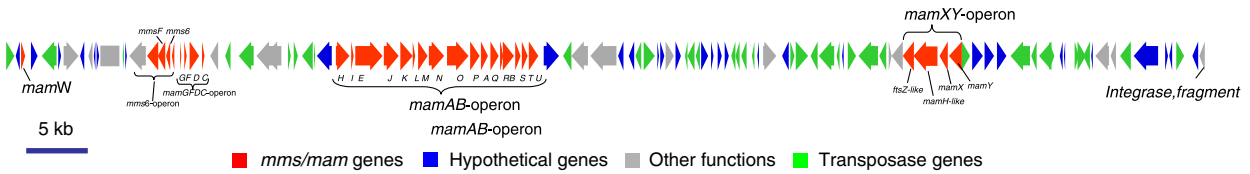

Fig. 8. Section from the genomic magnetosome island of *Magnetospirillum gryphiswaldense* comprising the genes encoding magnetosome genes. ORFs are shown as arrows in various colors.

have evolved only once, and different bacteria may have acquired magnetosome genes via horizontal gene transfer, which would have been facilitated by clustering all required genes within a compact MAI (Jogler & Schüler, 2006, 2007).

The *mam* and *mms* operons encoding most magnetosome proteins

In *M. gryphiswaldense* and other magnetospirilla most magnetosome proteins are encoded within the *mam*- (magnetosome membrane) and *mms*- (magnetic particle membrane-specific) operons, which are located within < 65 kb of the MAI (Ullrich *et al.*, 2005; Richter *et al.*, 2007) (Fig. 8). Single long polycistronic transcripts were experimentally demonstrated for the *mamAB*, *mamGFDC* and *mms6* operons, respectively (Schübbe *et al.*, 2006), and promoters were mapped closely upstream of the first genes in the operons, respectively. The presence of transcripts was independent of the growth phase, but expression of *mam* and *mms* genes was slightly upregulated under magnetite-forming conditions, i.e. during microaerobiosis and in the presence of iron (Schübbe *et al.*, 2006; Würdemann *et al.*, 2006). The operon organization of magnetosome genes is conserved among different *Magnetospirillum* strains and, to a lesser extent, also strain MC-1. In *M. gryphiswaldense*, the *mamAB* operon encompasses 17 collinear genes extending over 16.4 kb of DNA (Fig. 1). The 2.1-kb *mamGFDC* operon is located 15 kb upstream of the *mamAB* operon and comprises four genes. The 3.6-kb *mms6* operon is located 368 bp upstream of the *mamGFDC* operon and contains five genes. Further magnetosome proteins (MamX and MamY) are encoded by the *mamXY* operon within the MAI located about 30 kb downstream of the *mamAB* operon, which in addition comprises a putative and *ftsZ*-like gene and a further *mamH*-like gene with a C-terminal ferric reductase domain (Richter *et al.*, 2007). Another magnetosome protein is encoded within the MAI by the monocistronic *mamW* gene. Two further magnetosome proteins, MtxA and MmeA, are encoded outside the MAI. *mtxA* is part of a conserved operon-like cluster, which also comprises a TPR-like gene (*mgr0206*) and a gene (*mgr0207*) with both an adenylate cyclase and a CHASE2 domain, which are characteristic for transmembrane receptors that function as sensors for environmental cues. Therefore, the *mtxA* operon was implicated in signal transduction and magnetotaxis (Richter *et al.*, 2007).

Other proteins putatively involved in magnetosome formation

In addition to the proteins described above, the possible roles and contribution to magnetosome formation of several other genes, whose function either has not established unambiguously, or has a broader or nonspecific function in the cell, will be discussed in the following.

TPR proteins

MamA is an abundant protein of the MM in *M. gryphiswaldense* (Grünberg *et al.*, 2001) and other magnetospirilla (Okuda *et al.*, 1996) and contains four to five copies of the TPR motifs, which have been identified in a growing number of proteins with diverse functions and are known to mediate protein–protein interactions (Blatch & Lassle, 1999). It therefore has been speculated that MamA (named Mam22 in *M. magnetotacticum*) 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 & Fukumori, 2001). However, a MamA-deficient mutant of *M. magneticum* had only a weak phenotype and was not affected in the formation of magnetosome vesicles, but continued to produce magnetite crystals identical in shape and alignment to those in the wild type, albeit at reduced numbers. These observations indicate that MamA is not essential for magnetosome biomineralization, but has a so-far uncharacterized function, which might be in the 'activation' of magnetosome vesicles as mentioned above (Komeili *et al.*, 2004).

PDZ proteins

Another conspicuous characteristic of MTB is the presence of several magnetosome-associated proteins with sequence similarity to PDZ-domain-containing HtrA-like serine proteases, and the abundance of further related genes in the genome. The putative serine proteases MamE and MamO, which share only relatively weak (31%) sequence similarity to each other, were identified in the MM of *M. gryphiswaldense* and are encoded within the *mamAB* operon along with the *mamP* gene, encoding a further member of this family. 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 magnetosome proteins during MM assembly (Grünberg *et al.*, 2001).

Further gene functions with potential relevance for magnetotaxis

Random mutagenesis by conjugational transfer of Tn5 transposons into *M. magneticum* (Matsunaga *et al.*, 1992) was used to identify genes controlling magnetosome formation (Kawaguchi *et al.*, 1992; Wahyudi *et al.*, 2001, 2003;

Calugay *et al*., 2004; Matsunaga *et al*., 2005). For example, of 5762 identified Tn*5* insertants, 69 were found to be defective for magnetosome formation. Disrupted genes were distributed over the entire genome and categorized into various functional groups, including signal transduction, energy metabolism, cell envelope biogenesis, cell motility, and unknown functions (Matsunaga *et al*., 2005). For instance, Tn*5* mutagenesis led to the destruction of genes encoding an aldehyde ferredoxin oxidoreductase or a periplasmic transport binding protein kinase. However, none of the mutants have been complemented so far, and thus the roles of these genes require further clarification, given the high spontaneous mutability of the magnetic phenotype which bears the risk of obscuring mutagenesis experiments. Surprisingly, neither of the previously identified *mam* and *mms* genes, which are essential for magnetosome synthesis, nor other genes located within these operons were among the affected genes in the studies mentioned above. In contrast, all transposon mutants obtained in an independent study on the same organism resulted from insertions in the *mamAB* operon (Komeili *et al*., 2004). Likewise, Li *et al*. (2005) reported a nonmagnetic transposon mutant of *M. gryphiswaldense*, which mapped within the close neighborhood of *mamW* inside the MAI. The disrupted gene shows similarity to a CheIII-like protein, supporting the notion that putative taxis-related genes are essential for the magnetic phenotype. In addition to the characteristics discussed above, genome analysis of various MTB revealed some unusual and conspicuous characteristics compared with nonmagnetic prokaryotes, which might be significant for the magnetotactic phenotype. For example, the genomes of *Magnetospirillum* strains and the magnetic coccus MC-1 contain 30 or more hemerythrin-like genes, which appear to be the highest number of this gene family among all sequenced prokaryotes (Frankel *et al*., 2006). Hemerythrins are a group of oxygen-handling proteins, which are involved in O_2 transport in several eukaryotes, more recently they were also identified in prokaryotes, although their function in the latter group is unclear (Frankel *et al*., 2006). Because of the prevalence of hemerythrin-like genes in the genome of all MTB, including those located within the magnetosome island, their potential to bind oxygen (and iron), and their suggested roles in oxygen sensing, it has been speculated that hemerythrins might play a role in magnetite biomineralization and/or magnetotaxis, which however remains to be determined experimentally (Ullrich *et al*., 2005; Frankel *et al*., 2006). Another intriguing feature of all MTB is the genomic occurrence of unusually high numbers of chemotaxis transducers and other proteins potentially involved in cellular signaling and bacterial taxis, and it has been speculated that this might be related to the regulation and control of magnetotaxis (Alexandre *et al*., 2004; Matsunaga *et al*., 2005). Although none of these genes has been studied experimentally, these high numbers suggest the presence of an extraordinary complex and potentially redundant pathway of signal transduction in the MTB, which might reflect an adaptation to their lifestyle adapted to complex chemical gradients in their natural environments.

In summary, these studies seem to suggest that besides genetic determinants specifically involved in magnetosome formation encoded by the MAI, a so far undefined number of general or accessory metabolic functions are required for magnetosome biomineralization and magnetotaxis. Although we have just begun to understand their individual functions, it can be concluded from the available evidence that the magnetotactic phenotype is among the most complex prokaryotic traits with respect to cellular organization and genetic control.

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