Journal of Oceanology and Limnology
Vol. 39 No. 6, P. 2070-2081, 2021
https://doi.org/10.1007/s00343-021-0422-5

Redox control of magnetosome biomineralization*

Yingjie LI**

State Key Laboratory of Microbial Technology, *Shandong University*, *Qingdao 266237*, *China*

Received Nov. 3, 2020; accepted in principle Dec. 15, 2020; accepted for publication Dec. 27, 2020

Abstract Magnetotactic bacteria can orientate in the Earth's magnetic field to search for their preferred microoxic environments, which is achieved by their unique organelles, the magnetosomes. Magnetosomes contain nanometer-sized crystal particles of magnetic iron minerals, which are only synthesized in low-oxygen environments. Although the mechanism of aerobic repression for magnetosome biomineralization has not yet fully understood, a series of studies have verified that redox modulation is pivotal for magnetosome formation. In this review, these advances in redox modulation for magnetosome biosynthesis are highlighted, mainly including respiration pathway enzymes, specific magnetosome-associated redox proteins, and oxygen- or nitrate-sensing regulators. Furthermore, their relationship during magnetosome biomineralization is discussed to give insight into redox control and biomineralization and inspire potential solutions for the application of respiration pathways to improve the yields of magnetosome.

Keyword: magnetotactic bacteria; magnetosome; biomineralization; respiration; redox control

1 INTRODUCTION

Magnetotactic bacteria (MTB) biomineralize crystals of the iron oxide magnetite (Fe_3O_4) or the iron sulfide greigite (Fe_3S_4) inside membrane-enveloped organelles termed magnetosomes (Fig.1). These mineral particles have inherent magnetic properties, and therefore are exploited by MTB for orientating the earth's geomagnetic field to search for their preferred low oxygen environment (Jogler and Schüler, 2009). A huge diversity of the shape and size of magnetite or greigite nanoparticles is biosynthesized by different MTB (Faivre and Schüler, 2008; Schüler, 2008). Due to their relative ease of growth in the laboratory and genetic tractability, two closely related alphaproteobacteria, *Magnetospirillum gryphiswaldense* MSR-1 (MSR-1) and *Magnetospirillum magneticum* AMB-1 (AMB-1), which produce single chains of cuboctahedral magnetite particles, have been used as model organisms for the mechanistic understanding of biomineralization. Over the past decades, genetic studies by using these two *Magnetospirillum* species make significant progress in the identification of genes involved in magnetosome formation, which are clustered in a more than 100-kb genomic region, magnetosome island (MAI; Ullrich et al., 2005; Murat et al., 2010; Lohße et al., 2011). Although the mechanism of magnetosome formation has not been completely elucidated, a stepwise process for the synthesis and assembly of magnetite nanoparticles has been proposed: (i) invagination of magnetosome vesicles from cytoplasmic membrane, (ii) iron uptake, and (iii) magnetosome biomineralization and chain formation. Different proteins arrested at various stages of magnetosome formation by individual deletion of each gene are summarized in some excellent reviews (Uebe and Schüler, 2016; McCausland and Komeili, 2020). In addition, it is suggested that the synthesis of mixed-valence iron oxide magnetite could occur by coprecipitation of ferric and ferrous iron in supersaturating concentrations, which thereby requires a proper ratio of ferrous and ferric iron (Mann et al., 1990; Faivre et al., 2004, 2007). This may be the reason why magnetite crystals are only biomineralized under microaerobic and anaerobic conditions, whereas

* Supported by the National Natural Science Foundation of China (No. 41706165) and the Fundamental Research Funds of Shandong University (No. 2019HW022)
** Corresponding author: yingjie.li@sdu.edu.cn

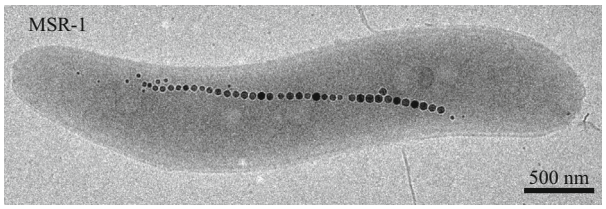

Fig.1 ***Magnetospirillum gryphiswaldense*** **MSR-1 and magnetosomes**

atmospheric oxygen concentrations entirely inhibit the biosynthesis of magnetosomes. In this review, the author highlights the developments in understanding of the relationship between redox processes and biomineralization, and describes the mechanisms of redox associated proteins affecting magnetosome formation.

2 EFFECTS OF ENVIRONMENTAL OXYGEN ON MAGNETOSOME FORMATION

Since magnetosomes were early found to comprise of magnetite, oxygen molecules of Fe_3O_4 was firstly proposed to come from air. Therefore, early hypotheses focused on whether a competition for oxygen occurs between respiration and biomineralization (Blakemore et al., 1985). However, later isotope experiments demonstrate that oxygen molecules bound in biologically synthesized Fe_3O_4 are not derived from O_2 but water (Mandernack et al., 1999). In spite of this, instead of being directly incorporated into Fe_3O_4, O_2 appears to be a crucial environmental factor for magnetite biomineralization. This speculation has been proposed based on the observations that magnetite crystals are only produced in low-oxygen environment while aerobic conditions completely inhibit their formation. Moreover, in *Magnetospirillum magnetotacticum* (MS-1), it has been found that changes of O_2 concentration clearly affect the synthesis of some proteins. For example, an increase of O_2 content resulted in enhanced activity of a manganese-type superoxide dismutase compared to an iron-type superoxide dismutase (Short and Blakemore, 1989). Also in the same strain, Sakaguchi et al. (1993) found that the presence of O_2 repressed the synthesis of a 140-kDa membrane protein. An O_2-shift growth experiment on MSR-1 also showed some links between oxygen concentrations and magnetosome formation: after shifting aerobic MSR-1 strain to microaerobic conditions, cells remained non-magnetic for about 2 h. When MSR-1 cells were shifted from aerobic to anaerobic conditions, cells started to display a magnetic response only after about 1 h, which is faster than those shifted to microaerobic conditions (Supplementary Fig.S1). Therefore, it was initially speculated that the observed lag in magnetite biomineralization might be directly caused by perturbed redox conditions for iron oxidation, or alternatively, by deregulated expression of proteins involved in magnetosome formation.

However, further studies on MSR-1 verified that the expression of key magnetosome proteins encoded in the genomic magnetosome island is not likely regulated by the concentrations of oxygen, a conclusion drawn based on the following observations: (i) the transcription of genes encoding magnetosome membrane proteins (Mam and Mms) is not regulated by oxygen (Schübbe et al., 2006; Wang et al., 2016); (ii) all tested magnetosome proteins, including MamA, MamC, MamK, and MamM are observed within the cell under aerobic conditions, and when quantified by immunodetection, these proteins show similar expression levels under different oxygen conditions (Supplementary Fig.S2); (iii) nonmagnetic MSR-1 cells cultured under high O_2 conditions still form empty vesicles, while no electron-dense nanoparticles are formed (Raschdorf et al., 2016). Therefore, the question arose as to whether the aerobic repression for magnetite biomineralization is indirectly caused by changed cellular redox homeostasis, which thereby alters the redox state of the mineral.

3 DENITRIFICATION AND MAGNETITE BIOMINERALIZATION

In many anaerobic and microaerophilic bacteria such as MTB, one of the major redox pathways is the denitrification pathway, which stepwise reduces nitrate to nitrogen gas. So far, all cultured magnetospirilla have been shown to be capable of denitrification with nitrate as an alternative terminal electron acceptor, but not with Fe(III), sulfate, or fumarate (Bazylinski and Blakemore, 1983; Bazylinski and Williams, 2006; Lower and Bazylinski, 2013). A potential link between denitrification and magnetite biomineralization has been realized for decades. For instance, in MS-1 more cells were found to contain magnetosomes in the presence of nitrate than its absence (Bazylinski and Blakemore, 1983). Also in AMB-1 nitrate supports magnetite biomineralization at low oxygen concentrations (Matsunaga et al., 1991; Matsunaga and Tsujimura, 1993; Yang et al., 2001). However, no genetic

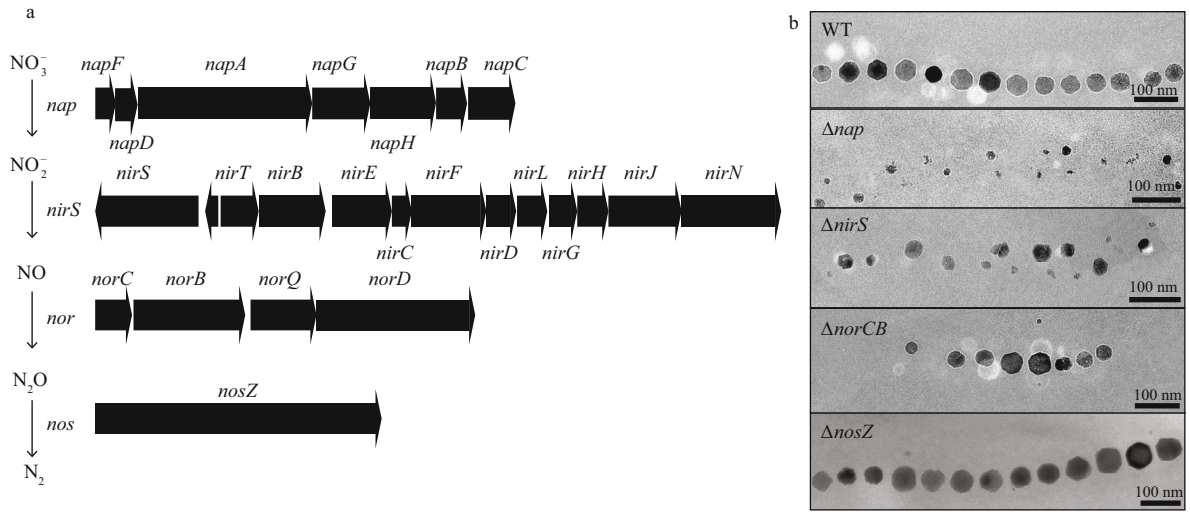

Fig.2 Denitrification pathways are involved in magnetite biomineralization

a. molecular organization of denitrification genes in MSR-1; b. magnetosomes from MSR-1 wild type (WT), Δ*nap*, Δ*nirS*, Δ*norCB*, and Δ*nosZ* strains. The images are adapted from Li et al. (2012, 2013) and amended with permission from American Society for Microbiology.

evidence was available to elucidate the relationship between denitrification and magnetosome formation. Later, studies upon genetic analyses of denitrification pathway in MSR-1 revealed a complete denitrification pathway including genes encoding nitrate (*nap* operon), nitrite (*nirS*), nitric oxide (*nor* operon), and nitrous oxide (*nosZ*) reduction (Li et al., 2012, 2013) (Fig.2a), which are explained in detail in the following.

3.1 The nitrate reductase Nap poises an optimum redox conditions for biomineralization

In magnetospirilla, nitrate is reduced to nitrite by periplasmic nitrate reductase Nap, which serves as the major energy-generating step. It is essential for anaerobic growth, while the subsequent steps of denitrification do not support anoxic growth (Li et al., 2012). In addition, inactivation of Nap delayed aerobic growth and severely impacted magnetite biomineralization during denitrification and microaerobic oxygen reduction. The Δ*nap* mutant strain only produced irregular and smaller magnetosome particles and formed a loose chain (Li et al., 2012) (Fig.2b). Therefore, a role independent of nitrate reductase is speculated in magnetite biomineralization, that is to maintenance of the intracellular redox balance, thereby poising an optimum redox potential for magnetite synthesis (Li et al., 2012).

3.2 The nitrite reductase NirS oxidizes ferrous iron for anaerobic magnetosome formation and requires NirN for proper d_1 heme assembly

NirS, a cytochrome cd_1 nitrate reductase is not absolutely required for anaerobic growth and the deletion of *nirS* did not completely abolish growth without oxygen (Li et al., 2013). In 1995, NirS was for the first time suggested to be involved in magnetosome formation since NirS purified from MS-1 was able to oxidize ferrous iron in the absence of oxygen (Yamazaki et al., 1995). This hypothesis is demonstrated in MSR-1, and the deletion of *nirS* gene caused a defect of biomineralization: fewer, irregular, and smaller nanoparticles (Li et al., 2013) (Fig.2b). During nitrite reduction, NirS displays an activity of ferrous iron oxidation directly for magnetite biosynthesis (Li et al., 2013). In addition, similar phenotypes (poor anaerobic growth and impaired magnetite synthesis) were observed in AMB-1 upon concomitant interruption of *norB* and *nirS* (*amb1395*) (Wang et al., 2011). Different from one *nirS* in MSR-1, two *nirS* genes *amb4165* and *amb1395* are present in the genome of AMB-1, and the deletion of *amb1395* completely repressed the reduction of nitrite to nitric oxide (Wang et al., 2011). However, the expression of both *amb1395* and *amb4165* from AMB-1 under the control of the MSR-1 *nirS* promoter can completely rescue the growth and magnetosome formation of MSR-1 Δ*nirS* strain, indicating that the observed lacking function of *amb4165* in AMB-1 might be caused by transcriptional inactivity under tested conditions (Li et al., 2013).

NirN, a protein with previously unknown function shares a conspicuous sequence similarity to NirS. This raised the question whether NirN can also catalyze the reduction of nitrite to nitric oxide. However, despite the surficially similar phenotypes in

growth and magnetosome formation, closer inspection revealed that Δ*nirN* strain is still capable of nitrite reduction upon prolonged incubation, and NirN alone is not able to support nitrite reduction in the absence of the NirS (Li et al., 2013). It has been further shown that proper assembly of the d_1 heme in *holo*-NirS depends on the functional interaction with the NirN, thereby providing the first evidence for a physiological function of NirN in vivo (Li et al., 2013). Consistent with this observation, in *Pseudomonas aeruginosa* the cofactor content of NirS in Δ*nirN* mutant strain was found to be different from that in the wild type (WT) strain by UV-visible absorption spectroscopy of periplasmic fractions (Nicke et al., 2013). Later, NirN in *P. aeruginosa* has been demonstrated to act as a novel electron-bifurcating dehydrogenase catalyzing the last step of heme d_1 synthesis, and the deletion of *nirN* resulted in the accumulation of dihydro-heme d_1, which finally led to a different form of *holo*-NirS and decreased enzymatic activity (Adamczack et al., 2014; Klünemann et al., 2019).

3.3 The nitric oxide reductase Nor is important for biomineralization by yet-unknown functions

The NO reductase Nor also participates in magnetosome formation by yet unknown functions. In both MSR-1 and AMB-1, genetic inactivation of Nor resulted in the formation of fewer magnetosome particles during denitrification pathway (Wang et al., 2011; Li et al., 2012) (Fig.2b). In addition, deletion of *norCB* also led to thicker, shorter cell morphology, which may be caused by accumulated NO stress (Li et al., 2012). Differently, in *P. aeruginosa* PAO1 NO stress was shown to be the major cause for cell elongation and subsequent anaerobic biofilm formation (Hamada et al., 2014).

3.4 Nitrous oxide reductase Nos

Although in MSR-1 the deletion of *nosZ* does not affect magnetosome biosynthesis, a putative periplasmic Fe(II) oxidase identified in *Magnetovibrio blakemorei* strain MV-1 using N_2O as the terminal electron acceptor, was proposed as N_2O reductase NosZ (Bazylinski and Williams, 2006). Thus, N_2O reductase NosZ might be also involved in magnetite biomineralization.

Overall, denitrification enzymes, especially nitrate reductase Nap, nitrite reductase Nir, and nitric oxide reductase Nor, have important but non-essential functions in magnetite biomineralization, and phenotypes of these deletions' TEM micrographs are summarized in Fig.2b. However, their role in magnetosome formation has not yet been fully elucidated. For example, it has remained unknown which gene(s) of the *nap* operon is involved in redox homeostasis for biomineralization. Siponen et al. (2012) have suggested that NapC (a Nap protein essential for nitrate reduction, Li and Schüler, unpublished data) might be required to transfer electrons from the quinone pool to magnetochrome containing proteins. Moreover, it is worth mentioning that all sequenced MTB belonging to *Alphaproteobacteria* contain homologues of *nap* and *nor* clusters, although some of them are not capable of denitrification, further implying a key role of Nap and Nor in magnetosome formation (e.g. redox maintenance), which is independent of denitrification pathway. In other clades of MTB, distinct anaerobic respiratory enzymes may be evolved in biomineralization and resemble function with denitrification enzymes.

4 AEROBIC RESPIRATION AND MAGNETOSOME FORMATION

In addition to being an important environmental factor to affect magnetosome biosynthesis, O_2 mainly functions as a preferred electron acceptor for respiration and energy generation used for aerobic respiration by all magnetospirilla under microaerobic and aerobic conditions. By visible absorption spectroscopy, cytochromes for respiration were identified in MS-1, including *a*-, a_1-, *b*-, *c*-, cd_1-, and *o*-type hemes (O'Brien et al., 1987). More than 85% of the detected cytochromes belong to the *c*-type, which are mostly soluble, while the *a*- and *b*-type cytochromes are mainly found in membrane fractions. Since a_1 hemes ('low aeration' cytochrome oxidase) and *o* hemes ('high aeration' cytochrome oxidase) were simultaneously identified in MS-1, O'Brien et al. (1987) proposed that the aerobic respiration chain is branched. Later, a novel 'cytochrome a_1-like' hemoprotein was purified from MS-1, which displayed weak cytochrome *c* oxidase activity in vitro (Tamegai et al., 1993). The observation that the hemoprotein is present in higher amounts in magnetic compared to nonmagnetic cells suggests that it might be involved in magnetosome formation (Tamegai et al., 1993). In the same organism, Tamegai and Fukumori (1994) identified a novel *cbb*-type cytochrome *c* oxidase, which is assumed to function as the terminal oxidase for O_2 respiration under

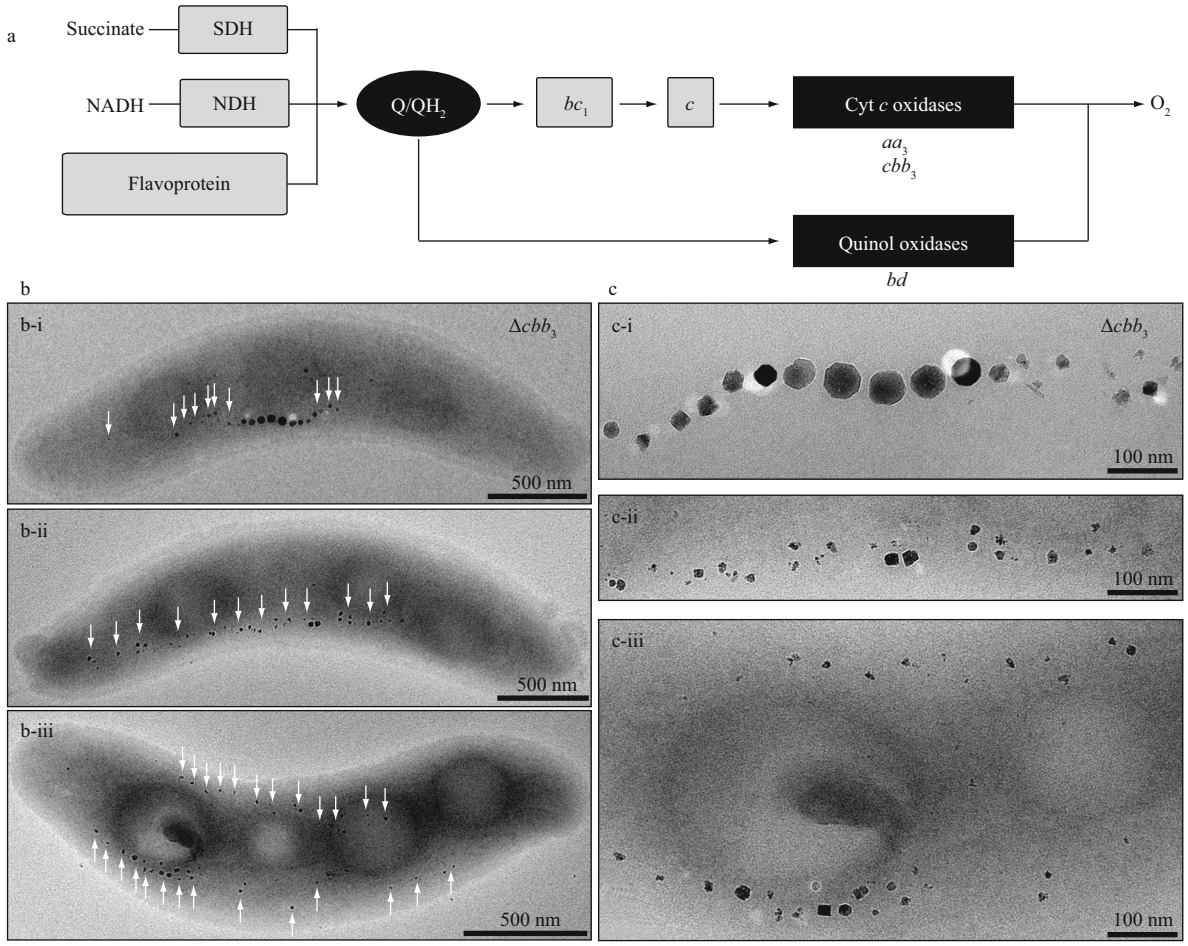

Fig.3 The *cbb*$_3$ deficient strain of MSR-1 shows various magnetosome phenotypes under microaerobic conditions in the presence of nitrate

a. aerobic respiration pathway in MSR-1; b. different magnetosome phenotypes and chain assembly in Δ*cbb*$_3$ strain; c. close-up views of the magnetosome particles shown in panel b. images in panel b and c are adapted from Li et al. (2014a) and amended with permission from American Society for Microbiology. NADH: nicotinamide adenine dinucleotide; SDH: succinate dehydrogenase; NDH: NADH dehydrogenase; Q: ubiquinone; QH2: ubiquinol. Irregularly shaped particles are indicated by white arrows.

microaerobic conditions.

Studies on oxygen terminal oxidases provide mechanistic insight into the function of aerobic respiration on magnetite biomineralization. Although in the genome of MSR-1 three operons encoding putative terminal *cbb*$_3$-type, *aa*$_3$-type, and *bd*-type oxidases are present (Fig.3a), only *cbb*$_3$ and *bd* were found to be essential for aerobic respiration, whereas *aa*$_3$ oxidase had no physiological significance under tested conditions (Li et al., 2014a). The *bd* oxidase is only required for O_2-dependent growth in the absence of *cbb*$_3$, and not involved in magnetosome formation. The *cbb*$_3$ oxidase appears to be the most important enzymes for not only O_2-dependent growth, but also denitrification and biomineralization. For example, *cbb*$_3$ mutant strains are incapable of simultaneous nitrate and O_2 reduction, but reduce O_2 firstly followed by nitrate reduction (Li et al., 2014a). With respect to biomineralization, loss of *cbb*$_3$ leads to subtle effects on biomineralization in the absence of nitrate under microaerobic conditions. However, pleiotropic effects on magnetosome formation and organization were observed in the presence of nitrate, mainly including (i) WT-like particles in the center of magnetosome chain, accompanied by smaller and irregularly shaped particles at each end, (ii) small and irregular particles arranged in loose chains, and (iii) irregular magnetosomes present two loose chains bent and adapted to the inner and outer curvatures (Fig.3b–c). The most conspicuous phenotype was that *cbb*$_3$ deficient strains form two loose magnetosome chains at each side of the cell, resembling those in Δ*mamY* mutant cells (Toro-Nahuelpan et al., 2019). Therefore, a potential link may be present between *cbb*$_3$ oxidase and MamY. Furthermore, reduced redox states in *cbb*$_3$ deficient strains indicate that observed defects in

magnetosome biosynthesis and organization probably results from the disturbed redox balance required for magnetosome biomineralization (Li et al., 2014a). In agreement with this, dynamic light scattering shows that MamY oligomerization in vitro is controlled by pH changed and fully reversible (Toro-Nahuelpan et al., 2019). Therefore, cbb_3 oxidase probably provides an initial redox environment to facilitate the function of magnetosome proteins (e.g. MamY) for biomineralization and magnetosome organization.

5 MAGNETOSOME-ASSOCIATED PROTEINS INVOLVED IN REDOX CONTROL OF BIOMINERALIZATION

Once soluble iron ions are obtained by MTB, they must be precipitated chemically to become insoluble magnetite crystal deposits, which requires a balanced ratio of ferrous and ferric iron (Mann et al., 1990; Faivre et al., 2004, 2007). Faivre et al. (2004) found that the formation of abiotic magnetite requires a rather high pH and high concentration of iron. In addition to general respiration pathways, some magnetosome-associated proteins are also suggested to be involved in redox control for magnetite biomineralization (Raschdorf et al., 2013; Müller et al., 2014). Some of these proteins, such as MamZ and FtsZm, display different effects on iron biomineralization between in the presence of nitrate and its absence (Raschdorf et al., 2013; Müller et al., 2014). MamZ even show similar but independent roles in redox control of magnetite synthesis with the mentioned Nap (Raschdorf et al., 2013). In contrast, FtsZm is involved in magnetosome formation only under nitrate deprivation (Müller et al., 2014). Recently, Wang et al. (2019a) found that FtsZm is capable of polymerization and subsequently recruits MamY, MamX, and MamZ.

In addition, MTB also contain a set of special redox proteins including MamE, MamP, MamT, and MamX, which are encoded by genes within magnetosome island (Siponen et al., 2012; Raschdorf et al., 2013). These proteins share a unique configuration of two close CXXCH heme-binding motifs (Grünberg et al., 2004), termed the magnetochrome domain, and spectral and redox characteristics of purified MamE and MamP further confirms that the identical magnetochrome domain is a cytochrome *c*-like domain (Siponen et al., 2012). Indeed, deletions of these genes resulted in the formation of irregular magnetosome particles (Murat et al., 2010; Raschdorf et al., 2013; Yang et al., 2013; Lohße et al., 2014). In addition, an allele of *mamE* with a point mutation in the CXXCH motif only partially complemented magnetite formation in the *mamE* deficient strain, further indicating a role for heme-binding domain in magnetite biomineralization (Quinlan et al., 2011). Loss of MamX putative magnetochrome domains also caused an identical biomineralization defect, demonstrating again, this CXXCH motif is associated with crystal formation (Raschdorf et al., 2013). Because some of these proteins including MamP and MamE also contain protein-interaction PDZ domain, Siponen et al. (2012) suggested that these proteins may form a protein complex, which serves as an electron transport chain to further regulate electron flow for magnetosome biomineralization. Furthermore, the crystal structure of MamP from marine magnetotactic ovoidal bacterium MO-1 provided the first insight into the role of this new type of cytochromes in iron biomineralization (Siponen et al., 2013). Studies of MamP and MamT in AMB-1 further verified that double CXXCH heme-binding motifs of MamP and MamT are essential for magnetite formation, supporting a physiological function for redox process (Jones et al., 2015).

6 REGULATION OF REDOX CONTROL FOR MAGNETOSOME FORMATION

As mentioned above, more and more observations imply that MTB need to control the redox state of the minerals by using multiply electron transport chains or pathways. Since MTB seem to employ general respiratory enzymes for magnetite biomineralization, it might be a common feature of bacterial biomineralization, which has been proposed by Rahn-Lee and Komeili (2013). If so, the modulation or redox control of magnetite biosynthesis may be accomplished by respiration pathways. In *Escherichia coli* and other bacteria, regulatory switch between aerobic and microaerobic metabolism is primarily governed by an Fnr (fumarate and nitrate reduction) regulator (Unden et al., 1995; Bueno et al., 2012). In MSR-1, inactivation of the Fnr homologue MgFnr not only decreased N_2 production due to reduced N_2O reductase activity, but also impaired magnetite biosynthesis under microaerobic conditions in the presence of nitrate (Li et al., 2014b). When MgFnr is overexpressed in the WT, cells would synthesize smaller magnetite nanoparticles during denitrifying growth, suggesting that its proper expression is

critical for WT-like magnetite formation (Li et al., 2014b). Analyses of transcriptional *gusA* reporter fusions revealed that MgFnr is involved in governing the expression of denitrification genes and thereby plays an indirect role in keeping proper redox conditions required for magnetosome formation (Li et al., 2014b). Recently, another Fnr homologue Mg2046 is also shown to be involved in biomineralization. The Δ*mg2046* strain displayed similar growth with WT cells, but synthesized fewer and smaller magnetosomes (Wang et al., 2019b). Further transcriptional analysis revealed that Mg2046 severs as a redox regulator to modulate genes coding for iron uptake, oxygen respiration, and denitrification. In the same organism, a global carbon and energy metabolism regulator Crp (3′-5′-cyclic adenosine monophosphate receptor protein) was found to play an important role in magnetosome biosynthesis (Wen et al., 2016). Disruption of *crp* dramatically reduced intracellular iron content and resulted in fewer magnetosome particles produced in the mutant strains. Transcription expression profile analyses showed that lack of *crp* not only affected the expression of both genes involving in carbon and energy metabolism, but also downregulated the transcription of all tested MAI genes, including *mamJ*, *mamC*, *feoB1*, *mms6*, and *ftsZm* (Wen et al., 2016). In 2017, a novel OxyR homologue OxyR-Like was identified to be involved in the regulation of carbon metabolism, and its deletion led to magnetosome consisting of not only magnetite, but also α-Fe_2O_3 and ε-Fe_2O_3 (Zhang et al., 2017). In addition to genes encoding tricarboxylic acid (TCA) cycle, MAI genes (*mamJ*, *mamC*, *mms6*, and *ftsZm*) are also controlled by OxyR-Like, and disruption of OxyR-Like caused a reduced expression of these genes (Zhang et al., 2017).

However, since their relative subtle effects on oxygen-dependent magnetosome formation, none of these identified regulators can account for the observed complete inhibition to produce magnetite particles under aerobic conditions. In this case, magnetite biomineralization is probably governed by other unknown O_2 sensors through controlling intracellular redox conditions. The global transcriptome analysis between aerobic- and microaerobic-culturing MSR-1 may provide some candidate sensors (e.g. Anr-Dnr, DnaA, and PvdS) responsible for redox control, which exhibit different expression under different oxygen conditions (Wang et al., 2016). Therefore, a more expanded investigation for regulators involved in redox control may gain insights into the mechanism of oxygen-repressed magnetosome formation.

7 OUTLOOK

7.1 What is the role of denitrification and oxygen respiration in magnetosome formation?

There is no doubt that general respiratory pathways, such as denitrification and oxygen respiration play a vital role in redox control for magnetosome formation. Based on current data, I propose that respiratory enzymes, such as denitrification enzymes Nap and O_2 terminal oxidase cbb_3, act as the primary contributors controlling intracellular redox conditions. In addition, magnetochrome proteins (MamE, MamP, and MamT) fine-tune microenvironmental redox balance for magnetosome synthesis. In turn, the suitable redox environment promotes other redox-associated magnetosome proteins, such as MamY and FtsZm, to polymerize and recruit other players for magnetosome synthesis and chain formation (Fig.4b). From the schematic overview, it becomes apparent that a core cofactor, heme, is present in most proteins involved in redox modulation (Fig.4, yellow rectangles). On the other hand, heme as a versatile and indispensable cofactor in vivo, can catalyze a wide range of redox reaction in the company of different proteins (Mayfield et al., 2011). Although transcription and translation of magnetochrome proteins are not modulated by oxygen, their cofactor heme is able to mediate electron transfer in response to different redox conditions. Therefore, it is possible that heme is the major player controlling redox process for magnetosome formation, which may be the reason why the global iron regulator Fur plays only a rather minor role in magnetite synthesis in MSR-1 (Uebe et al., 2010; Qi et al., 2012). Alternatively, there are three *irr* homologues (*irrA*, *irrB*, and *irrC*) present in the genome of MSR-1, which mainly perceive and respond to the synthesis of heme (Chandrangsu et al., 2017). These Irr regulators are probably involved in the control of redox process for biomineralization. The deletion of *irrB* resulted in not only impaired growth and magnetosome synthesis, but also decreased expression of genes coding for iron transport and storage, heme biosynthesis, and Fe-S cluster assembly (Wang et al., 2015). However, the links among the three Irr regulators, as well as between heme and biomineralization, are yet little known. A comprehensive analysis of the relationship between

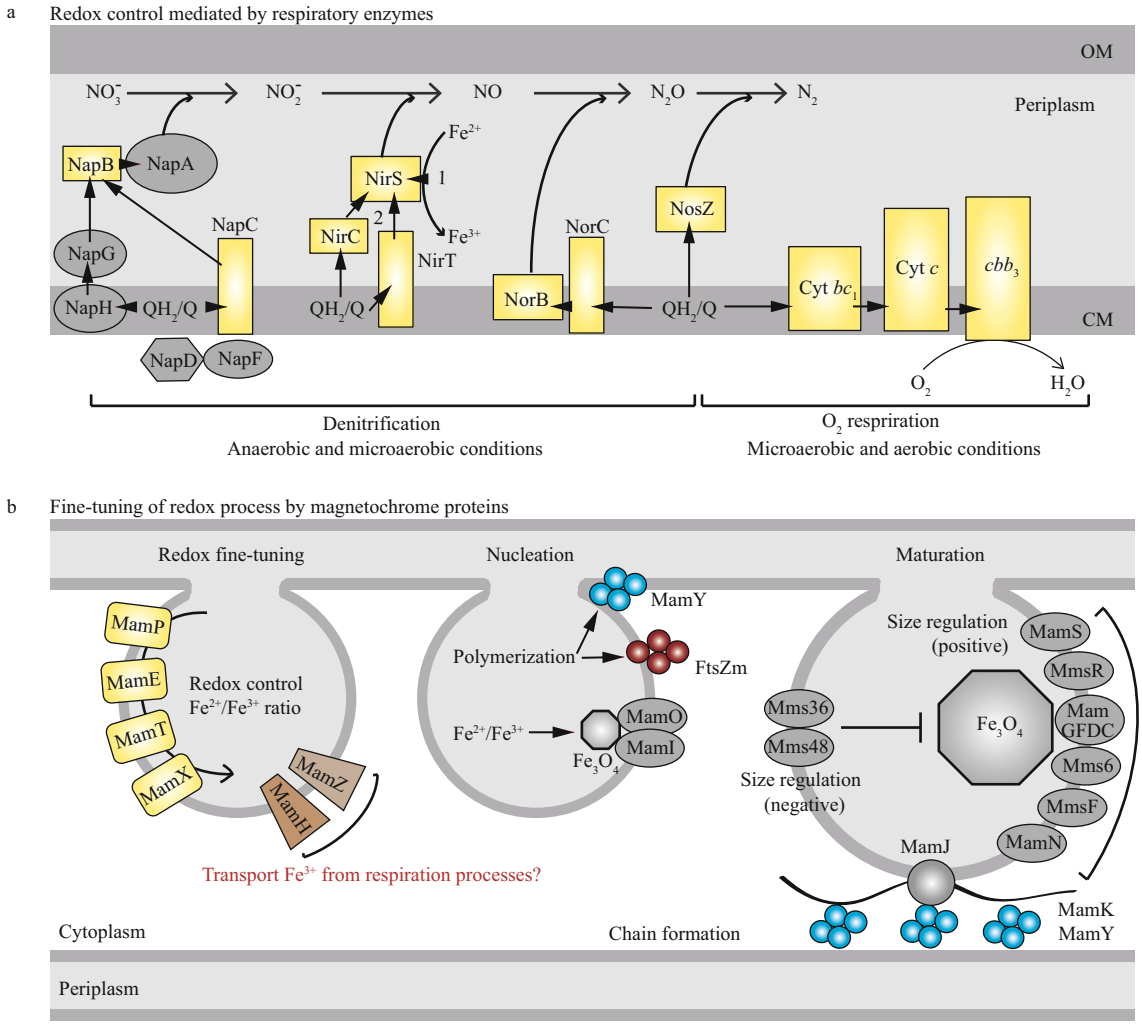

Fig.4 Proposed link between redox processes and biomineralization

a. respiratory pathways including denitrification and O_2 reduction provide the initial redox hemostasis; b. magnetochrome proteins (MamP, MamE, MamT, and MamX) subsequently fine-tune redox conditions in magnetosomal vesicle. This redox control facilitates the polymerization of MamY and FtsZm, and a suitable ratio of Fe^{2+} and Fe^{3+} for magnetosome nucleation. In addition, MamH and MamZ may transport Fe^{3+} from respiration pathways. Heme-binding proteins are indicated in yellow rectangles; OM, outer membrane; CM, cytoplasmic membrane.

Irrs orthologs and heme might provide new insight into the role of heme in redox control and magnetosome formation.

7.2 Is it possible to engineer denitrification pathway or oxygen respiration to improve magnetosome yields?

Due to their proved biocompatibility, magnetosomes were found to be outstanding magnetic nanoparticles in various biotechnological and biomedical applications. However, most magnetotactic bacteria are recalcitrant to grow in the laboratory, and furthermore, even ones capable of cultivating, as mentioned above are only to synthesize magnetosomes under microoxic conditions with poor yields of magnetosomes. Therefore, a major challenge for potential applications is the requirement of the enormous amount of magnetosomes by mass cultivation. In 2014, Schüler and colleagues (Kolinko et al., 2014) for the first time successfully developed a "magnetized non-magnetotactic bacterium" *Rhodospirillum rubrum* by heteroexpressing genes associated with magnetosome formation. A subsequent study revealed that overexpression of magnetosome genes in MSR-1 by genomic amplification of gene clusters via sequential chromosomal insertion dramatically improves magnetosome yields, a potential strategy for the design and mass production of size adjusted nanoparticles with tuned magnetic properties (Lohße et al., 2016). Here there are also some clues as to the genetic factors during respiration pathways that play

an important role in biomineralization. For example, because the conversion of nitrate to nitrite catalyzed by periplasmic reductase Nap is the major energy-generating step during denitrification, the use of stronger promoter in front of *nap* cluster or more energy-producing membrane nitrate reductase Nar might possibly lead to increased growth, and consequently to supply more energy required for magnetosome biosynthesis. For example, the incomplete denitrifier *Ensifer meliloti* 1021 cannot grow anoxically due to low expression level of *nap* despite of containing a complete set of denitrification genes, while the overexpression of *nap* cluster rescues the capability of anaerobic growth in the presence of nitrate (Torres et al., 2018). Therefore, it may be possible to improve cultivation and magnetosome yield by genetic engineering of redox pathways in the future.

8 DATA AVAILABILITY STATEMENT

All data generated and analyzed during the current study are available from the corresponding author upon request.

References

Adamczack J, Hoffmann M, Papke U, Haufschildt K, Nicke T, Bröring M, Sezer M, Weimar R, Kuhlmann U, Hildebrandt P, Layer G. 2014. NirN protein from *Pseudomonas aeruginosa* is a novel electron-bifurcating dehydrogenase catalyzing the last step of heme d_1 biosynthesis. *The Journal of Biological Chemistry*, **289**(44): 30753-30762, https://doi.org/10.1074/jbc.M114.603886.

Bazylinski D A, Blakemore R P. 1983. Denitrification and assimilatory nitrate reduction in *Aquaspirillum magnetotacticum*. *Applied and Environmental Microbiology*, **46**(5): 1118-1124, https://doi.org/10.1128/AEM.46.5.1118-1124.1983.

Bazylinski D A, Williams T J. 2006. Ecophysiology of magnetotactic bacteria. *In*: Schüler D ed. Magnetoreception and Magnetosomes in Bacteria. Springer, Heidelberg. p.37-75, https://doi.org/10.1007/7171_038.

Blakemore R P, Short K A, Bazylinski D A, Rosenblatt C, Frankel R B. 1985. Microaerobic conditions are required for magnetite formation within *Aquaspirillum magnetotacticum*. *Geomicrobiology Journal*, **4**(1): 53-71, https://doi.org/10.1080/01490458509385920.

Bueno E, Mesa S, Bedmar E J, Richardson D J, Delgado M J. 2012. Bacterial adaptation of respiration from oxic to microoxic and anoxic conditions: redox control. *Antioxidants & Redox Signaling*, **16**(8): 819-852, https://doi.org/10.1089/ars.2011.4051.

Chandrangsu P, Rensing C, Helmann J D. 2017. Metal homeostasis and resistance in bacteria. *Nature Reviews Microbiology*, **15**(6): 338-350, https://doi.org/10.1038/nrmicro.2017.15.

Faivre D, Agrinier P, Menguy N, Zuddas P, Pachana K, Gloter A, Laval J Y, Guyot F. 2004. Mineralogical and isotopic properties of inorganic nanocrystalline magnetites. *Geochimica et Cosmochimica Acta*, **68**(21): 4395-4403, https://doi.org/10.1016/j.gca.2004.03.016.

Faivre D, Böttger L H, Matzanke B F, Schüler D. 2007. Intracellular magnetite biomineralization in bacteria proceeds by a distinct pathway involving membrane-bound ferritin and an iron(II) species. *Angewandte Chemie International Edition*, **46**(44): 8495-8499, https://doi.org/10.1002/anie.200700927.

Faivre D, Schüler D. 2008. Magnetotactic bacteria and magnetosomes. *Chemical Reviews*, **108**(11): 4875-4898, https://doi.org/10.1021/cr078258w.

Grünberg K, Müller E C, Otto A, Reszka R, Linder D, Kube M, Reinhardt R, Schüler D. 2004. Biochemical and proteomic analysis of the magnetosome membrane in *Magnetospirillum gryphiswaldense*. *Applied and Environmental Microbiology*, **70**(2): 1040-1050, https://doi.org/10.1128/aem.70.2.1040-1050.2004.

Hamada M, Toyofuku M, Miyano T, Nomura N. 2014. cbb_3-type cytochrome *c* oxidases, aerobic respiratory enzymes, impact the anaerobic life of *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology*, **196**(22): 3881-3889, https://doi.org/10.1128/JB.01978-14.

Jogler C, Schüler D. 2009. Genomics, genetics, and cell biology of magnetosome formation. *Annual Review of Microbiology*, **63**: 501-521, https://doi.org/10.1146/annurev.micro.62.081307.162908.

Jones S R, Wilson T D, Brown M E, Rahn-Lee L, Yu Y, Fredriksen L L, Ozyamak E, Komeili A, Chang M C Y. 2015. Genetic and biochemical investigations of the role of MamP in redox control of iron biomineralization in *Magnetospirillum magneticum*. *Proceedings of the National Academy of Sciences of the United States of America*, **112**(13): 3904-3909, https://doi.org/10.1073/pnas.1417614112.

Klünemann T, Preuß A, Adamczack J, Rosa L F M, Harnisch F, Layer G, Blankenfeldt W. 2019. Crystal structure of dihydro-heme d_1 dehydrogenase NirN from *Pseudomonas aeruginosa* reveals amino acid residues essential for catalysis. *Journal of Molecular Biology*, **431**(17): 3246-3260, https://doi.org/10.1016/j.jmb.2019.05.046.

Kolinko I, Lohße A, Borg S, Raschdorf O, Jogler C, Tu Q, Pósfai M, Tompa É, Plitzko J M, Brachmann A, Wanner G, Müller R, Zhang Y M, Schüler D. 2014. Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters. *Nature Nanotechnology*, **9**(3): 193-197, https://doi.org/10.1038/nnano.2014.13.

Li Y J, Bali S, Borg S, Schüler D. 2013. Cytochrome cd_1 nitrite reductase NirS is involved in anaerobic magnetite biomineralization in *Magnetospirillum gryphiswaldense* and requires NirN for proper d_1 heme assembly. *Journal*

of Bacteriology, **195**(18): 4297-4309, https://doi.org/10.1128/JB.00686-13.

Li Y J, Katzmann E, Borg S, Schüler D. 2012. The periplasmic nitrate reductase Nap is required for anaerobic growth and involved in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. *Journal of Bacteriology*, **194**(18): 4847-4856, https://doi.org/10.1128/JB.00903-12.

Li Y J, Raschdorf O, Silva K T, Schüler D. 2014a. The terminal oxidase cbb_3 functions in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. *Journal of Bacteriology*, **196**(14): 2552-2562, https://doi.org/10.1128/JB.01652-14.

Li Y J, Sabaty M, Borg S, Silva K T, Pignol D, Schüler D. 2014b. The oxygen sensor MgFnr controls magnetite biomineralization by regulation of denitrification in *Magnetospirillum gryphiswaldense*. *BMC Microbiology*, **14**: 153, https://doi.org/10.1186/1471-2180-14-153.

Lohße A, Borg S, Raschdorf O, Kolinko I, Tompa É, Pósfai M, Faivre D, Baumgartner J, Schüler D. 2014. Genetic dissection of the *mamAB* and *mms6* operons reveals a gene set essential for magnetosome biogenesis in *Magnetospirillum gryphiswaldense*. *Journal of Bacteriology*, **196**(14): 2658-2669, https://doi.org/10.1128/JB.01716-14.

Lohße A, Kolinko I, Raschdorf O, Uebe R, Borg S, Brachmann A, Plitzko J M, Müller R, Zhang Y M, Schüler D, Vieille C. 2016. Overproduction of magnetosomes by genomic amplification of biosynthesis-related gene clusters in a magnetotactic bacterium. *Applied and Environmental Microbiology*, **82**(10): 3032-3041, https://doi.org/10.1128/AEM.03860-15.

Lohße A, Ullrich S, Katzmann E, Borg S, Wanner G, Richter M, Voigt B, Schweder T, Schüler D. 2011. Functional analysis of the magnetosome island in *Magnetospirillum gryphiswaldense*: the *mamAB* operon is sufficient for magnetite biomineralization. *PLoS One*, **6**(10): e25561, https://doi.org/10.1371/journal.pone.0025561.

Lower B H, Bazylinski D A. 2013. The bacterial magnetosome: a unique prokaryotic organelle. *Journal of Molecular Microbiology and Biotechnology*, **23**(1-2): 63-80, https://doi.org/10.1159/000346543.

Mandernack K W, Bazylinski D A, Shanks III W C, Bullen T W. 1999. Oxygen and iron isotope studies of magnetite produced by magnetotactic bacteria. *Science*, **285**(5435): 1892-1896, https://doi.org/10.1126/science.285.5435.1892.

Mann S, Sparks N H C, Board R G. 1990. Magnetotactic bacteria: microbiology, biomineralization, palaeomagnetism and biotechnology. *Advances in Microbial Physiology*, **31**: 125-181, https://doi.org/10.1016/S0065-2911(08)60121-6.

Matsunaga T, Sakaguchi T, Tadakoro F. 1991. Magnetite formation by a magnetic bacterium capable of growing aerobically. *Applied Microbiology and Biotechnology*, **35**(5): 651-655, https://doi.org/10.1007/BF00169632.

Matsunaga T, Tsujimura N. 1993. Respiratory inhibitors of a magnetic bacterium *Magnetospirillum* sp. AMB-1 capable of growing aerobically. *Applied Microbiology and Biotechnology*, **39**(3): 368-371, https://doi.org/10.1007/BF00192094.

Mayfield J A, Dehner C A, DuBois J L. 2011. Recent advances in bacterial heme protein biochemistry. *Current Opinion in Chemical Biology*, **15**(2): 260-266, https://doi.org/10.1016/j.cbpa.2011.02.002.

McCausland H C, Komeili A. 2020. Magnetic genes: studying the genetics of biomineralization in magnetotactic bacteria. *PLoS Genetics*, **16**(2): e1008499, https://doi.org/10.1371/journal.pgen.1008499.

Müller F D, Raschdorf O, Nudelman H, Messerer M, Katzmann E, Plitzko J M, Zarivach R, Schüler D. 2014. The FtsZ-Like protein FtsZm of *Magnetospirillum gryphiswaldense* likely interacts with its generic homolog and is required for biomineralization under nitrate deprivation. *Journal of Bacteriology*, **196**(3): 650-659, https://doi.org/10.1128/JB.00804-13.

Murat D, Quinlan A, Vali H, Komeili A. 2010. Comprehensive genetic dissection of the magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(12): 5593-5598, https://doi.org/10.1073/pnas.0914439107.

Nicke T, Schnitzer T, Münch K, Adamczack J, Haufschildt K, Buchmeier S, Kucklick M, Felgenträger U, Jänsch L, Riedel K, Layer G. 2013. Maturation of the cytochrome cd_1 nitrite reductase NirS from *Pseudomonas aeruginosa* requires transient interactions between the three proteins NirS, NirN and NirF. *Bioscience Reports*, **33**(3): e00048, https://doi.org/10.1042/BSR20130043.

O'Brien W, Paoletti L C, Blakemore R P. 1987. Spectral analysis of cytochromes in *Aquaspirillum magnetotacticum*. *Current Microbiology*, **15**(3): 121-127, https://doi.org/10.1007/BF01577258.

Qi L, Li J, Zhang W J, Liu J N, Rong C B, Li Y, Wu L F. 2012. Fur in *Magnetospirillum gryphiswaldense* influences magnetosomes formation and directly regulates the genes involved in iron and oxygen metabolism. *PLoS One*, **7**(1): e29572, https://doi.org/10.1371/journal.pone.0029572.

Quinlan A, Murat D, Vali H, Komeili A. 2011. The HtrA/DegP family protease MamE is a bifunctional protein with roles in magnetosome protein localization and magnetite biomineralization. *Molecular Microbiology*, **80**(4): 1075-1087, https://doi.org/10.1111/j.1365-2958.2011. 07631.x.

Rahn-Lee L, Komeili A. 2013. The magnetosome model: insights into the mechanisms of bacterial biomineralization. *Frontiers in Microbiology*, **4**: 352, https://doi.org/10.3389/fmicb.2013.00352.

Raschdorf O, Forstner Y, Kolinko I, Uebe R, Plitzko J M, Schüler D. 2016. Genetic and ultrastructural analysis reveals the key players and initial steps of bacterial magnetosome membrane biogenesis. *PLoS Genetics*, **12**(6): e1006101, https://doi.org/10.1371/journal.pgen.1006101.

Raschdorf O, Müller F D, Pósfai M, Plitzko J M, Schüler D.

2013. The magnetosome proteins MamX, MamZ and MamH are involved in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. *Molecular Microbiology*, **89**(5): 872-886, https://doi.org/10.1111/mmi.12317.

Sakaguchi H, Hagiwara H, Fukumori Y, Tamaura Y, Funaki M, Hirose S. 1993. Oxygen concentration-dependent induction of a 140-kDa protein in magnetic bacterium *Magnetospirillum magnetotacticum* MS-1. *FEMS Microbiology Letters*, **107**(2-3): 169-173, https://doi.org/10.1111/j.1574-6968.1993.tb06025.x.

Schübbe S, Wurdemann C, Peplies J, Heyen U, Wawer C, Glöckner F O, Schüler D. 2006. Transcriptional organization and regulation of magnetosome operons in *Magnetospirillum gryphiswaldense*. *Applied and Environmental Microbiology*, **72**(9): 5757-5765, https://doi.org/10.1128/AEM.00201-06.

Schüler D. 2008. Genetics and cell biology of magnetosome formation in magnetotactic bacteria. *FEMS Microbiology Reviews*, **32**(4): 654-672, https://doi.org/10.1111/j.1574-6976.2008.00116.x.

Short K A, Blakemore R P. 1989. Periplasmic superoxide dismutases in *Aquaspirillum magnetotacticum*. *Archives of Microbiology*, **152**(4): 342-346, https://doi.org/10.1007/BF00425171.

Siponen M I, Adryanczyk G, Ginet N, Arnoux P, Pignol D. 2012. Magnetochrome: a *c*-type cytochrome domain specific to magnetotatic bacteria. *Biochemical Society Transactions*, **40**(6): 1319-1323, https://doi.org/10.1042/BST20120104.

Siponen M I, Legrand P, Widdrat M, Jones S R, Zhang W J, Chang M C Y, Faivre D, Arnoux P, Pignol D. 2013. Structural insight into magnetochrome-mediated magnetite biomineralization. *Nature*, **502**(7473): 681-684, https://doi.org/10.1038/nature12573.

Tamegai H, Fukumori Y. 1994. Purification, and some molecular and enzymatic features of a novel *ccb*-type cytochrome *c* oxidase from a microaerobic denitrifier, *Magnetospirillum magnetotacticum*. *FEBS Letters*, **347**(1): 22-26, https://doi.org/10.1016/0014-5793(94)00500-1.

Tamegai H, Yamanaka T, Fukumori Y. 1993. Purification and properties of a 'cytochrome a_1'-like hemoprotein from a magnetotactic bacterium, *Aquaspirillum magnetotacticum*. *Biochimica et Biophysica Acta* (*BBA*) - *General Subjects*, **1158**(3): 237-243, https://doi.org/10.1016/0304-4165(93)90020-9.

Toro-Nahuelpan M, Giacomelli G, Raschdorf O, Borg S, Plitzko J M, Bramkamp M, Schüler D, Müller F D. 2019. MamY is a membrane-bound protein that aligns magnetosomes and the motility axis of helical magnetotactic bacteria. *Nature Microbiology*, **4**(11): 1978-1989, https://doi.org/10.1038/s41564-019-0512-8.

Torres M J, Avila S, Bedmar E J, Delgado M J. 2018. Overexpression of the periplasmic nitrate reductase supports anaerobic growth by *Ensifer meliloti*. *FEMS Microbiology Letters*, **365**(7): fny041, https://doi.org/10.1093/femsle/fny041.

Uebe R, Schüler D. 2016. Magnetosome biogenesis in magnetotactic bacteria. *Nature Reviews Microbiology*, **14**(10): 621-637, https://doi.org/10.1038/nrmicro.2016.99.

Uebe R, Voigt B, Schweder T, Albrecht D, Katzmann E, Lang C, Böttger L, Matzanke B, Schüler D. 2010. Deletion of a *fur*-like gene affects iron homeostasis and magnetosome formation in *Magnetospirillum gryphiswaldense*. *Journal of Bacteriology*, **192**(16): 4192-4204, https://doi.org/10.1128/JB.00319-10.

Ullrich S, Kube M, Schübbe S, Reinhardt R, Schüler D. 2005. A hypervariable 130-kilobase genomic region of *Magnetospirillum gryphiswaldense* comprises a magnetosome island which undergoes frequent rearrangements during stationary growth. *Journal of Bacteriology*, **187**(21): 7176-7184, https://doi.org/10.1128/JB.187.21.7176-7184.2005.

Unden G, Becker S, Bongaerts J, Holighaus G, Schirawski J, Six S. 1995. O_2-sensing and O_2-dependent gene regulation in facultatively anaerobic bacteria. *Archives of Microbiology*, **164**(2): 81-90, https://doi.org/10.1007/BF02525312.

Wang K, Ge X, Bo T, Chen Q, Chen G, Liu W. 2011. Interruption of the denitrification pathway influences cell growth and magnetosome formation in *Magnetospirillum magneticum* AMB-1. *Letters in Applied Microbiology*, **53**(1): 55-62, https://doi.org/10.1111/j.1472-765X.2011.03063.x.

Wang Q, Wang M W, Wang X, Guan G H, Li Y, Peng Y L, Li J L. 2015. Iron response regulator protein IrrB in *Magnetospirillum gryphiswaldense* MSR-1 helps control the iron/oxygen balance, oxidative stress tolerance, and magnetosome formation. *Applied and Environmental Microbiology*, **81**(23): 8044-8053, https://doi.org/10.1128/AEM.02585-15.

Wang Q, Wu S, Li X Y, Zhang T W, Yang J, Wang X, Li F, Li Y, Peng Y L, Li J L. 2019a. Work patterns of MamXY proteins during magnetosome formation in *Magnetospirillum gryphiswaldense* MSR-1. *Applied and Environmental Microbiology*, **85**(2): e02394-18, https://doi.org/10.1128/AEM.02394-18.

Wang X, Wang Q, Zhang Y, Wang Y J, Zhou Y, Zhang W J, Wen T, Li L, Zuo M Q, Zhang Z D, Tian J S, Jiang W, Li Y, Wang L, Li J L. 2016. Transcriptome analysis reveals physiological characteristics required for magnetosome formation in *Magnetospirillum gryphiswaldense* MSR-1. *Environmental Microbiology Reports*, **8**(3): 371-381, https://doi.org/10.1111/1758-2229.12395.

Wang X, Zheng H L, Wang Q, Jiang W, Wen Y, Tian J S, Sun J B, Li Y, Li J L. 2019b. Novel protein Mg2046 regulates magnetosome synthesis in *Magnetospirillum gryphiswaldense* MSR-1 by modulating a proper redox status. *Frontiers in Microbiology*, **10**: 1478, https://doi.org/10.3389/fmicb.2019.01478.

Wen T, Guo F F, Zhang Y P, Tian J S, Li Y, Li J L, Jiang W. 2016. A novel role for Crp in controlling magnetosome

biosynthesis in *Magnetospirillum gryphiswaldense* MSR-1. *Scientific Reports*, **6**: 21156, https://doi.org/10.1038/srep21156.

Yamazaki T, Oyanagi H, Fujiwara T, Fukumori Y. 1995. Nitrite reductase from the magnetotactic bacterium *Magnetospirillum magnetotacticum*. *European Journal of Biochemistry*, **233**(2): 665-671, https://doi.org/10.1111/j.1432-1033.1995.665_2.x.

Yang C D, Takeyama H, Tanaka T, Matsunaga T. 2001. Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant *Magnetospirillum magneticum* AMB-1. *Enzyme and Microbial Technology*, **29**(1): 13-19, https://doi.org/10.1016/S0141-0229(01)00343-X.

Yang J, Li S Q, Huang X L, Li J H, Li L, Pan Y X, Li Y. 2013. MamX encoded by the mamXY operon is involved in control of magnetosome maturation in *Magnetospirillum gryphiswaldense* MSR-1. *BMC Microbiology*, **13**: 203, https://doi.org/10.1186/1471-2180-13-203.

Zhang Y P, Wen T, Guo F F, Geng Y Y, Liu J Q, Peng T, Guan G H, Tian J S, Li Y, Li J L, Ju J, Jiang W. 2017. The disruption of an OxyR-Like protein impairs intracellular magnetite biomineralization in *Magnetospirillum gryphiswaldense* MSR-1. *Frontiers in Microbiology*, **8**: 208, https://doi.org/10.3389/fmicb.2017.00208.

Electronic supplementary material

Supplementary material (Supplementary Figs.S1–S2) is available in the online version of this article at https://doi.org/10.1007/s00343-021-0422-5.