MMS6 Protein Regulates Crystal Morphology during Nano-sized Magnetite Biomineralization \textit{in Vivo}\footnote{This work was funded by a Grant-in-Aid for Scientific Research (A) (No. 20246119) from the Japan Society for the Promotion of Science (JSPS), and the Grant-in-Aid for Scientific Research on Innovative Areas of “Fusion Materials: Creative Development of Materials and Exploration of Their Function through Molecular Control” (No. 2206) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).}

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Masayoshi Tanaka, Eri Mazuyama, Atsushi Arakaki, and Tadashi Matsunaga

From the Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

Biominalization, the process by which minerals are deposited by organisms, has attracted considerable attention because this mechanism has shown great potential to inspire bottom-up material syntheses. To understand the mechanism for morphological regulation that occurs during biomineralization, many regulatory proteins have been isolated from various biominerals. However, the molecular mechanisms that regulate the morphology of biominerals remain unclear because there is a lack of \textit{in vivo} evidence. Magnetotactic bacteria synthesize intracellular magnetosomes that comprise membrane-enveloped single crystalline magnetite (Fe$_3$O$_4$). These nano-sized magnetite crystals (<100 nm) are bacterial species dependent in shape and size. Mms6 is a protein that is tightly associated with magnetite crystals. Based on \textit{in vitro} experiments, this protein was first implicated in morphological regulation during nano-sized magnetite biomineralization. In this study, we analyzed the \textit{mms6} gene deletion mutant (\textit{\Delta}mms6) of \textit{Magnetospirillum magneticum} (\textit{M. magneticum}) AMB-1. Surprisingly, the \textit{\Delta}mms6 strain was found to synthesize the smaller magnetite crystals with uncommon crystal faces, while the wild-type and complementation strains synthesized highly ordered cubo-octahedral crystals. Furthermore, deletion of \textit{mms6} gene led to drastic changes in the profiles of the proteins tightly bound to magnetite crystals. It was found that Mms6 plays a role in the \textit{in vivo} regulation of the crystal structure to impart the cubo-octahedral morphology to the crystals during biomineralization in magnetotactic bacteria. Magnetotactic bacteria synthesize magnetite crystals under ambient conditions via a highly controlled morphological regulation system that uses biological molecules.

The control of morphology and size of nano-sized crystals by biological systems under ambient conditions has the potential to be adapted to wide-ranging industrial, biomedical, and electronic applications (1\textendash}2). Living organisms perform various mineral depositions in construction of structures such as shells, teeth, and bones in which morphologies are strictly controlled using biological molecules in biomineralization processes (3\textendash}5). These biomineralization mechanisms have captured the imagination of material scientists engaged in development of bottom-up syntheses of various materials under mild conditions. Ansocalcin, a protein isolated from goose eggshell matrix, induces the construction of spherical calcite polycrystalline aggregates (6). Osteopontin, a protein that is abundant in urine, interacts with the specific crystal edges of calcium oxalate and influences the morphology of the crystals (7). In recent years, it has been determined that hierarchical organized structures with highly uniform pseudo-hexagonal structures are involved in the formation of the sturdy nacreous layer of mollusk shells and that rhombohedral nano-sized crystals are involved in the development of egg shells (3, 8\textendash}9). However, the functions of proteins in the development of morphology and/or size control during biomineralization were mainly demonstrated by \textit{in vitro} studies of chemical synthesis using purified proteins or synthetic peptides. Direct evidence of the protein-mediated control \textit{in vivo} has not been elucidated to date. In particular, the morphology and size regulation mechanisms of single crystalline nano-sized biominerals are still unclear.

Magnetotactic bacteria provide an ideal model organism for investigating the mechanism of nano-sized biomineral formation including size and morphological regulation, because the cells synthesize highly controlled single crystalline magnetites (magnetosomes). These microorganisms are thought to orient their cells in the geomagnetic field and to swim toward their preferred microaerobic environment. Previous molecular studies have revealed that magnetosomes are formed by a unique set of proteins in magnetotactic bacteria. Briefly, magnetosome vesicles are formed from the cytoplasmic membrane through an invagination process (10\textendash}11), and the vesicles are then aligned along the actin-like filamentous protein within the cells (11\textendash}12). Magnetite crystals are formed within magnetosomes in a highly controlled manner. The crystal formation is also presumed to involve several processes, such as nucleation, growth, and morphological regulation.

Within the magnetotactic bacteria, various magnetite crystals with narrow and species-specific sizes and morphologies including cubo-octahedra, elongated hexahedra, and bullet shapes have been observed under various environmental conditions (13\textendash}15). This has led to the proposal that magnetotactic bacteria exert significant regulation of the magnetite biomineralization process using specifically produced biological molecules. In our previous study, we identified a series of pro-

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teins, which are tightly bound to magnetite crystals in *Magnetospirillum magneticum* AMB-1. These proteins include Mms5, Mms6, Mms7/MamD, and Mms13/MamC (16). Among these proteins, Mms6 possesses a hydrophobic Leucine-Glycine repeat motif at the N terminus and the hydrophilic region of the C terminus, which is thought to interact with magnetite crystal and iron ions (17–18). The recombinant Mms6 protein has been shown to mediate the formation of uniform magnetite crystals during *in vitro* chemical synthesis (16). Furthermore, the primary protein function has been suggested to be morphological regulation at the crystallographic level in nano-sized magnetite biomineralization, based on the results of the *in vitro* experiment. Magnetite crystals consisting of (1 0 0) and (1 1 1) faces were obtained in the presence of Mms6 protein, while the crystals consisting of mainly the (1 1 1) face were formed in the absence of this protein (17). A similar observation was reported in an investigation using synthetic peptides mimicking the characteristic amino acids of Mms6 protein (18).

On the other hand, in the closely related species *Magnetospirillum gryphiswaldense* (M. gryphiswaldense) MSR-1, the *mamGFDC* gene operon, which includes the *mms7/mamD* and *mms13/mamC* genes was proposed to play a role in controlling the size of the magnetite crystal (19). In a recent study using gene deletion mutants, MamP, R, S, and T were also shown to function in controlling the size and number of magnetite crystals in *M. magneticum* AMB-1 (20). Although several proteins, including Mms6, have been hypothesized to relate to magnetite crystallization, a detailed *in vivo* functional analysis of this protein in nano-sized magnetite crystallization has not yet been performed.

In this study, to understand the role of Mms6 protein during magnetosome formation, we constructed and analyzed a Δ*mms6* strain of *M. magneticum* AMB-1. The crystallographic study of nano-sized magnetite crystals synthesized *in vitro* was conducted by high-resolution transmission electron microscopy (HRTEM).²

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions**—Strains, plasmids, and primers are described in detail in **supplemental Table S1**. *Escherichia coli* (E. coli) strains NEB turbo (New England Biolabs, Canada) and Top10 (Invitrogen) were used for gene cloning. *E. coli* cells were cultured in LB medium at 37 °C after addition of appropriate antibiotics. For conjugation experiments, *E. coli* strain S17-1 was used as a donor and cultivated as previously described (21). *M. magneticum* AMB-1 (ATCC700264) was anaerobically grown in an 8-liter fermentor (22–23). Colonies of *M. magneticum* AMB-1 were obtained on magnetic spirillum growth medium (MSGM) that was incubated microaerobically at 28 °C.

**Nonpolar Deletion of mms6 Gene**—The mms6 sequence was obtained from NCBI (YP_420381.1). The counter selectable suicide vector for the in-frame mms6 gene deletion was constructed using the methods described in the **supplemental information**. For genetic complementation of the Δ*mms6* strain, a pUMG (24) based plasmid harboring the mms6 (pUMBL/M6B) was generated (25). For Mms6 protein overexpression in wild-type strain, pUMP16mms6–6H was generated and transformed in the cell.

**Sizes, Shape Factors, and Crystallographic Analysis of Magnetite Crystal with TEM in Magnetotactic Bacteria**—Low magnification TEM analysis was performed using a conventional TEM (JEM1200-EX, JEOL Ltd., Japan) at 80 or 100 kV. In this study, by measuring at least 220 crystals for each strain, the crystal size and shape factor were evaluated. HR-TEM analysis was performed using TEM (H-9000NAR, Hitachi, Japan) at 300 kV, which has a point-to-point resolution of ~0.18 nm. The crystal faces were identified by indexing of the diffraction patterns (JCPDS-International Centre for Diffraction Data, 2001). Using the symmetry of the planar distance and the diffractograms, the diffraction spots of each diffractograms are indexed. The preparation and observation of thin-sectioned samples were previously described (26).

**Protein Extraction and Gel Electrophoresis of Magnetosomes from Δ*mms6* Strain**—The magnetosome protein extraction and the proteomic study were conducted according to previously described methods (10, 16). Gels were stained with BioSafe Coomassie G-250 (Bio-Rad).

**RESULTS**

**Characterization of the mms6 Gene Deletion Mutant by Transmission Electron Microscopy**—mms6 gene is presumably located in an operon with 2 other uncharacterized genes in the magnetosome island (MAI), a genome region containing several genes specific to magnetotactic bacteria (27–28). To avoid the polar effect, an in-frame gene deletion was carried out for the mms6 gene mutation in this study. The growth curves of the experimental strains (the Δ*mms6* strain, the complementation strain harboring pUMBL/M6B) and control (wild-type) were charted, and the results revealed that the gene deletion and complementation had no effect on bacterial growth (data not shown). The aligned magnetite crystal chain was observed in the Δ*mms6* strain (Fig. 1). The average number of crystals per cell in the Δ*mms6* strain was found to be similar to that of the wild type (≈20). The crystals within the chain are occasionally irregularly aligned and spaced, while similar average sizes of magnetosome vesicles (≈64 nm) were observed in the thin-sectioned samples (supplemental Fig. S1). This observation suggests that the structure of the magnetosome vesicles is unaffected by the deletion of *mms6* gene. The average distance between the centers of the crystals was found to be 63 ± 21 nm (average ± S.D.) and 58 ± 11 nm in the Δ*mms6* strain and wild type, respectively. The slightly increased distance between the crystals and the standard deviation of the Δ*mms6* strain might be due to weak magnetic interactions between the smaller magnetite crystals in the magnetosome chain. To further identify the morphological characteristics of the magnetite crystals, the size and shape factor of crystals between the Δ*mms6* and wild-type strains were compared based on the electron micrographs. Magnetite crystals synthesized in the Δ*mms6* strain had distinctly differ-

² The abbreviations used are: HRTEM, high-resolution scanning transmission electron microscopy; TEM, transmission electron microscopy; MSGM, magnetic spirillum growth medium; MAI, magnetosome island.
ent sizes and morphologies relative to the crystals of the wild-type strain (Fig. 1). The \( \Delta mms6 \) strain contained significantly smaller crystals \((\text{minor axis} + \text{major axis})/2 = 27.4 \pm 8.9 \) than the wild type \((48.3 \pm 12.5) \) (Fig. 2A). As a noteworthy difference in this evaluation, the maximum size of magnetite crystals in the \( \Delta mms6 \) strain was 63.7 nm. In contrast, the maximum size in the wild-type strain was 89.3 nm. Because of the deletion of the \( mms6 \) gene, a lower shape factor \((0.74 \pm 0.23) \) was also observed within the cells. The shape factor was found to be \( 0.92 \pm 0.16 \) in the wild-type strain (Fig. 2B).

Crystallographic Analysis by HRTEM of Magnetite Crystals Synthesized within the \( \Delta mms6 \) Strain—Many magnetite crystals in the \( \Delta mms6 \) strain did not have the cubic-octahedral structure. Instead, the crystals had various indefinite shapes within the cells (Fig. 1). Among the magnetite crystals of the \( \Delta mms6 \) strain, there were frequent examples of 2 different- or similar-sized crystals aggregating as shown in Fig. 1. The twinning magnetite crystal, which is considered to represent a defect of crystallization, has been reported in magnetotactic bacteria (29–30). Though the twinning structure was also occasionally found both in the \( \Delta mms6 \) and wild-type strains, the majority structures of magnetite crystals found in the \( \Delta mms6 \) strain were distinctly different.

For further evaluation of magnetite crystals in the \( \Delta mms6 \) strain, HRTEM experiments were performed on the magnetite crystals and the crystal faces were identified (Fig. 3, supplemental Fig. S2 and Table 1). The observed continuous crystal lattice, which does not have any irregular boundaries, clearly indicates that the crystals within the \( \Delta mms6 \) strain are single crystals (Fig. 3 and supplemental Fig. S2). In addition, the representative magnetite crystals found in the middle of the chain within the \( \Delta mms6 \) strain revealed an elongated structure toward the \( <111> \) direction (supplemental Fig. S3). The crystallographic direction, which is coincident with the long axis of the cell has also been observed in the wild-type strain (30). These observations indicate that the crystallographic direction of magnetite crystals within the cell is unaffected by the deletion of \( mms6 \) gene.

The data also indicate the prevailing projected image of the magnetite crystal in the wild type (Fig. 3a). A typical crystal of the wild type was determined to have \((1\,1\,1)\) and \((1\,0\,0)\) crystal faces. Similar results have been reported previously with respect to \( M.\,magnetica \) AMB-1 and \textit{in vitro} magnetite synthesis with recombinant Mms6 protein (16). In contrast, uncommon crystal faces, such as \((2\,1\,0)\), \((2\,1\,1)\), and \((3\,1\,1)\), were also identified in the \( \Delta mms6 \) strain at a higher rate and frequency, while these faces were not found in the wild-type strain (Table 1). All 9 crystals in the \( \Delta mms6 \) strain revealed the presence of the \((1\,1\,0)\) face on the magnetite crystal surface. As the \((1\,1\,0)\) crystal surface energy is higher than the \((1\,1\,1)\) and \((1\,0\,0)\) faces (31), such an unstable crystal surface generally disappears from the surface during crystal growth. The presence of the \((1\,1\,0)\) crystal face in the \( \Delta mms6 \) strain suggests that the crystal growth in this strain is incomplete.

On the other hand, the complementation strain harboring pUMBL/M6B, was found to have 
~26% of crystals within the cells with cubo-octahedron-like morphology comprising \((1\,1\,1)\) and \((1\,0\,0)\) crystal faces with similar sizes in wild type (supplemental Fig. S4). Some cells did not exhibit complete complementation, probably because of the expression of heterologous peptide-tagged Mms6 using a different promoter (P16; promoter of Mms16) (32).

**Figure 1.** Transmission electron micrographs of the \( \Delta mms6 \) and wild-type strains. The inset images are magnifications of the square area in the whole cell images. The scale bars are 800 nm for the main image and 200 nm for the inset.

**Figure 2.** Characterization of magnetite crystals within the \( \Delta mms6 \) and wild-type strains. Crystal size (A) and shape factor (B) distributions of magnetite crystals within the \( \Delta mms6 \) (black bar) and wild-type (white bar) strains. The particle size of a crystal is defined as the average value of the major axis and minor axis of the crystal. On the other hand, the shape factor of any crystal was determined as the minor axis/major axis due to the characteristics of these dimensions. These crystal size and shape factor were evaluated by measuring at least 220 crystals in 20 cells for each strain.
To evaluate the overexpression of the Mms6 protein in *M. magneticum* AMB-1 (wild-type strain), the AMB-1 transformant harboring the pUMP16mms6–6H (plasmid for the overexpression of the histidine-tagged Mms6 protein) was used and the phenotypic changes, including magnetite crystal morphology, were observed by using TEM. However, no significant changes in the magnetite crystal morphology and size were found in the *M. magneticum* AMB-1 transformant.

### Protein Profiles of Magnetosomes from the Δmms6 Strain

To verify the mms6 gene deletion, the protein profiles within both fractions of the magnetosome membrane and proteins tightly bound to magnetite crystals were analyzed for Δmms6 by SDS-PAGE (Fig. 4, A and B). As the magnetosome vesicles containing peripheral and transmembrane protein are dissolved by the first treatment with urea- and CHAPS-based solution, the proteins directly associating with magnetite crystals were obtained by a subsequent boiling SDS treatment (16). While the profiles of magnetosome membrane protein

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**TABLE 1**  
Crystal faces identified in magnetite crystal within magnetotactic bacteria

| Magnetite crystal face | (100) | (110) | (111) | (210) | (211) | (311) |
|------------------------|-------|-------|-------|-------|-------|-------|
| **Wild-type**          |       |       |       |       |       |       |
| a                      | ○     |       | ○     |       |       |       |
| a’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| Δmms6                  |       |       |       |       |       |       |
| b                      |       |       |       |       |       |       |
| b’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| c                      | ○     | ○     | ○     | ○     | ○     | ○     |
| c’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| d                      | ○     | ○     | ○     | ○     | ○     | ○     |
| d’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| e                      | ○     | ○     | ○     | ○     | ○     | ○     |
| e’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| f                      | ○     | ○     | ○     | ○     | ○     | ○     |
| f’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| g                      | ○     | ○     | ○     | ○     | ○     | ○     |
| g’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| h                      | ○     | ○     | ○     | ○     | ○     | ○     |
| h’                     | ○     | ○     | ○     | ○     | ○     | ○     |
| i                      | ○     | ○     | ○     | ○     | ○     | ○     |
| i’                     | ○     | ○     | ○     | ○     | ○     | ○     |

*○*: identified.  
*○*: not detected.  

*Previously reported crystal faces in Magnetospirillum species (wild type) (Devouard et al., 29, Amemiya et al., 17, Alphandery et al., 30). HRTEM images and the corresponding diffraction patterns of underlined samples are described in supplemental Fig. S2.*

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**FIGURE 3.** HRTEM images (a, b, c, and d) and the corresponding diffraction patterns (a’, b’, c’, and d’) from the inserted square of magnetite crystals in the wild-type strains (a–a’) and Δmms6 (b–b’, c–c’, and d–d’). Based on the diffractograms, the crystals are indexed using the symmetry of the diffractograms, and the planar distance the corresponding crystal faces were revealed in the HRTEM images. Schematic diagram of magnetite crystal in wild-type strain (a”).

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fractions were quite similar between the Δmms6 and wild-type strains, the profiles of the proteins tightly bound to magnetite crystals were significantly different (Fig. 4). In particular, the levels of low molecular weight proteins containing the Mms6 and Mms13 proteins were found to be decreased in the Δmms6 strain (Fig. 4B). To clearly show the protein profiles in the Δmms6 strain, two-dimensional gel electrophoresis (2DE) was conducted (supplemental Fig. S5). By the comparison of protein profiles in gel images (n = 3), we not only confirmed the deletion of Mms6 protein, but also verified the drastic decrease of the Mms5, Mms7, and Mms13 protein expressions by <6%, <9%, and <17% respectively, in the protein fraction obtained from the magnetite crystal surface in the Δmms6 strain. These results suggest that Mms6 protein interacts with these proteins and co-locates on the magnetite crystal surface.

DISCUSSION

In this study, because of the deficiency of Mms6 protein, M. magneticum AMB-1 synthesized magnetite crystals that had a lower aspect ratio, a smaller size, and an indefinite morphology consisting of uncommon crystal faces. Magnetite crystal formation is presumed to involve several steps such as nucleation, growth (size regulation), and morphological control. By the deletion of mms6 gene in M. magneticum AMB-1, the number of magnetite crystals remained unaffected. This observation clearly indicates that Mms6 does not function in the nucleation step of magnetite biomineralization. Although 2 distinct stages in magnetite crystal growth were recently suggested in magnetotactic bacteria (33–35), whether or not the Mms6 protein functions in a specific stage of magnetite crystal growth remains unclear.

In the previous reports on the crystallographic influence of proteins during biomineralization, the direct interactions of proteins with specific crystallographic faces and round edges of calcium oxalate have been observed (36–37). Because of this interaction between the protein and crystals, the crystal growth kinetics and thermodynamics change and can consequently alter the morphology of crystals in calcium-based biomineralization. While the calcium-based biomineralization attains the amorphous phase and contributes to the final biomineral habit (38–39), there is no direct evidence of the amorphous phase during magnetite biomineralization in magnetotactic bacteria. The morphological regulation in magnetite biomineralization might occur via a mechanism other than calcium-based biomineralization, but the actual mechanism remains largely unknown.

In our previous examination of in vitro chemical magnetite synthesis, the Mms6 protein was shown to mediate the formation of uniform magnetite crystals with cubo-octahedral morphology and a narrow size distribution (16–17). The reaction conditions of the in vitro chemical magnetite synthesis and magnetite formation in bacterial cells are significantly different. However, both processes indicated that Mms6 mediates the formation of uniform magnetite crystals with cubo-octahedral morphology consisting of (1 1 1) and (1 0 0) crystal faces. In contrast, in the absence of Mms6 protein, magnetite crystals mainly consisting of (1 1 1) faces were formed in vitro, while during in vivo magnetite formation, indefinite crystal morphologies were formed in the Δmms6 strain. This may be rationalized on the basis of the differences of the reaction conditions used in the in vivo and in vitro. The observed crystal defects and reduced size in the Δmms6 strain also suggests alternative functions of Mms6, such as the surface stabilization of magnetite crystal growth. Another potential function of the Mms6 protein could be to scaffold/stabilize these protein complexes to properly localize them onto the magnetite crystal surfaces. The lack of the Mms6 protein on the magnetite crystals may delocalize and/or enhance the degradation of other proteins.

Various morphologies with high-index crystal faces were identified in the Δmms6 strain. Such faces usually possess higher surface energy and tend to form on the crystal surface to increase the portion of the low-index faces (40). The observation in the Δmms6 strain was therefore unexpected, and this is probably due to the effects provided by cellular components and/or peculiar conditions within the magnetosome vesicle. It is also interesting that the crystals were observed to elongate toward the \(<1 1 1>\) direction in the Δmms6 strain. If the Mms6 protein (complex) regulates simple homogeneous crystal growth, the deficiency is likely to cause formation of the spherical or homogeneously minimized crystals. As the elongated crystal was revealed based on the mms6 gene deletion, we suggest that the biological molecule could regulate the orientation of crystal growth at the nano-sized scale and another unknown biological molecule might induce the magnetite crystal to grow toward the \(<1 1 1>\) direction in magnetotactic bacteria.

Four proteins (Mms5, Mms6, Mms7/MamD, and Mms13/MamC) have been identified on the magnetite crystal surface (16). The majority of these proteins were extracted by treatment using 1% SDS with boiling. The tight association of proteins with the magnetite surface was considered to be due to the presence of acidic amino acids, and their specific association with magnetite crystal. In addition, the common N-terminal hydrophobic regions of proteins have been considered to play a role in self-assembly of these proteins on the magnetite crystal surface to accelerate and/or direct the shape and
size (16). The result of this study revealed that the deletion of the \textit{mms6} gene significantly reduces the homeostasis of the other proteins on the crystal surface. Furthermore, the absence of these proteins resulted in formation of magnetite crystals with reduced sizes and undefined crystal morphologies. This result is consistent with our previous observation (16), and strongly suggests that these proteins are associated with each other, and that they may co-localize on the surface of magnetite in the form of protein complexes. In addition, these proteins are considered to have similar functions and to cooperate in the formation of magnetite with a consistent crystal surface. To fully understand the interaction and function of these protein complexes, further studies that include crystallographic structures of other gene mutants and multiple gene mutations combined with \textit{mms6} will be required.

The Mms7/MamD and Mms13/MamC protein functions have been also analyzed using the mutant \textit{mamGFDC} gene operon in closely related species (19). It has been proposed that in \textit{M. gryphiswaldense} MSR-1, the genes encoding Mms7/MamD and Mms13/MamC play a role in controlling the size of the magnetite crystals. Their protein homologs are also found in \textit{M. magnetotacticum} AMB-1. Although the magnetite crystals were not studied in detail in the \textit{mamGFDC} mutant of \textit{M. gryphiswaldense} MSR-1, it was observed that the size of the magnetite crystals had clearly decreased. In our study, the \textit{mms6} gene deletion from the \textit{M. magnetotacticum} AMB-1 genome caused a drastic decrease in protein expression levels, including the levels of the Mms7/MamD and Mms13/MamC proteins in the magnetite crystal surface (Fig. 4 and \textit{supplemental Fig. S5}), and a decrease in the crystal size (Fig. 2). This observation clearly shows the contribution of Mms6 (complexes) in magnetite bio-mineralization in magnetotactic bacteria.

Recent genome sequence analyses have clarified that the genes encoding magnetosome membrane proteins are conserved in phylogenetically diverse magnetotactic bacteria within the form of genomic island known as MAI. From the analyses of the MAI gene region, the proteins presumed to be involved in morphological regulation and possessing the Leucine-Glycine repeat motif and Mms13/MamC were commonly identified in 5 strains belonging to \textit{\alpha}-Proteobacteria (\textit{M. magnetotacticum} strain AMB-1, \textit{M. magnetotacticum} strain MS-1, \textit{M. gryphiswaldense} strain MSR-1, marine magnetotactic vibrio strain MV-1, and marine magnetotactic coccus strain MC-1), although the gene components and their amino acid sequences exhibit some variety among different strains (41–42). Strains AMB-1, MSR-1, MV-1, and MC-1 that form cubo-octahedral magnetite crystals consisting of (1 0 0) and (1 1 1) faces possess 4, 3, 3, and 3 putative proteins similar to the presumed morphological regulation protein in MAI, respectively (41, 43–44). In contrast, the genes encoding these proteins are absent in the genome of \textit{Desulfovibrio magneticus} RS-1 that synthesizes irregular bullet-shaped magnetite crystals (45–46). This observation also suggests that the genes are common in magnetotactic bacteria, which form magnetite crystals with cubo-octahedral morphology, and that strain RS-1 may have different morphological control factors in its magnetosome synthesis mechanism. Further identification of these genetic factors in other magnetotactic bacteria, which synthesize various magnetite crystals with different sizes and morphologies will allow us to design and control magnetic materials \textit{in vivo} and \textit{in vitro}.

In conclusion, the \textit{\textit{mms6}} strain synthesizes magnetite crystals of smaller sizes and lower shape factors relative to the wild-type strain and the complementation strain. The crystals revealed that the crystal faces (2 1 0), (2 1 1), and (3 1 1) are produced by the \textit{\textit{mms6}} strain, while the wild type synthesizes magnetite crystals having (1 1 1) and (1 0 0) faces. We, therefore, conclude that Mms6 functions in the regulation of crystal surfaces to control the magnetite crystal morphology during crystal growth in magnetotactic bacterial cells. This is the first example of a protein being involved in the regulation of a nano-sized crystallographic structure in \textit{in vivo} bio-mineralization.

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