Studies on a protein-protein interaction in the bacterial magnetic organelle “magnetosome”

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細菌の磁気オルガネラ「マグネトソーム」におけるタンパク質間相互作用に関する研究

Division of Life Science

Graduate School of Natural Science and Technology

Kanazawa University

NGUYEN VIET HOANG

Abstract

Magnetosomes are membrane-enveloped magnetic particles that found in magnetotactic bacteria and function as a cellular magnetic sensor that assists the cells to navigate and swim along the geomagnetic field. Magnetosomes use a suite of proteins, for their synthesis and maintenance. These proteins have specific individual functions, but some of them can interact with each other to form supramolecular complexes within the magnetosome. However, the detailed protein organization in magnetosome is still unresolved. MamA is one of the most abundant protein in magnetosome, and consists of tetratricopeptide repeat (TPR) motifs used for protein-protein interactions. It was proposed that MamA is anchored to the surface of magnetosome through protein-protein interactions. In this study, I found that MamA binds to a magnetosome membrane protein Mms6. Two different molecular masses of Mms6, 14.5-kDa and 6.0-kDa, were associated with the magnetosomes. I identified that the 14.5-kDa Mms6 interacts with MamA by pull-down, immunoprecipitation and size-exclusion chromatography experiments. Moreover, to detail into MamA-Mms6 interaction, I used truncated mutants of Mms6 to examine the MamA binding site in Mms6. Prior to this, Mms6 was assumed to be only involved in magnetite biomineralization, however, these results suggested that Mms6 has an additional responsibility, binding to MamA.

Main text

Magnetosomes are membrane-enveloped bacterial organelles which are found in magnetotactic bacteria (MTB). Magnetosomes consist of nano-sized magnetic crystals, magnetite (Fe_3O_4) or greigite (Fe_3S_4), that function as a magnetic sensor to

allow MTB to align and swim along the geomagnetic field when MTB move to find a favorable microaerobic habitat. Here, magnetosomes from *Magnetospirillum magneticum* AMB-1 was purified and studied because AMB-1 is now used as a model species among a diverse group of MTB.

Localized with each magnetosome is a suite of proteins involved in the synthesis, maintenance and functionalization of the organelle, however the detailed molecular organization of the proteins in magnetosomes is unresolved. One of the most abundant proteins in magnetosome is MamA. In 1996, MamA is the first protein which is identified from the purified magnetosomes, and determined amino acid sequence and found to consist of tetratricopeptide repeat (TPR) motifs which are known to mediate protein-protein interactions. Up until now, many studies on MamA have been reported. For example, the function of MamA has been proposed that MamA appears to function in activating or priming preformed magnetosomes for biomineralization. On the other hand, the detailed localization of MamA in the magnetosome structure has been studied. Even though MamA is a soluble cytoplasmic protein, previous studies using transmission electron microscopy (TEM) or atomic force microscopy (AFM) clearly demonstrated that MamA localizes in the magnetosome matrix, a proteinaceous layer surrounding magnetosome vesicles, of *Magnetospirillum* species (Figure 1).

Recently, the X-ray crystal structures of MamA from different MTB species have been determined. MamA consists of multiple TPR motifs (five TPR motifs and one putative TPR motif) which provide a super-helix structure. The super-helix structure yields a pair of concave and convex curved surfaces that function as binding sites for protein-protein interactions to form multiprotein complexes (Figure 2). However, the idea that MamA interacts with other magnetosome-associated protein(s) still remains

undetermined. The goal of this study is to identify the MamA binding partner in magnetosome. The soluble MamA proteins need to bind to other magnetosome proteins in order to anchor the magnetosome (Figure 3). The interaction between MamA and other magnetosome-associated proteins provide a clue to answer the question of how MamA binds to magnetosomes.

In this study, after screening the MamA binding proteins from the magnetosome protein extracts by affinity chromatography and finding that Mms6, a magnetosome membrane protein, is one candidate of MamA binding partners, I focused on study on the interaction between MamA and Mms6. Mms6 is one of the well-studied magnetosome-associated proteins that involved in biomineralizing magnetite crystals in *Magnetospirillum* species. By using affinity chromatography, the screening of MamA binding proteins showed that the 14.5-kDa Mms6 is one of the binding candidates. In previous report, Mms6 was identified as a 6.0-kDa mature protein consisting of 59 amino acids, however the *mms6* gene sequence shows that the full-length of the Mms6 protein is 133 amino acids. In order to clarify why the 14.5-kDa Mms6 is found in magnetosome protein extract and it might bind to MamA, I first want to confirm the presence of the 14.5-kDa version of Mms6 in magnetosomes. The immunoblotting analysis of AMB-1 cellular fractions using anti-Mms6$^{1\text{-}133}$ polyclonal antibodies showed that two bands of Mms6, one at 14.5-kDa and another at 6.0-kDa, were specifically localized in the magnetosome fraction (Figure 4).

To prove the protein-protein interaction between MamA and 14.5-kDa Mms6 (Mms6$^{1\text{-}133}$), biochemical methods of immunoprecipitation, pull-down and size-exclusion chromatography were used to examine such protein-protein interaction. Results obtained from these all methods showed that MamA interacts with 14.5-kDa

Mms6 ($Mms6^{1-133}$) (Table 1).

Moreover, the detailed protein-protein interaction between MamA and Mms6 was investigated to further understand the protein organization of two proteins in magnetosome. I performed the mutational dissection of Mms6 to identify the MamA binding site in Mms6. Three different truncated Mms6 peptides, $Mms6^{1-111}$ which lacks the C-terminus, $Mms6^{75-133}$ (6.0-kDa Mms6) which lacks the N-terminus and $Mms6^{1-88}$ which lacks the C-terminus and transmembrane region, were used to examine the interaction with MamA (Table 1). According to size-exclusion chromatography, both $Mms6^{1-111}$ and $Mms6^{75-133}$ containing transmembrane region (a. a. 89 to 111) formed the large oligomer with over 1,000-kDa. In contrast, $Mms6^{1-88}$, lacking the transmembrane region, did not form the large oligomer, and was eluted as a trimer with molecular mass approximate 30.0-kDa (Table 1). Interestingly, $Mms6^{1-111}$ oligomer and $Mms6^{75-133}$ oligomer were determined to interact with MamA oligomer. However, $Mms6^{1-88}$ neither formed the large oligomer nor interacted with MamA. These results suggested that the transmembrane region is needed for oligomerization of Mms6. The Mms6 oligomerization may be necessary for the interaction with MamA.

In this study, I propose a model for a MamA binding site in Mms6 oligomer (Figure 5). Mms6 works as the factor to anchor MamA in magnetosomes. Two types of Mms6, 14.5-kDa Mms6 ($Mms6^{1-133}$) and 6.0-kDa Mms6 ($Mms6^{75-133}$), located on the magnetosome membrane in roughly equal amounts. The C-terminal part of Mms6 is within the magnetosome vesicle because the C-terminal region of Mms6 contains the putative iron binding site for magnetite synthesis as described in previous reports. The N-terminal cytosolic part of Mms6 is predicted to provide the binding site which attaches MamA. For the interaction with MamA, two regions of Mms6 are involved in

such protein-protein interaction. First, the transmembrane regions are needed for Mms6 self-oligomerization. Second, after Mms6-oligomerization, the cytosolic regions with a. a. 75 to 88 seem to provide a binding site for the interaction with MamA oligomer to form the multiprotein complex in magnetosomes.

There are at least 30 proteins associated with the magnetosome, one of which is MamA, a key protein for the process of constructing the organelle. By proving the fact that Mms6 interacts with MamA, I found a major piece of the puzzle, which allows other researchers to continue the work on MamA and other magnetosome-associated proteins. This study provides a new protein-protein interaction in magnetosome and inspires further studies into the protein-protein interactions in magnetosome to more understand the formation of bacterial magnetic organelles.

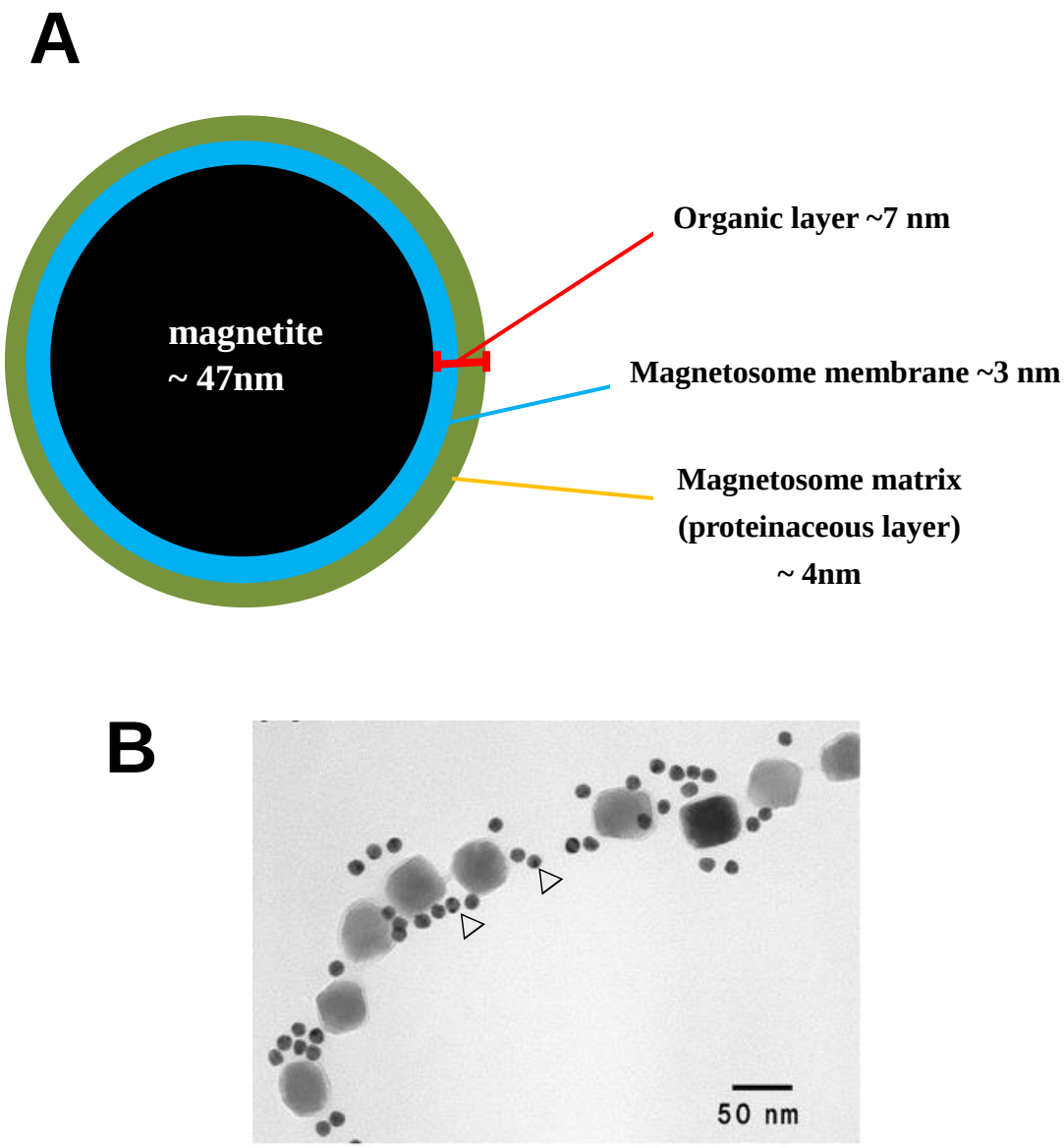


Figure 1. Spatial localization of MamA in the magnetosomes. (A) Schematic drawing of magnetosome. The individual magnetite crystal is surrounded by an organic layer (Yamamoto et al.). Magnetosomal matrix is a proteinaceous layer surrounding magnetosome vesicles. (B) Transmission electron micrograph of purified magnetosomes which were labelled with immunogold. Purified magnetosomes were incubated with polyclonal anti-MamA antibodies, followed by incubation with 15 nm gold-conjugated goat anti-rabbit IgG. Gold particles represented MamA localized on magnetosome matrix (open arrowheads), indicating the localization of MamA (Taoka et al.).

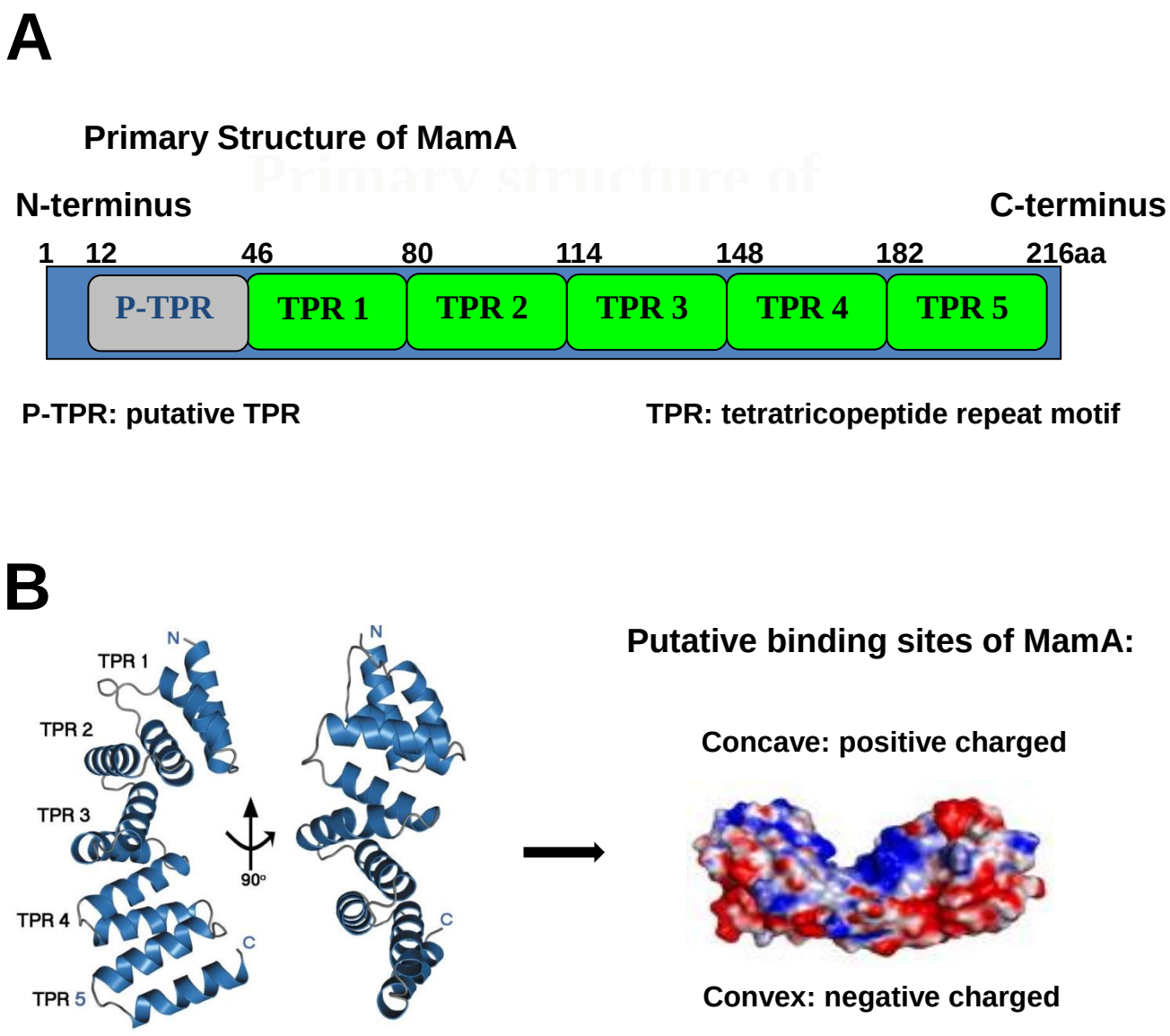


Figure 2. (A) The primary structure of MamA. MamA consists of five TPR motifs and one putative TPR motif as previous described by Okuda et al. (B) Ribbon structure of MamAΔ41 (without the putative TPR) monomer showed that 5 TPR motifs of MamA yields concave and convex surfaces. According to electrostatic potential calculation, the concave surface is positive charged and the convex surface is negative charged which possibly mediate the protein-protein interaction as described by Zeytuni et al.

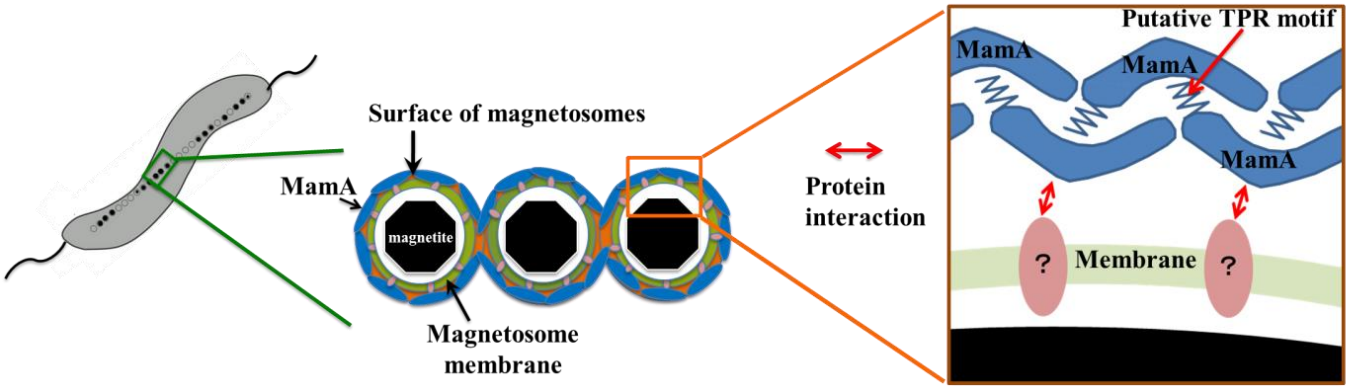


Figure 3. Model of the protein-protein interaction between MamA and other magnetosome associated proteins in magnetosomes. It has been proposed by Zeytuni et al. that MamA contains at least three protein binding sites, a putative TPR binding site, a concave binding site, and a convex binding site. The speculation is that the putative TPR motifs bind to the concave sites of other MamA monomers to form a homo-oligomer. The other site could bind other magnetosome-associated proteins because the soluble MamA proteins need to bind to other magnetosome proteins in order to anchor in magnetosomes. The goal of this study is to identify the MamA binding partners in magnetosomes.

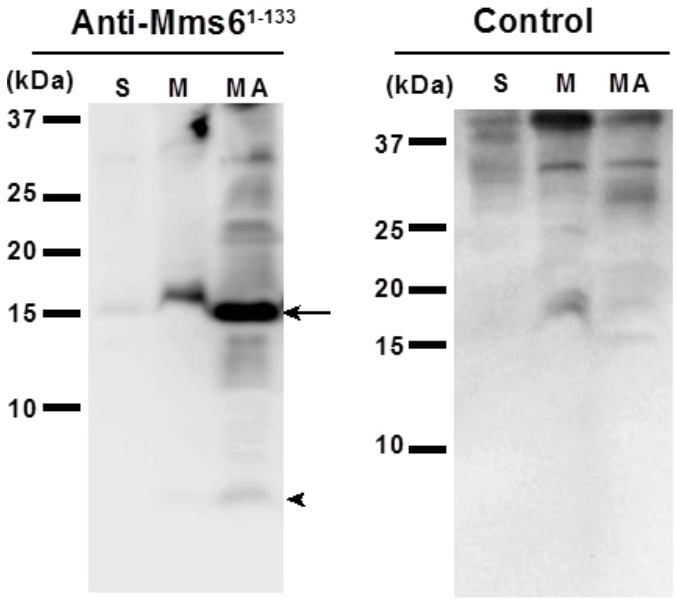


Figure 4. Immunoblotting of *M. magneticum* AMB-1 extracts labeled with anti-Mms6$^{1-133}$ polyclonal antibodies [left]. Two different Mms6 bands are evident, one at 14.5-kDa (arrow) and the other at 6.0-kDa (arrowhead). In the control experiment, the immunoblotting was carried out with an excess amount of Mms6$^{1-133}$ antigen. In the control, the 14.5-kDa and 6.0-kDa bands were not detected [right]. S: soluble fraction; M: membrane fraction; MA: magnetosome fraction.

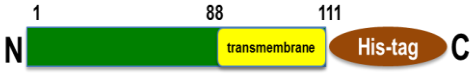
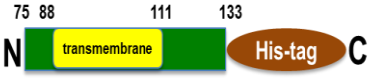
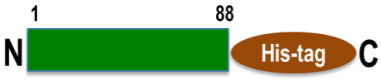
Schematic representation of Mms6 peptides	Oligomerization	Interact with MamA	Protein-protein interaction experiments
	**> 1,000 kDa**	**+** **+** **+**	**SEC** **IP** **Pull-down**
	**> 1,000 kDa**	**+** **+** **+**	**SEC** **IP** **Pull-down**
	**> 1,000 kDa**	**+** **+** **+**	**SEC** **IP** **Pull-down**
	**~ 30 kDa**	**-** **-** **-**	**SEC** **IP** **Pull-down**

Table 1. Summary of protein-protein interactions between MamA and different Mms6 peptides. Symbol "+" and "–" account for positive and negative interactions. SEC is size-exclusion chromatography; IP is immunoprecipitation assay; Pull-down is Ni-NTA pull down assay. The Mms6 peptides containing transmembrane region, $Mms6^{1-133}$, $Mms6^{1-111}$, and $Mms6^{75-133}$, formed the oligomer with over 1,000-kDa but $Mms6^{1-88}$ peptide lacking the transmembrane region did not form the large oligomer. This indicates that the transmembrane region in Mms6 functions in self-assembly to form large oligomer. MamA oligomer (~500-kDa) interacts with $Mms6^{1-133}$, $Mms6^{1-111}$, or $Mms6^{75-133}$ oligomers, but not with $Mms6^{1-88}$, suggesting that the oligomerization of Mms6 may be necessary for the interaction with MamA.

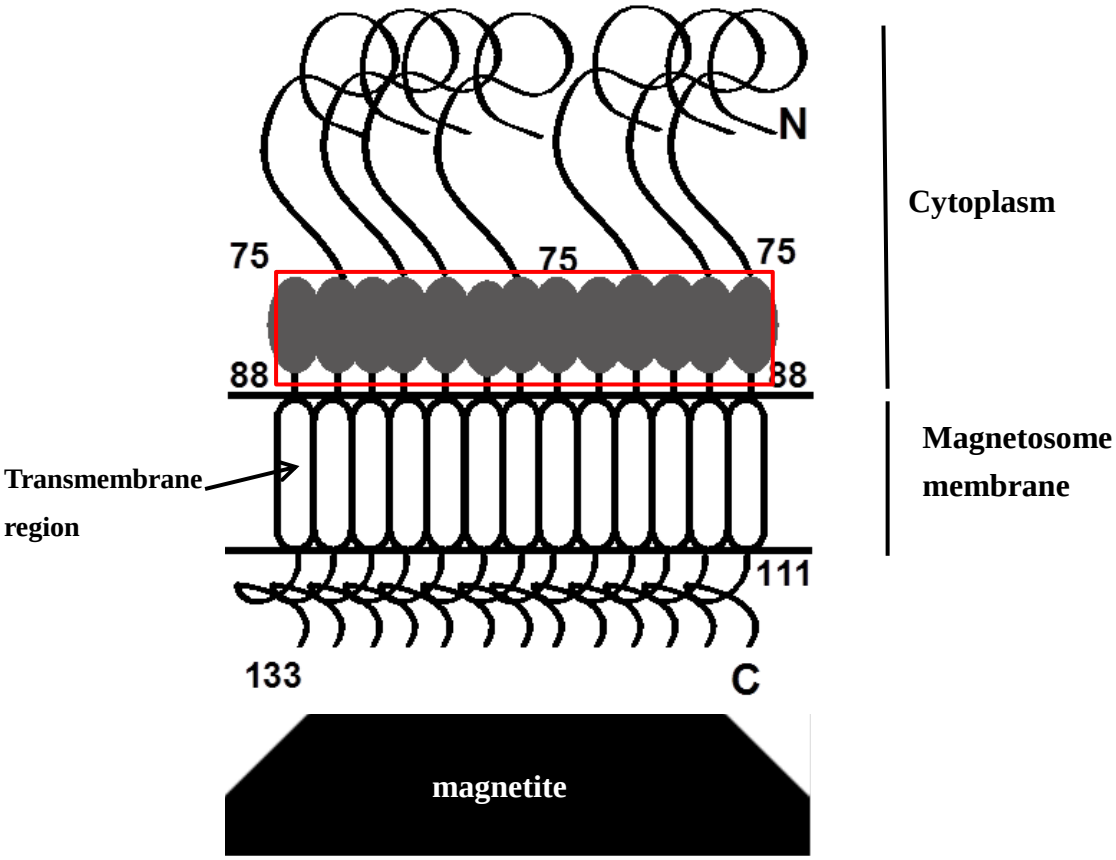


Figure 4. Schematic model for the Mms6 oligomerization which provides the MamA binding site. Two types of Mms6, the 14.5-kDa Mms6$^{1\text{-}133}$ and the 6.0-kDa Mms6$^{75\text{-}133}$, exit in magnetosome membrane in roughly equal amounts. The C-terminal parts of Mms6 are inside the magnetosome vesicle because the C-terminal region of Mms6 contains the putative iron binding site for magnetite synthesis. Mms6 proteins interact with each other by transmembrane region to form the large oligomer in magnetosome membrane. After oligomerization, the N-terminal parts of Mms6 (a. a. 75 to 88) in the cytosol are predicted to provide the binding site which attaches MamA oligomer in magnetosome. The red box indicates the MamA binding site of Mms6 oligomer.

平成２８年　７月２６日

学位論文審査報告書（甲）

１．学位論文題目（外国語の場合は和訳を付けること。）

Studies on a protein-protein interaction in the bacterial magnetic organelle "magnetosome"（細菌の磁気オルガネラ「マグネトソーム」におけるタンパク質間相互作用に関する研究）

２．論文提出者　(1) 所　　属　生命科学　専攻

(2) 氏　　名（ふりがな）　Nguyen Viet Hoang（ぐえん　ういえっと　ほあん）

３．審査結果の要旨（600～650字）

磁性細菌は、磁気センサーとして機能するマグネトソームとよばれる原核細胞オルガネラを形成する。マグネトソームは、磁性細菌に保存されマグネトソームに特異的に局在する一群の蛋白質と、リン脂質膜小胞、磁鉄鉱結晶によって構成されるナノサイズの超分子複合体であるが、マグネトソーム内における具体的な蛋白質間相互作用は明らかにされていない。本論文では、マグネトソームの最外殻を構成する蛋白質であるMamAが、マグネトソーム膜に局在するMms6蛋白質と相互作用することを明らかにした。MamA及びMms6蛋白質を大腸菌で発現、精製し、両者の相互作用を、免疫沈降実験、プルダウンアッセイ、ゲルろ過により示した。次に、C末端ドメインや膜貫通領域を取り除いた３種類の変異型 Mms6を作製し、Mms6におけるMamA相互作用領域を生化学的に検証した。また、分子サイズの異なる２種類の Mms6 がマグネトソームに局在することを初めて示した。Mms6 は、これまで磁鉄鉱の結晶形調節に関わる蛋白質として知られていたが、本研究により MamA との相互作用を介した新たな機能が示唆された。本研究は、マグネトソームを構成する蛋白質間の具体的な相互作用を初めて明らかにしたもので、マグネトソームを構成する蛋白質の機能とマグネトソームの形成機構の解明のために重要な発見であり、当該研究分野の発展に寄与する。以上をふまえ、本論文は博士（理学）の学位論文として相応しいと判定した。

４．審査結果　(1) 判　　定（いずれかに○印）　（合　格）・　不合格

(2) 授与学位　　博　士（　理学　）