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Supporting Online Material for

Magnetosomes Are Cell Membrane Invaginations Organized by the Actin-Like Protein MamK

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Materials and Methods References

Other Supporting Online Material for this manuscript includes the following: (available at www.sciencemag.org/cgi/content/full/1123231/DC1)

Movies S1 to S4

Supporting Online Material:

Materials and methods:

Culture growth conditions

Cultures of *Magnetospirillum magneticum* sp. AMB-1 were grown in MG medium containing per liter: 5 mL Wolfe's mineral solution (prepared without iron), 0.68 g potassium phosphate, 0.12 g sodium nitrate, 0.07 g sodium acetate, 0.035 g ascorbic acid, 0.37 g tartaric acid, 0.37 g succinic acid and 0.05 g sodium thiosulfate with the final pH adjusted to 6.9 prior to autoclaving. Prior to inoculation, Wolfe's vitamin solution was added to 1X final concentration and for iron-rich conditions cultures were amended with $30-50 \mu$ M ferric malate (*1*).

Spontaneous non-magnetic mutant

The spontaneous non-magnetic mutant was obtained in one of our other studies. This strain was found by PCR to be missing all of following genes: *mamC*, *mamD*, *mamE*, *mamK* and *mamA*.

Electron cryotomography

Four strains of *Magnetospirillum sp.* AMB-1 were imaged: wild-type, $\Delta mamK$, $\Delta mamK$ complemented with the plasmid pBBR1MCS-2 expressing MamK-GFP, and $\Delta mamK$ carrying the control plasmid pBBR1MCS-2. Cells were imaged from cultures grown both with and without iron in the medium. In all cases, 5-µL aliquots of culture were applied to glow-discharged Quantifoil (Quantifoil Micro Tools GmbH) or "lacy carbon

film" grids and then plunge-frozen in liquid ethane using a Vitrobot (FEI). Ten-nm colloidal gold particles were both dried onto the grids and mixed into cell cultures before freezing to serve as fiducial markers. Energy-filtered, dual-axis image tilt-series were collected of individual cells on a 2k x 2k Gatan CCD camera in an FEG G2 Polara FEI transmission electron microscope operating at 300 keV using the new "flip-flop" cryorotation holder (2). Tilt-series were collected automatically from -60° to +60° at 1.5° intervals along both axes using the UCSF predictive tomography software (3). Liquid nitrogen was used as the cryogen, the energy slit-width was 20 eV, the defocus was ~16 μ m, the total dose for both tilt-series was 120 e⁻/Å₂, and the magnification was set such that each CCD pixel corresponded to 1.2 nm at the specimen level.

Image processing

Images were binned 2-fold, and then dual-axis tomographic reconstruction was performed using the IMOD package (4). When the two single-axis volumes were combined, the warping residual ranged from 0.75 to 1.2. Briefly, images were aligned, R-weighted, and back-projected, resulting in one tomogram for each single tilt series. The two tomograms were registered to one another using local 3D cross-correlation between patches from the two volumes and then combined in Fourier space. The Amira software package (Mercury Computer Systems, Inc.) was used for 3D visualization. Membranes, magnetosomes and filaments were segmented manually. No denoising was used.

Quantification of Membrane Invaginations

A very rigid standard was used to classify a magnetosome feature as an inner membrane invagination. Only cases as clear as those presented in Fig.1 B-E were counted as invaginations. A clear pattern emerged, in that the magnetosomes along the sides of the cells were evidently invaginations, but the connectivity of those on the "top" and "bottom" of the cells (with respect to the electron beam) was unclear, suggesting that the ambiguity was simply a result of the missing pyramid of data.

Phylogenetic Analysis

To build the phylogenetic tree the following sequences were downloaded from Genbank: *Magnetospirillum magnetotacticum* MS-1 MamK (ZP_00054405) and MreB (ZP_00055538), *Magnetococcus* str. MC-1 MamK (EAN27146) and MreB (EAN29762), Caulobacter *crescentus* CB-15 MreB (AAK23522), *Escherichia coli* MreB (AAC76283), *Bacillus subtilis* MreB (D69660), *Escherichia coli* Plasmid R100 ParM/StbA (NP_052909), *Escherichia coli* plasmid pCoo ParM/StbA (AAT07423) and ACT1 from *Saccharomyces cerevisiae* (NP_116614). MreB homologs of MS-1 and MC-1 were chosen based on their high similarity to MreB from *E. coli* and *C. crescentus* as well as the presence of other *mre* genes next to them in the genome. These sequences were aligned using ClustalX and unrooted trees were generated using the Phylip server at http://bioweb.pasteur.fr/seqanal/interfaces/drawtree-simple.html.

GFP fusion to MamK

mamK was PCR amplified from AMB-1 with the following primers: ORF3GFP1 (5' GGCGAATTCATGAGTGAAGGTGAAGGCCAGGCCA 3') and ORF3GFP2 (5' GGCGGATCCCGAGCCGGAGACGTCTCCAAGCTG 3'). Due to the presence of restriction sites at the ends of these two primers the resulting PCR product was flanked by *EcoRI* and *BamHI* sites. This PCR product was digested by these two enzymes and ligated into plasmid pAK20 to create pAK22. This plasmid is a pBBR1MCS-based plasmid carries a kanamycin resistance gene and expresses the *mamK*-GFP fusion from a *tac* promoter. Fluorescence microscopy was performed as described previously (1).

Generation of *AmamK*

The same method used to create a deletion of *mamA* was used to generate a non-polar deletion of *mamK* (1). The upstream region flanking *mamK* was amplified with primers to generate an approximately 1.2 kb fragment and the downstream region was amplified with primers to generate a 0.9 kb PCR fragment. A fusion PCR reaction was carried out with these two fragments and the two outside primers to generate a ~2.1 kb fragment that was cloned into the pAKO plasmid's *SpeI* sites. First-step integrants were obtained by selection on kanamycin MG plates. These were grown for several generations in medium lacking kanamycin and subsequently plated on MG sucrose plates (1-3%) to select for recombinants that had lost the integrated plasmid. The resulting sucrose resistant strains were screened by PCR to find the *AmamK* strain. This strain was checked for the absence of *mamK* as well as the presence of other *mam* genes. The deletion region was also sequenced to ensure proper recombination had occurred.

Complementation of *∆mamK*

pAK22 was used to complement *AmamK* with the parent plasmid, pBBR1MCS2, serving as the negative control. The relative abundance of MamK in the cells was checked with fluorescence microscopy and it was found that anywhere from 30-50 % of the cells did not express the protein. This could be due to the stability of the plasmid or the amount of expression from the *tac* promoter. This posed a problem when ECT was used to assess complementation since only small numbers of cells can be imaged. Thus, prior to the collection of tilt-series, the cells were scanned for the length of their magnetosome chains. All of the negative control cells surveyed by this method had only small chains with gaps in them whereas $\sim 15\%$ of the complemented cells (6 out of 40) contained long chains of magnetite. Two of the complemented cells that contained long chains of magnetite were subsequently imaged by ECT and were found to contain straight chains flanked by filaments, as in the wild-type. Two other observations further indicated that only mamK (and not other downstream genes within the mamAB operon) was disrupted in this strain. First, the sequencing of the deletion region in $\Delta mamK$ showed that the reading frame of the mRNA was not disturbed. Second, polar insertions in genes immediately downstream of *mamK* result in completely non-magnetic cells (data not shown) and $\Delta mam K$ cells were still magnetic. Collectively, these results indicate that the phenotypes observed in the $\Delta mamK$ strain were due solely to loss of mamK.

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Supplementary Figure Legends:

Movie S1. Aligned, dual-axis image tilt-series of a wild-type AMB-1 cell grown under iron-rich conditions. Each frame of the movie contains one image of one of the tilt-series.

Movie S2. Three-dimensional reconstruction of an AMB-1 cell from the images in Movie S1. Each frame of the movie contains one 12-nm thick slice from the reconstruction, progressing through the cell from top to bottom.

Movie S3. Three dimensional reconstruction of an AMB-1 cell grown in iron-poor conditions.

Movie S4. Three-dimensional reconstruction of a $\Delta mamK$ cell.