## Short Communication

# Cell division in magnetotactic bacteria splits magnetosome chain in half

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Cell division in magnetotactic bacteria has attracted much interest, speculation and hypothesis with respect to the biomineralised chains of magnetic iron-oxide particles known as magnetosomes. Here we report direct Transmission Electron Microscopy (TEM) evidence that division occurs at a central point of the cell and the chain, cleaving the magnetosome chain in two. Additionally, the new magnetosome chain relocates rapidly to the centre of the daughter cell and the number of magnetosomes is directly proportional to the cell length, even during the division part of the cell cycle.

## Introduction

Since their published discovery over 30 years ago [1, 2], magnetotactic bacteria have enjoyed a large amount of research interest, mainly due to the fact that these bacteria biomineralise nanoparticles of magnetic iron minerals (most commonly magnetite) within a phospholipidic vesicle, known as magnetosomes. Magnetosomes have been found in a range of bacterial phenotypes including cocci, spirilla and rods, and they enable the cells to align in a magnetic field. They are arranged in a chain motif, with the number of chains (1, 2 or several), numbers of particles in the chains (commonly around 20 but can be up to 1000), size of particles (30–100 nm across) and shape (cuboidal to bullet shaped) of the magnetosomes all being strictly controlled within each strain but varying from strain to strain [3, 4].

Research to date has established that the most probable magnetosomes formation mechanism is: the magnetosome vesicles are first invaginated from the cytoplasmic membrane [5], cells uptake large quantity of iron ions [6], subsequently proteins specific to the magnetosome membrane nucleate and control magnetite

crystallisation [7, 8]. Recent work has identified an actin like filamentous protein (MamK) [9] to which the individual magnetosomes are attached in conjunction with the protein MamJ [10, 11] and this ordinates and aligns the magnetosomes forming a cytoskeletal chain structure down the cell [9–11].

Although magnetotactic bacteria are the subject of increased research activity, there are still several basic questions that have not yet been fully answered. For example, what happens to the magnetosome chain during cell division? Cell and magnetosome division mechanisms have been proposed, although these have been speculative and often as an aside to another study, referring to incidental data and as yet no conclusive study exists. For example, Scheffel and Schüler [11] noted (while studying MamJ) that wild-type cells have a tighter distribution of the number of magnetosomes per cell than the Δ*mamJ* mutant (with no chain structure) and ascribed this to the fact that the aggregated magnetosomes in the mutant resulted in an "all or nothing" asymmetric distributions after division as opposed to a more symmetric division assumed to take place in the wild-type. However, evidence or an explanation as to how symmetric this should be in the wild-type was not given [11]. Therefore we still do not know if the division of the chain is controlled to be central or if it is arbitrary (Fig. 1a)? Another study of the cell cycle of *Magnetospirillum magneticum* AMB-1 [12] showed an

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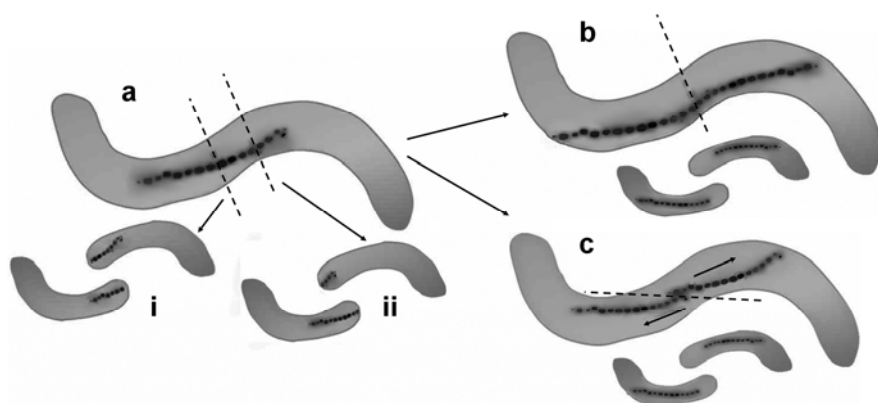**Phone:** +44 113 343 3399
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**Figure 1.** Diagram to show different division hypothesis. (a) Symmetric or asymmetrically cleaved down the cell. (b) Increased magnetosome synthesis prior to cell division [12]. (c) Replicated chains within the parent cell "slide" into daughter cell [13].

elongated cell buckled and presumable about to divide. However, this cell had unusual smaller segmented magnetosome chains throughout the cell length and therefore little can be said about what would happen to a usual central single magnetosome chain [12]. The above paper also suggested a rapid increase in magnetosome number prior to cell division in order to provide the daughter cells with mature chains (Fig. 1b). However the few data points and associated errors rendered the results inconclusive [12]. Erglis *et al.* [13] also suggested the build-up of magnetosomes directly prior to cell division while studying the dynamics of rotating magnetotactic bacteria in a magnetic field. They noticed several cells with the beginning of doubling chains (Fig. 2b i)) and suggested that this represented the chain side-by-side replication prior to cell division, hence the increased number of magnetosomes before division. They proposed that these two chains "slide" apart into the daughter cells (Fig. 1c). They also noted that both daughter cells still rotated in the field showing that both of them contained some magnetosomes, but no evidence to support the magnetosome division hypothesis was presented.

From the literature to date it seems that both daughter cells are magnetic so each does inherent some magnetosome, but how symmetric (controlled) or asymmetric (arbitrary) this process is with respect to the magnetosomes and the cells is still unclear. Is there a rapid increase in magnetosomes immediately prior to cell division? How are two chains obtained from one? Are they directly cleaved or do the chains replicate side-by-side then slides apart?

These questions have not yet been directly answered but instead are still being posed in the literature [14], and until now there is no compelling photographic evidence of the magnetosome chain behaviour during cell division. Here we can begin to answer these questions by presenting TEM studies of *Magnetospirillum gryphiswaldense* cells undergoing cellular division.

## Materials and methods

### Bacterial strain and growth conditions

*Magnetospirillum gryphiswaldense* (DSM 6361) [15] was used in this study. *M. gryphiswaldense* was cultured in a flask standard medium (FSM) [16]. For micro-aerobic cultivation, the culture flasks were sealed before autoclaving with butyl-rubber stoppers and microaerobic conditions arose in the medium at higher cell densities by oxygen consumption of cells [6]. The micro-aerobic cultures were inoculated by injection through the stopper and incubated at 28 °C, without agitation. For aerobic cultivation, cells were incubated in free exchange with air at 28 °C and agitated at 100 r.p.m. in an incubator shaker [16].

### Transmission electron microscopy and image analysis

Electron microscopy studies were performed on whole cells from different growth phases; samples of whole cells were prepared for electron microscopy by deposition of a drop of washed cells directly onto a copper TEM mesh grid covered with a carbon film and the grid was allowed to air dry. No additional staining (i.e. contrast enhancement) was used. All samples were imaged using a JEOL 1200 EX transmission electron microscope at an accelerating voltage of 80 kV. Cell images were digitized from scanned, low-magnification TEM and the photomicrographs analysed using an analytical software for processing digitized electron microscope images (ImageJ). Statistical analysis of the dimensions (cell
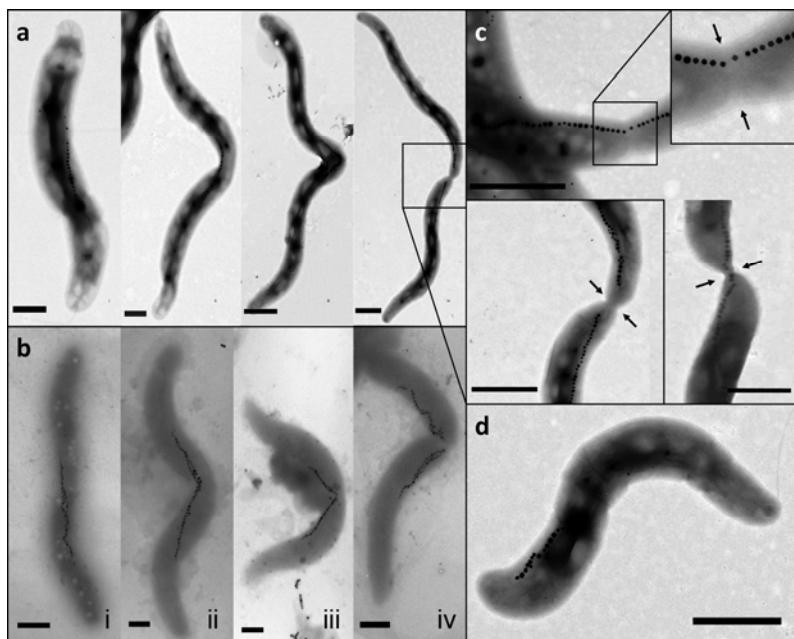
**Figure 2.** TEM images of *M. gryphiswaldense* cells during cellular division. (a and b) shows a series of cells undergoing the same phases of a symmetric cellular division: i) mother cell, ii) cell elongation and bending, iii) cell bending up to an acute angle forcing the magnetosome chain to bend as the division is progressing, and iv) separation of the two magnetosome chain halves, inherited by each daughter cell as the cell divides. (a) Cell series with a single and (b) with a double magnetosome chains. (c) Single-chain cell showing the initiation of the septum formation (arrows) and subsequently the cleavage of the magnetosome chain. (d) *M. gryphiswaldense* cell immediately after division (Scale bar = 1 μm).

length, chain length, number of magnetosomes and chain position) of between 100 and 220 cells were performed using standard statistical methods.

## Results and discussion

### Cell division splits magnetosome chains in two

TEM studies of *Magnetospirillum gryphiswaldense* cells showed that during cell division this magnetotactic bacterium divides symmetrically down the centre of the cell (division at 0.505 of cell length, SD 0.024), cleaving the magnetosome chain in two. Fig. 2 shows a series of cells dividing in this way. Fig. 2a shows clearly in a series of TEM images (i–iv) how the cells buckle-up and bend to an acute angle typical of spirilla bacterial division [17] and the magnetosome chain bends also just prior to division (Fig. 2a iii)). This bent cell shape was also observed in the Yang *et al.* [12] cell cycle study. Subsequently, in our study the cell begins to divide as the magnetosome chain is cleaved (Fig. 2c). Our TEM studies also revealed a small number of cells with sections of double chains, the presence of which was discussed as a possible division method [13]. However, we found this was most improbable due to the fact that these double chains also bent centrally and divided in

the same way as the single chain cells, with double chains inherited by the daughter cells, not single chains that slide apart as it was proposed (Fig. 2b).

### Statistical analysis shows no rapid increase in magnetosome number prior to cell division

A detailed statistical analysis of the cell length, number and position of magnetosomes in many hundreds of cells showed a standard cell length population for magnetospirilla cells with an average length of approximately 6 μm (5.83, SD 1.49) (Fig. 3a). The analysis also revealed a direct linear relationship between cell length and numbers of magnetosomes (for cells over 5 μm, one additional magnetosomes per $0.13 \pm 0.007$ μm of cell growth), suggesting a gradual growth of magnetosomes number that is proportional to cell elongation (Fig. 3b). This linear relationship applies throughout the cell cycle, even just prior to and during cell division, showing there is no rapid increase in magnetosome number prior to division as was previously suggested [12, 13].

Analysis of the magnetosome chain position within the cell showed it to be central, regardless of the cell length (Fig. 3c). From the schematic in Fig. 1a it can be seen that immediately after division the newly formed half chain would be located at cell poles. However, only
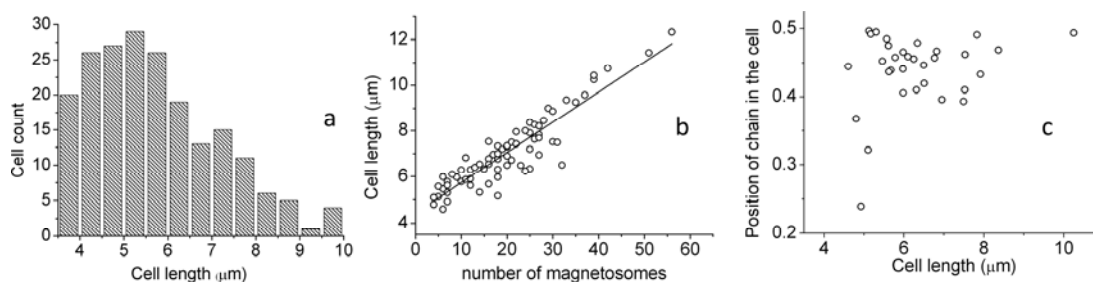
**Figure 3.** Graphs of the cell length, magnetosome chain number and position analysis of the TEM images. (a) Cell length distribution (average cell length ≈ 6 μm), (b) linear relationship of cell length with the number of magnetosomes, and (c) relationship between cell length and the position of the chain within the cell (position = $d_1/(d_1 + d_2)$, where $d_1$ and $d_2$ = the distance from the end of the chain to the nearest end of the cell on both sides).

2−3 examples of chains significantly off-centre were found amongst the entire cells analysed (Fig. 3c) and these were all of the smallest cell size, in keeping with the theory that these are new daughter cells, imaged very soon after division. An example of one of these is shown in Fig. 2d. This sudden jump in position and the very notable lack of a population of cells with off-centred chains suggests that the chain structure moves rapidly after being cleaved along the cytoplasmic membrane to the centre of the cell. This demonstrates that the chain structure is dynamic and mobile for this short time during and after division has occurred.

### Symmetrical halving of magnetosome chains under aerobic conditions

In order to further test the central chain cleaving result, we conducted an experiment to observe the symmetric halving of the chain length upon cell division.

Magnetotactic bacteria only produce magnetosomes under depleted oxygen conditions (micro-aerobic) or in the absence of oxygen (anaerobic). However, for some strains, including *M. gryphiswaldense*, cells can grow in more oxygen rich conditions but in this case they do not produce magnetosomes [16]. Therefore a micro-aerobic magnetic cell culture was transferred and further grown under increased $O_2$ "non-magnetosome formation" conditions (i.e., aerobic), and periodically sampled over 2 d. During a period of 40 h the cells changed from being magnetic and dark grey to non-magnetic and white. Over this time course the close to symmetric halving in magnetosome chain length was clearly observed (Fig. 4). Assuming that no magneto some formation occurred after the $O_2$ tension was increased ($t = 0$; Fig. 4a), then the halving of the numbers of magnetosomes per chain can be directly related to the number of cell divisions. The cells started with an
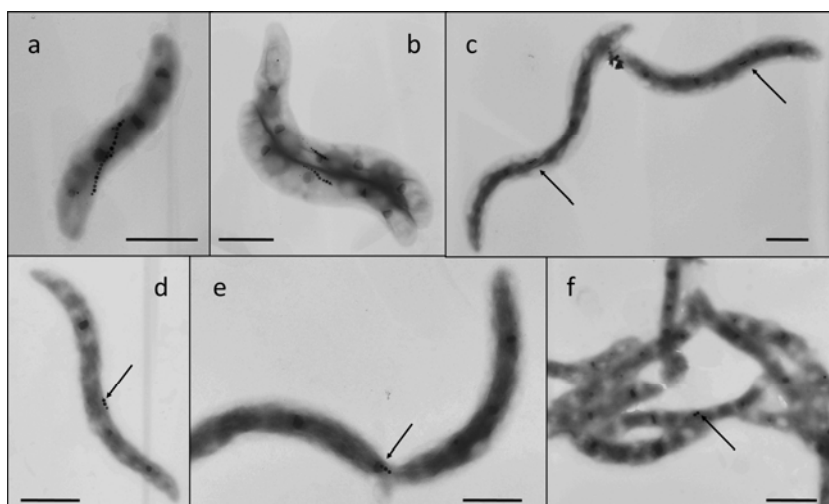


**Figure 4.** TEM images of *M. gryphiswaldense* cells at given time intervals (t) after aerobic growth was initiated (to inhibit magnetosome growth). (a) Control cell at $t = 0$ containing 21 magnetosomes; (b) cells at $t = 10$ hours (after 1 division) containing 8 and 11 magnetosomes. (c) Cells at $t = 24$ hours (after 2 divisions) both containing 5 magnetosomes. (d) Cell at $t = 30$ h (after 3 division) containing 3 magnetosomes. (e) Cell dividing with 3 magnetosomes in the centre of the division $t = 36$ h. (f) Cells at $t = 40$ h, all cell have 0 magnetosomes with the exception of one with 2 magnetosomes. (Scale bar = 1 μm)

average of 20 magnetosomes per cell at $t = 0$ (Fig. 4a) and therefore 4–5 divisions would render the cells non-magnetic (the number of magnetosomes per cell decreases from 20–10–5–2/3–2/1/0–1/0) (Fig. 4b–f). This would happen with a doubling time of 8–10 hours which is in agreement with the literature [12, 18].

## Concluding remarks

In summary, the images and image analysis of *M. gryphiswaldense* cells dividing as well as growing under aerobic conditions showed that the cells divided symmetrically down the middle of the cell and cleave the magnetosome chain in half as represented by Fig. 1a i. We propose that the smaller chains present in the daughter cells must move rapidly to the centres of the new cells and subsequent magnetosome synthesis will be re-established. This suggests that this chain cytoskeletal structure including magnetite particles, vesicles and the fibrous protein MamK, is dynamic over this part of the cell cycle. Further studies could investigate and track the location of empty vesicles present in the aerobic sample at either side of the reduced magnetosome chain (as well as the chains themselves) to assess the centering and dynamics of the mechanism further.

## Acknowledgements

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