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Synthesis of Bacterial Magnetic Particles During Cell Cycle of *Magnetospirillum magneticum* AMB-1

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Abstract

We investigated the relationship between the synthesis of bacterial magnetic particles (BMPs) and the transcription of *magA* gene–encoding iron transport protein using synchronous culture of *Magnetospirillum magneticum* AMB-1. Synchronously cultured cells were subjected to transmission electron microscopic observation and fluorescence *in situ* hybridization. The average number of BMPs slowly increased in the cell with increasing cell size. A sharp increase in BMPs occurred just before cell division and resulted in maximum BMP production of 30 particles/cell. The transcription of *magA* was regulated immediately before and after cell division.

Index Entries: Synchronous culture; *Magnetospirillum magneticum* AMB-1; bacterial magnetic particle; cell cycle; *magA* gene.

Introduction

Magnetic bacteria have been found in freshwater and marine sediment *(1)*. They produce magnetic particles, which are small (50–100 nm) and covered with a stable lipid membrane *(2)*. A magnetic bacterium, *Magnetospirillum magneticum* AMB-1, has been isolated *(3)* and the bacterial magnetic particles (BMPs) have been used for highly sensitive immunoassays in which enzymes and antibodies immobilized on BMPs were used *(4,5)*. Genetic analysis of BMP synthesis has led to the isolation of the *magA* gene from *M. magneticum* AMB-1, which encodes an iron transport membrane protein on the BMPs *(6)*. Functional proteins have been displayed on BMPs using MagA as an anchor protein through the gene fusion *(7)*. Protein A–

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displayed BMPs through the fusion with MagA protein has also been used in immunoassays *(8,9)*. These applications emphasize the need to enhance the production of BMPs for biotechnological applications.

To design cell cultivation processes and to achieve efficient production, several factors need to be considered. To overproduce BMPs, fed-batch culture of *M. magneticum* AMB-1 has been performed with continuous addition of nitric acid and iron to the medium *(10,11)*. The cell density during fed-batch cultures of AMB-1 was 10 times higher than that in batch culture; however, the yield of BMPs per cell was decreased. Our recent work shows that the yield of BMPs per cell decreases only after logarithmic growth in a fed-batch culture (unpublished results). The cell-cycle population is one of important factors, because the characteristics of the cells in phases may differ with respect to gene expression. The cell-cycle dependency of rice α-amylase production by a recombinant yeast has been reported *(12)*. Cyclic appearance of intracellular nitrogenase activity was observed in the synchronously grown marine cyanobacterium *Synechococcus* sp., revealing cell-cycle-dependent nitrogenase activity *(13)*. This characteristic has been used for efficient production of hydrogen mediated by nitrogenase in this cyanobacterium *(14)*. We previously described how a synchronous culture of *M. magneticum* AMB-1 was successfully established by repeated cold treatment of cells *(15)*.

In the present study, we investigated the relationship between *magA* expression and BMP synthesis during different cell-cycle phases. The results obtained in this study may contribute to control of cell-cycle phases for efficient BMP production in the strain AMB-1.

Materials and Methods

Bacterial Strain and Growth Medium

M. magneticum AMB-1 (ATCC700264) was cultured with 40 mL of magnetic spirillum growth medium in a 50-mL flask at 25°C *(16)*. The oxygen concentration in the gas phase was reduced to <1 ppm by repeated flushing with argon.

Synchronous Cultivation

Synchronous cultivation of AMB-1 cells was achieved by using repeated cold treatment at 5°C *(15)*. Precultured AMB-1 cells were inoculated into 40 mL of medium to a density of 1×10^5 cells/mL. The culture was incubated for 14 h in a constant 25°C (optimum growth temperature) water bath and then transferred between constant 5°C and 25°C water baths at 5-h intervals for five cold treatments. The cold-treated cells were inoculated into a 40-mL fresh medium to a density of 2×10^6 cells/mL and cultured at 25°C. The cell concentration was determined every hour by direct cell count using a hemocytometer. Cells immediately before division that were longer and had a narrow middle section were considered "doubling cells."

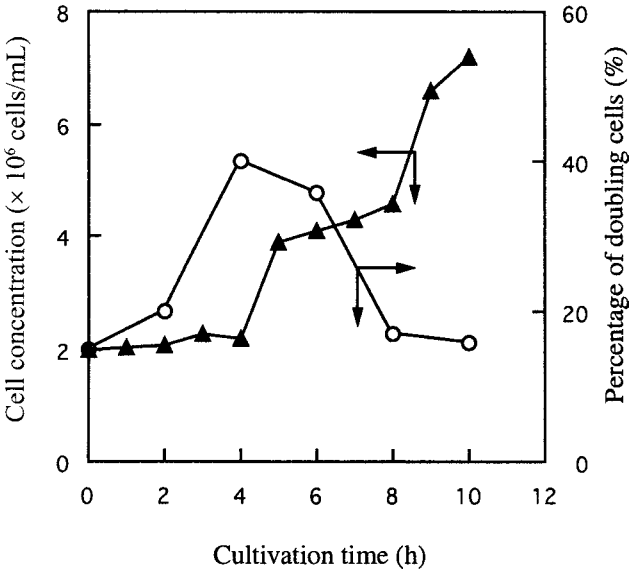

Fig. 1. Relationship between cell growth and percentage of doubling cells in synchronous culture. ▲, synchronous cell growth curve; ○, percentage of doubling cells.

Transmission Electron Microscopic Observation of Synchronous Culture

Synchronously cultivated cells were sampled at 2-h intervals and observed with a transmission electron microscope (Hitachi H700) operating at 150 kV. Electron-dense particles in the cells were counted and an average number of particles/cell were determined.

Fluorescence In Situ *Hybridization Analysis*

Synchronously cultivated cells were sampled at 2-h intervals and used for fluorescence *in situ* hybridization (FISH) analysis for *magA* expression. FISH was carried out using chemically synthesized fluorescein isothiocyanate (FITC)-labeled *magA* probe (FITC-5'-CAGATCGCGGAACGA ATGGACATG-3') according to the method of Chomczynski *(17)*.

Results and Discussion

Figure 1 shows the cell growth curve of synchronously grown AMB-1 at 25°C. Cell density remained constant for approx 4 h, doubled during a short period of about 1 h, then remained constant for another 4 h, which indicated synchronicity of cells. The percentage of doubling cells in culture increased from 20 to 40% rapidly and then decreased by 17%. This alteration of the proportion of doubling cells also indicated synchronized cell division. However, the percentage of doubling cells at dividing phase (4-h cultivation time) was 40%. In synchronously grown *Synechococcus* sp. using light/dark cycles, higher synchronicity (70–80% of doubling cells)

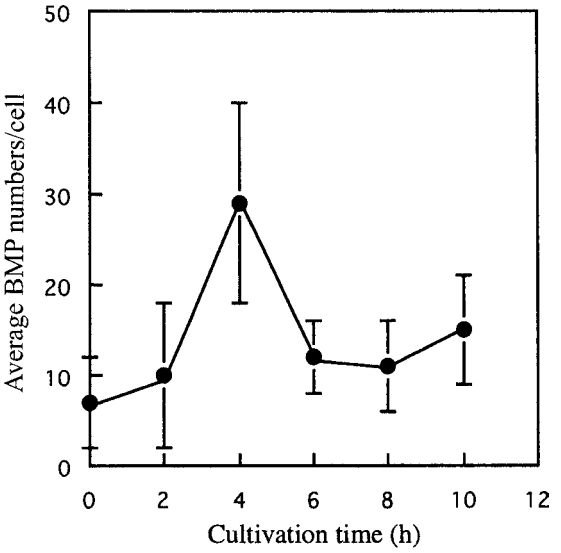

Fig. 2. Average BMPs/cell at various intervals of synchronous culture.

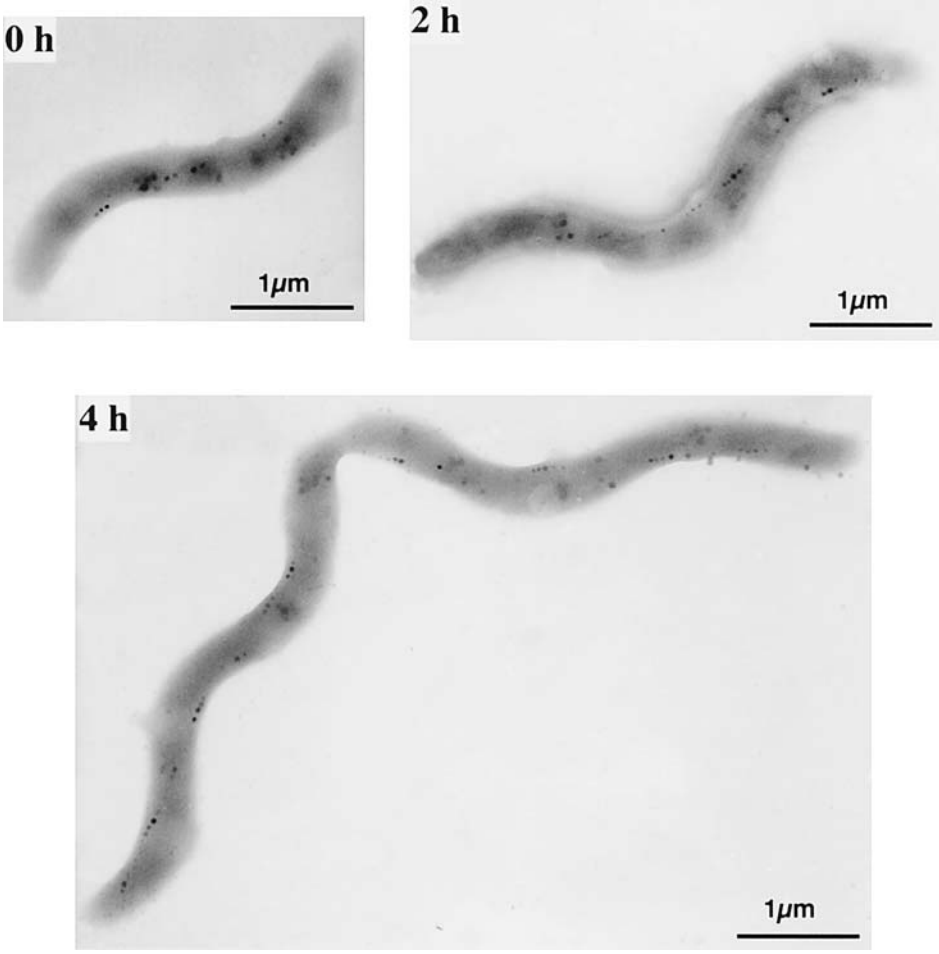

Fig. 3. Transmission electron micrographs of AMB-1 cells sampled at various intervals during synchronous growth. Bars = 1 μm.

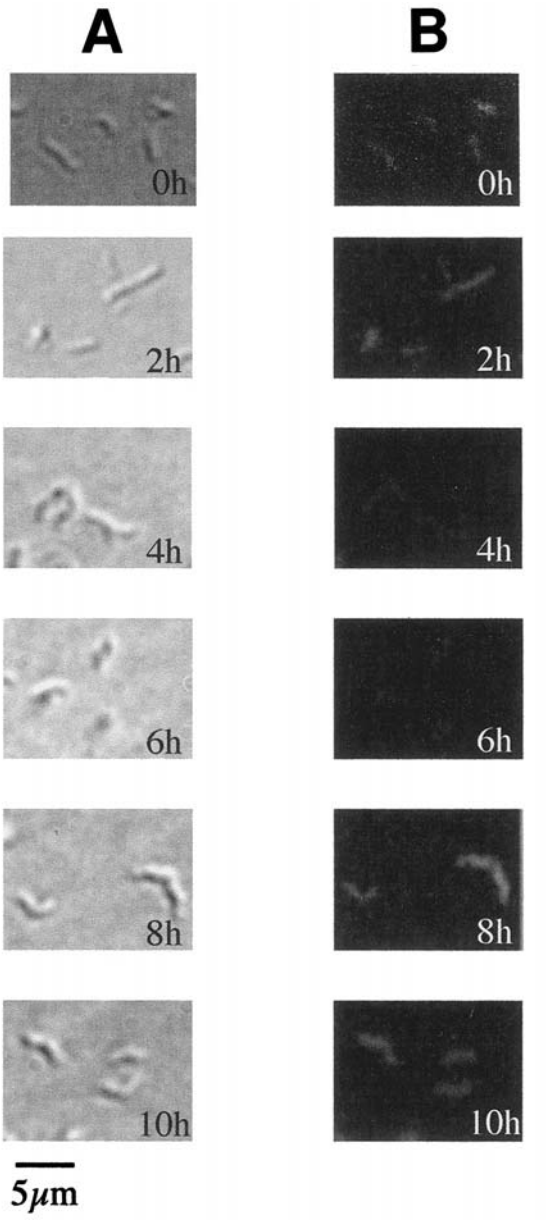

Fig. 4. Detection of *magA* mRNA in synchronous cells by FISH using FITC-labeled *magA* probe. **(A)** Difference interference contrast image; **(B)** fluorescence image.

was performed under continuous light conditions *(13)*. By contrast, lower synchronicity in AMB-1 may be caused by taking a time for temperature shift of medium and its shorter generation time than cyanobacteria.

To investigate BMP synthesis in synchronously grown AMB-1, change of the number of BMPs per cell was investigated. BMPs per cell gradually increased with increasing cell size, as shown in Figs. 2 and 3.

However, the number of BMPs sharply increased immediately before cell division in spite of cell size and reached a maximum number of 30 particles/cell at 4 h. The magnetic particles were equally divided to daughter cells. These data suggest that BMP synthesis by AMB-1 occurs just before cell division following a short period.

FISH gave strong fluorescence by FITC in whole cells at 0, 2, 8, and 10 h (Fig. 4). On the other hand, fluorescence signals in the cells were weak at 4 and 6 h. These results show that *magA* was transcribed during most of the cell cycle and repressed just before and after cell division. The repression of *magA* transcription at the phase of active BMP synthesis occurred, which may be caused by fluctuation of iron concentration in the cells. The transcription of *magA* is repressed during iron-sufficient conditions.

The BMP synthesis was found to rapidly increase just before cell division. The control of cell-cycle phase will contribute to enhanced BMP production in AMB-1. In future studies, we will design a new fed-batch culture system by controlling cell cycle for efficient BMP production.

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References

1. Blakemore, R. P. (1982), *Annu. Rev. Microbiol.* **36,** 217–238.
2. Balkwill, D. L., Maratea, D., and Blakemore, R. P. (1980), *J. Bacteriol.* **141,** 1399–1480.
3. Matsunaga, T., Sakaguchi, T., and Tadokoro, F. (1991), *Appl. Microbiol. Biotechnol.* **35,** 651–655.
4. Matsunaga, T., Kawasaki, M., Yu, X., Tsujimura, N., and Nakamura, N. (1996), *Anal. Chem.* **68,** 3551–3554.
5. Nakamura, N., Hashimoto, K., and Matsunaga, T. (1991), *Anal. Chem.* **63,** 268–272.
6. Nakamura, C., Burgess, J. G., and Matsunaga, T. (1995), *J. Biol. Chem.* **270,** 28,392–28,396.
7. Nakamura, C., Kikuchi, T., Burgess, J. G., and Matsunaga, T. (1995), *J. Biochem.* **118,** 23–27.
8. Matsunaga, T., Sato, R., Kamiya, S., Tanaka, T., and Takeyama, H. (1999), *J. Magn. Magn. Mater.* **194,** 126–131.
9. Tanaka, T. and Matsunaga, T. (2000), *Anal. Chem.* **72,** 3518–3522.
10. Matsunaga, T., Tsujimura, N., and Kamiya, S. (1996), *Biotechnol. Techniques* **10,** 495–500.
11. Matsunaga, T., Togo, H., Kikuchi, T., and Tanaka, T. *Biotechnol. Bioeng.*, in press.
12. Uchiyama, K. and Shioya, S. (1999), *J. Biotechnol.* **71,** 133–141.
13. Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Cao, S., and Arai, T. (1986), *Nature* **323,** 720–722.
14. Campbell, C., Takeyama, H., and Mitsui, A. (1994), *J. Mar. Biotechnol.* **2,** 39–43.
15. Sato, R., Miyagi, T., Kamiya, S., Sagaguchi, T., Thornhill, R. H., and Matsunaga, T. (1995), *FEMS Microbiol. Lett.* **128,** 15–20.
16. Blakemore, R. P., Maratea, D., and Wolfe, S. (1979), *J. Bacteriol.* **140,** 720–729.
17. Chomczynski, P. (1987), in *Current Protocols in Molecular Biology*, vol. 3, Ausuble, F. M., ed., Wiley, New York, pp. 14.1.1–14.7.11.