MAGNETITE AND MAGNETOTAXIS IN MICROORGANISMS

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INTRODUCTION

Magnetotactic bacteria include various species of aquatic micro-
organisms that orient and swim along magnetic field lines (Blakemore,
1975; 1982; Blakemore & Frankel, 1981; Moench & Konetzka, 1978). All
magnetotactic cells examined to date by electron microscopy contain
iron-rich, electron opaque particles (Balkwill et al. 1980; Towe &
Moench, 1981). In several and possibly all species of magnetotactic
bacteria, the particles consist of magnetite, Fe_3O_4 (Frankel et al.,
1979). In most species the particles are arranged in chains, which
impart a magnetic moment to the cell, parallel to the axis of motility.
The moment is sufficiently large that the bacterium is oriented in the
geomagnetic field at ambient temperature as it swims, i.e. the chain of
Fe_3O_4 particles functions as a biomagnetic compass (Frankel & Blakemore,
1980). By this means the organism propels itself along the geomagnetic
field lines. The direction of migration depends on the orientation of
the biomagnetic compass. Those with north-seeking pole forward migrate
north along the field lines. Those with the south-seeking pole forward
migrate south. It has been found that north-seeking bacteria predomi-
nate in the Northern Hemisphere while south-seeking bacteria predominate
in the Southern Hemisphere (Blakemore et al., 1980; Kirschvink, 1980).
The vertical component of the inclined geomagnetic field selects the
predominant polarity in each hemisphere by presumably favoring those
cells whose polarity causes them to be directed downward towards the
sediments and away from the toxic effects of the oxygen-rich surface
waters. At the geomagnetic equator where the vertical component is zero
both polarities coexist; presumably, horizontally directed motion is
equally beneficial to both polarities in reducing harmful upward
migration (Frankel et al., 1981).

The magnetotactic bacteria examined to date share certain characteristics in addition to magnetism. They all appear to be Gram negative, motile by means of flagella, and microaerophilic. It is possible that nonmotile and consequently nonmagnetotactic forms of magnetic bacteria exist or that soil or host-associated forms may eventually be found. However, the magnetic methods which have been used to recover these unusual microorganisms (Blakemore, 1975; Moench and Konetzka, 1978) have selected for aquatic and magnetotactic cells.

Magnetotactic bacteria are morphologically and metabolically diverse. Only one species, Aquaspirillum magnetotacticum, has been isolated, grown axenically and taxonomically characterized (Blakemore et al., 1979; Maratea and Blakemore, 1981). Cells of this species are denitrifying (Escalante-Semerena et al., 1980; Bazylinski and Blakemore, 1983), nitrogen fixing (Bazylinski and Blakemore, 1983), chemoheterotropic spirilla. A coccoid form has been proposed to be a new species of colorless sulfur-oxidizing bacterium, Thiococcus magnetotacticus (T.T. Moench manuscript submitted). Certain other forms appear similar to Ochrobium and other species of "iron bacteria." An especially fascinating magnetotactic "organism" has been recovered from sediments in Brazil (Farina et al., 1983) and in salt marshes in New England (unpublished). This is a spherical aggregate of from 7 to 20, or so, ovoid, flagellated, magnetic, prokaryotic cells arranged as a hollow or filled sphere. The assemblage functions as a coordinated, highly motile and magnetotactic "microcolony" consisting of apparently identical cells, e.g., it does not appear to be a consortium of more than one microbial species.

The diversity of magnetotactic cell types is illustrated in Figures 1-4. We interpret this variability to indicate widespread phylogenetic distribution of magnetotaxis.

MAGNETOSOMES

The permanent magnetic character of magnetotactic bacteria results from a striking and consistent cell structural feature which characterizes the group; the "magnetosome" (Balkwill et al., 1980). In forms in which they have been studied, magnetosomes are enveloped single crystals of the iron oxide magnetite (Frankel et al., 1979; Towe and Moench, 1981; Matsuda et al., 1983; Mann et al., 1984). Each is a single magnetic domain with a crystal size approximately 400 to 1000 Å, depending upon the species. Consequently, individual magnetosomes are not evident within cells observed with the light microscope. Their high iron content, however, renders them quite impenetrable by electrons and they are easily visualized even in unstained cells by means of electron microscopy. The structure and composition of the magnetosome envelope has not been studied. However, ferrihydrite, a second iron biomineral abundant in cells of the magnetotactic spirillum, co-purified with the cell magnetosome fraction (Frankel et al., 1983). This hydrated iron (III) oxide is possibly present in noncrystalline amorphous regions shown by high resolution electron microscopy to be intrinsically associated with magnetosome crystalline edges (Mann et al., 1984).

Magnetosomes within a given strain or cell type are homogeneous in grain size, and are uniform in shape and arrangement within the cell. The maximum size of the magnetosome within a given bacterial species is limited by an unknown mechanism. The number of magnetosomes per cell, however, can vary in response to culture conditions including iron
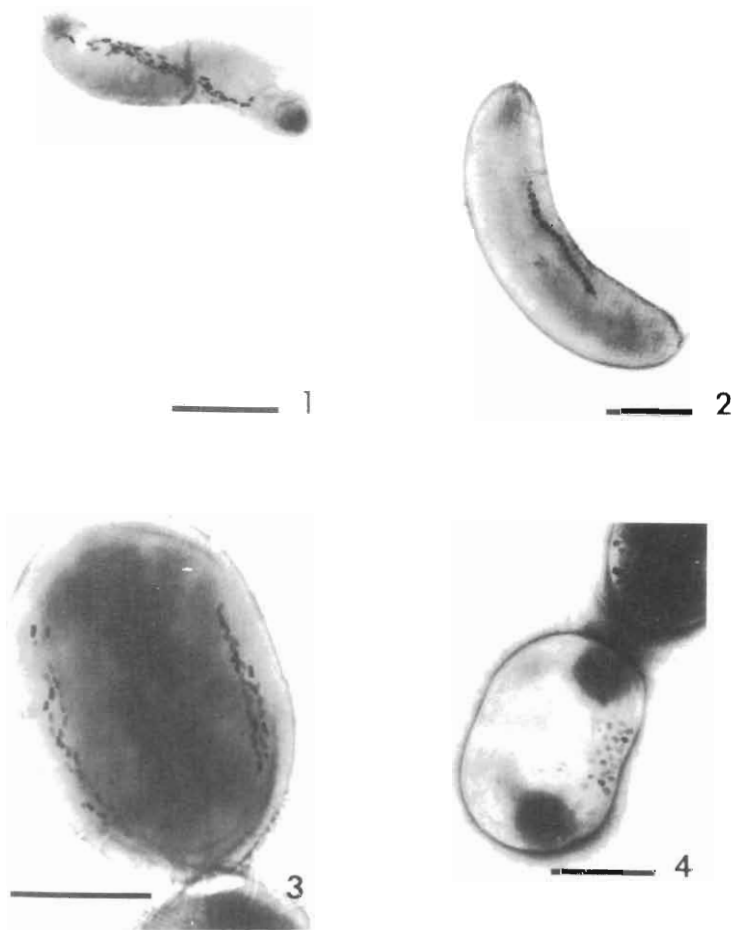
Fig. 1-4.   Transmission electron micrographs showing whole cells of
              magnetotactic bacteria. Cells were recovered from natural
              water samples in a magnetic field gradient. These as yet
              unnamed species are of diverse morphology and contain
              magnetosomes of different shapes.   Bars = 1 μm.

supply and dissolved oxygen. For instance, the average number of magnetosomes within cells of a magnetic spirillum varied from zero to 17 in response to culture Po_2 and optimal numbers were produced under microaerobic conditions (Blakemore et al., 1984).

Several morphologically distinct types of magnetosomes have been observed within various types of magnetotactic organisms. Some of these are illustrated in the selection of electron micrographs appearing in Figures 1-5. Cells were magnetically separated from sediments collected at various locations, negatively stained and examined by transmission electron microscopy. Magnetosomes within A. magnetotacticum are truncated octahedral prisms (Mann, Frankel and Blakemore, 1984). Magnetosomes within coccoid cells studied by Mann, Moench and Williams (1984) as well as those within an unidentified cell from a pond in Japan (Matsuda et al., 1983) were truncated hexagonal prisms. The prismatic crystals of either hexagonal or octahedral type were oriented with their easy axes of magnetization ([111] planes) along the chain axis. The crystal morphology of tear-drop or bullet shaped magnetosomes illustrated by Figures 1 and 3 is completely unknown.

In some cell types the magnetosomes occur in clusters predominantly at one side of the cell (Figure 4). In other species or types the magnetosomes occur as a string or chain of particles arranged along the motility axis of the cell. The magnetosomes situated at ends of such chains are often smaller (Figure 2). This suggests that magnetosome chains "grow" bidirectionally along their long axis as iron newly transported into the cell is transformed into magnetite. At cell division, whether they exist in chains or not, magnetosomes appear to be partitioned between each daughter cell.

Because they contain magnetosomes, cells of magnetotactic bacteria each have a permanent magnetic moment. The cell magnetic moment interacts with the local geomagnetic field tending to passively align the cell in the field (Frankel and Blakemore, 1980). Inasmuch as cell orientation and not absolute cell velocity is directly affected by the magnetic field, the observed behavior is a true taxis and not a klinokinesis. The geomagnetic field over most of the earth is inclined from the horizontal (e.g. it has an angle of dip). The vertical component of the local geomagnetic field exerts strong selective pressure on natural populations for cells with a direction of magnetization tending to direct them downward along the inclined field lines (Frankel and Blakemore, 1980; Blakemore and Frankel, 1981; Blakemore et al., 1980; Frankel et al., 1981). This was first evident with monopolarly flagellated forms which persistently swam forward and in the magnetic field direction (e.g. the direction indicated by the north-seeking end of a compass needle), and was further substantiated by field observations which revealed that cells in Southern hemisphere natural populations were of opposite magnetic polarity to those in the Northern hemisphere. Consequently, magnetotaxis tends to direct unidirectionally swimming cells downward in each hemisphere. Interestingly, this also applies to the colonial form of magnetotactic bacterium; the aggregates found in Brazil (Farina et al., 1983) swim south and down, whereas those from New England (our unpublished results) swim north and down. Some magnetotactic bacteria are bipolarly flagellated and swim principally along the inclined geomagnetic field lines but in either direction. The direction actually taken at any instant depends not only upon magnetism but also upon other "taxes." Aerotaxis, for instance, has been shown to override magnetotaxis in bipolarly flagellated magnetotactic spirilla (Spormann and Wolfe, 1984). The observed effect of Earth's magnetic field in orienting cells so that they may swim preferentially downward is consistent with their observed natural distribution. They are found

in sediments and in the sediment-water interface, not in surface films or the surface micro-layer.

As mentioned, magnetosome production appears to be a genetically stable character; a given cell type producing magnetosomes of a particular morphology and arrangement within the cell. Cultured in the laboratory, nonmagnetic mutants of magnetic spirilla survive many passages without producing magnetosomes. Since this trait can be lost, often abruptly, but with no obvious detrimental effect on cells, and since diverse species or morphological types of bacteria in natural environments possess magnetosomes, it would not be surprising if genes encoding magnetosome formation were carried on plasmids. Extensive efforts in several laboratories to detect plasmids within magnetotactic spirilla have met with negative results, however.

Fe_3O_4 PRECIPITATION IN MAGNETOSOMES

On the basis of extensive spectroscopic analysis, cells of A. magnetotacticum are known to contain ferrous ions, a low-density hydrous-ferric-oxide, a high-density hydrous-ferric-oxide (ferrihydrite) and Fe_3O_4. Additional experiments with cell fractions show that ferrihydrite in the magnetotactic cells is associated with the magnetosomes (Frankel et al., 1983). It has been proposed that A. magnetotacticum precipitates Fe_3O_4 in the sequence: Fe^{3+} quinate → $\overline{Fe^{2+}}$ → low-density hydrous-ferric-oxide → ferrihydrite → Fe_3O_4. In nonmagnetic cells the process stops with ferrihydrite. In cells of the cloned, nonmagnetotactic strain the process stops with low-density hydrous ferric oxide.

In the proposed sequences, iron enters the cell as Fe^{3+} chelated by quinic acid. Reduction to Fe^{2+} releases iron from the chelator. Fe^{2+} is reoxidized and accumulated as the low-density hydrous-iron-oxide. By analogy with the deposition of iron in the micellar cores of the protein ferritin, this oxidation step might involve molecular oxygen, which is required for Fe_3O_4 precipitation in A. magnetotacticum (Blakemore et al., 1984). Dehydration of the low-density hydrous-ferric-oxide results in ferrihydrite. Finally, partial reduction of ferrihydrite and further dehydration yields Fe_3O_4.

In high resolution TEM studies (Mann et al., 1984), no other crystalline phases in addition to Fe_3O_4 were detected. However, in some magnetosomes, noncrystalline material was found contiguous with the Fe_3O_4. This suggests that the hydrous-ferric-oxide phase is amorphous ferrihydrite, and that final crystallization of Fe_3O_4 occurs as a solution-reprecipitation process, possibly triggered by Fe^{2+} ions.

Additional experiments demonstrate that while the hydrous-ferric-oxide is primarily associated with magnetosomes, Fe^{2+} in the cell is very probably associated with the peptidoglycan wall layer of the cell (Ofer et al., 1984). This association could occur during the conversion from the iron quinate complex outside the cell to ferric iron and ultimately to Fe_3O_4 within the cell.

Fe_3O_4 is thermodynamically stable with respect to hematite and ferrihydrite at low E_H and high pH (Garrels and Christ, 1965). However, rapid transformation of ferrihydrite to magnetite appears to involve more than simple reduction and dehydration. While the degree of crystallinity of ferrihydrite can vary, in crystalline samples it has a structure related to hematite, with hexagonal close-packed oxygen atoms and Fe^{3+} octahedrally coordinated sites. Fe_3O_4 has a cubic, inverse spinel structure with Fe^{3+} in octahedral and tetrahedral sites, and Fe^{2+}

in octahedral sites. This, plus the fact that the precipitation process requires spatial segregation of regions of differing E_H and possibly pH, suggests that the process falls into the biomineralization category described by Lowenstam (1981) as "organic matrix mediated." Thus the magnetosome envelope is probably an integral element in the precipitation process, functioning as a locus for enzymatic activities, compartmentalizing constituents, providing control of E_H and pH, as well as comprising a structural element anchoring the Fe_3O_4 particles to the remainder of the cell.

MAGNETOTACTIC ALGAE

In addition to bacteria, Fe_3O_4 has been reported as a biomineralization product in eukaryotes including chitons, honeybees, pigeons, bobolinks, tuna and others (see e.g., Kirschvink, 1985). In these organisms, Fe_3O_4 has been identified magnetically or following extraction from the cell. Recently, Fe_3O_4 has been identified in vivo in magnetotactic euglenoid algal cells from brackish sediments in Brazil (Torres de Araujo et al., 1985). TEM of these organisms shows that they contain numerous Fe_3O_4 particles arranged in chains oriented more or less parallel to the long axis of the cell (Figure 5). Individual particles are arrowhead or tooth-shaped and are within the single magnetic domain size range for Fe_3O_4. Hence, each chain is a permanent magnetic dipole. If the moments of all the chains are oriented parallel to each other, a cell would have a magnetic dipole moment equal to the sum of the moments of all its particles. An estimate of the total magnetic moment M of algal cells gives M = 5×10^{-10} emu. This is about 1000 times the moment of a typical magnetic bacterium, and corresponds to a total of about 3×10^3 aligned particles of the observed dimensions.

The biological significance of magnetotaxis in these algae is not yet understood. However the highly ordered arrangement of the chains of particles in the cells suggests that they are chains of magnetosomes very much like the chains of magnetosomes in bacteria. Evidence for the presence of membranes enveloping the particles must await TEM of thin sections. However, the fact that the particles are separated from each other and not clumped is evidence that they are not free to move in the cells. Chains of free magnetic particles would lower their energy by moving together and eventually forming clumps.

Thus, eukaryotic cells as well as prokaryotic cells can produce Fe_3O_4 in the form of single magnetic domains as an intracellular biomineralization product. It will be interesting to compare the biomineralization process and the role(s) of membranes in these fundamentally different types of organisms.

MAGNETOSOME MEMBRANES

Previous studies (Balkwill et al., 1980) provided suggestive evidence for a lipid bilayer envelope surrounding the bacterial magnetosome. However, conclusive evidence had been lacking because of the difficulty in interpreting thin sections and the absence of data on purified magnetosomes. Recent data, obtained by freeze-etching and by thin sectioning both cells and magnetically extracted magnetosomes (Gorby et al., 1988), indicate the presence of a trilaminate membrane surrounding each magnetosome core. This membranous envelope was absent from purified magnetosomes treated with detergent to remove lipids and proteins. Trilaminate membrane vesicles with dimensional and spatial
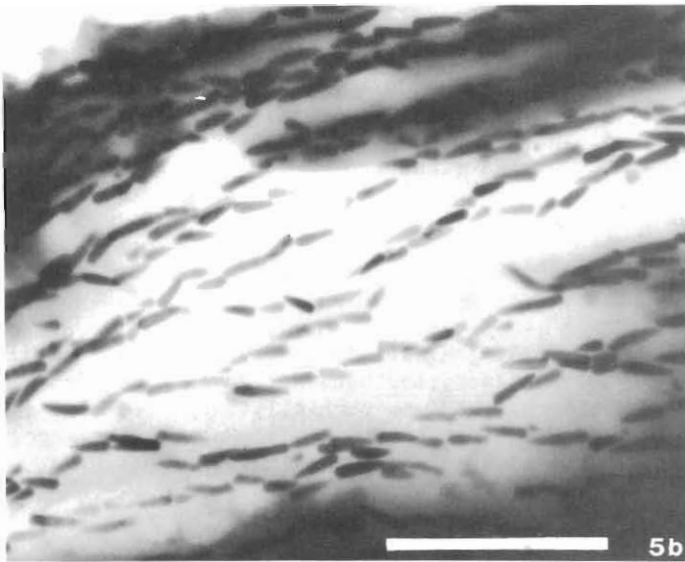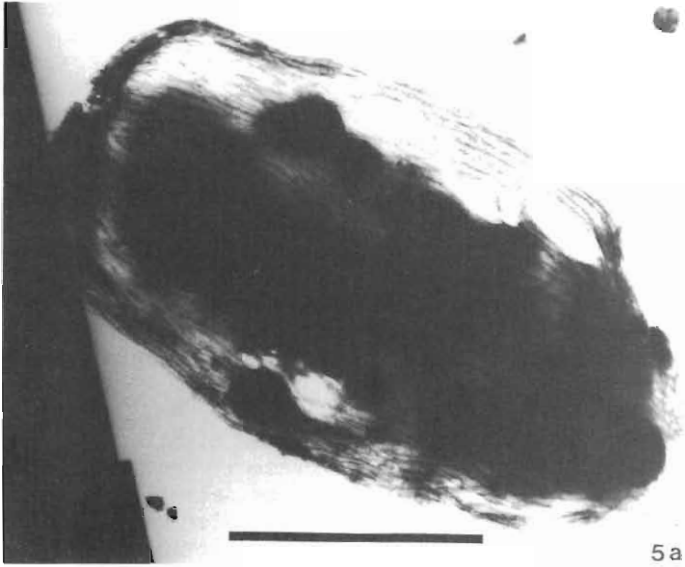
Fig. 5.   (a) Transmission electron micrograph of a negatively stained whole cell of the magnetotactic alga _Anisonena platysomum_. Bar = 10 µm.   (b) magnified region of the above cell showing numerous bullet-shaped magnetosomes arranged in chains. Bar = 1 µm.

characteristics of magnetosomes, but devoid of cores, were present in wild type magnetic cells grown without iron. Amorphous iron oxide was occasionally present in small quantity within these vesicles. Magnetosomes, vesicles with amorphous iron oxide, or empty vesicles were not present within cells of a nonmagnetic mutant strain. It is apparent, therefore, that these membranes are an integral part of magnetosomes and may be considered to be magnetosome boundary membranes.

Magnetosome membranes do not appear to be contiguous with the cytoplasmic membrane. Connections between the two membranes have never been observed in numerous thin sections, including stereo views, of magnetic cells. If the magnetosome membranes were invaginations of the cytoplasmic membrane, freeze-etching would be expected to reveal severed connections as pits in the inner surface of this membrane [as observed with freeze-etched preparations of cyanobacteria which possess photosynthetic membranes as vesicular intrusions of the cytoplasmic membrane [Lommen and Takemoto, 1978)]; it did not. Furthermore, when spheroplasts were made, they did not evert their magnetite crystals as would be expected of particles within surficial invaginations of the cytoplasmic membrane.

The magnetosome membrane does not appear significantly different in overall composition from other cell membranes. Proteins, free fatty acids, glycoproteins, and phospholipids were detected as components. The ratio of their abundance is that expected for a biological membrane (Rogers, 1983). Although most proteins detected in envelopes of purified magnetosomes were of similar mass (but not quantity) to those of the cytoplasmic membrane, two were uniquely with the magnetosome envelope. It is tempting to speculate that these could have a specific role in magnetite production. As enzymes, they could promote the accumulation of supersaturating quantities of iron oxide within the vesicles, serve to oxidize ferrous iron, or reduce and dehydrate the ferrihydrite precursor of bacterial magnetite. They could also be ferrihydrite-associated proteins such as bacterioferritin (Stiefel and Watt, 1979) apoprotein. As structural proteins, they might contribute to the compartmentalization deemed essential for "organic matrix-mediated" biomineralization (Lowenstam, 1981).

REFERENCES

Balkwill, D.L., Maratea, D., and Blakemore, R.P., 1980, Ultrastructure of a Magnetotactic Spirillum, J. Bacteriol. 141:1399 .
Bazylinski, D.A., and Blakemore, R.P., 1983, Denitrification and Assimilatory Nitrate Reduction in Aquaspirillum magnetotacticum, App. Environ. Microbiol. 46:1118.
Bazylinski, D.A., and Blakemore, R.P., 1983, Nitrogen Fixation (Acetylene Reduction) in Aquaspirillum magnetotacticum, Curr. Microbiol. 9:305.
Blakemore, R.P., 1975, Magnetotactic Bacteria, Science 190:377.
Blakemore, R.P., 1982, Magnetotactic Bacteria, Ann. Rev. Microbiol. 36:217.

Blakemore, R.P., and Frankel, R.B., 1981, Magnetic Navigation in
    Bacteria, Sci. Am. 245(b):58.
Blakemore, R.P., Maratea, D., and Wolfe, R.S., 1979, Isolation and Pure
    Culture of a Freshwater Magnetic Spirillum in Chemically Defined
    Medium, J. Bacteriol. 140:720.
Blakemore, R.P., Frankel, R.B., and Kalmijn, A.J., 1980, South-seeking
    Magnetotactic Bacteria in the Southern Hemisphere, Nature 286:384.
Blakemore, R.P., Short, K.A., Bazylinski, D.A., Rosenblatt, C., and
    Frankel, R.B., 1984, Microaerobic Conditions are Required for
    Magnetite Formation within Aquaspirillum magnetotacticum,
    Geomicrobiol. J. 4:62.
Escalente-Semerena, J.C., Blakemore, R.P., and Wolfe, R.S., 1980,
    Nitrate Dissimilation under Microaerophilic Conditions by a
    Magnetic Spirillum, Appl. Environ. Microbiol. 40:429.
Farina, M., Lins de Barros, H., Esquivel, D.M.S., and Danon, J., 1983,
    Ultrastructure of a Magnetotactic Microorganism, Biol. Cell 48:85.
Frankel, R.B., 1984, Magnetic Guidance of Organisms, Ann. Rev. Biophys.
    Bioeng. 13:85
Frankel, R.B., and Blakemore, R.P., 1980, Navigational Compass in
    Magnetic Bacteria, J. Magn. and Magn. Matls. 15-18:156.
Frankel, R.B., Blakemore, R.P., Torres de Araujo, F.F., Esquivel,
    D.M.S., and Danon, J., 1981, Magnetotactic Bacteria at the
    Geomagnetic Equator, Science 212:1269.
Frankel, R.B., Papaefthymiou, G.C., Blakemore, R.P., and O'Brien, W.,
    1983, Fe_3O_4 Precipitation in Magnetotactic Bacteria, Biochim.
    Biophys. Acta 763:147.
Garrels, R.M., and Christ, C.L., 1965, "Solution, Minerals and
    Equilibrium," Harper and Row, New York.
Gorby, Y.A., Beveridge, T.J. and Blakemore, R.P., 1988, Character of the
    Bacterial Magnetosome Membrane, J. Bacteriol. 170:0000.
Kirschvink, J.L., Jones, D.S., and MacFadden, B.J., Eds., 1985,
    "Magnetic Biomineralization and Magnetoreception in Organisms,"
    Plenum, New York.
Lommen, M.A., and Takemoto, S., 1978, Comparison, by Freeze-Fracture
    Electron Microscopy, of Chromatophores, Spheroplast-derived
    Membrane Vesicles, and Whole Cells of Rhodopseudomonas spheroides,
    J. Bacteriol. 136:730.
Lowenstam, H.A., 1981, Minerals Formed by Organisms, Science 211:1126.
Mann, S., Frankel, R.B., and Blakemore, R.P., 1984, Structure, Morph-
    ology and Crystal Growth of Bacterial Magnetite, Nature 310:405.
Mann, S., Moench, T.T., and Williams, R.J.P., 1984, A High Resolution
    Electron Microscopic Investigation of Bacterial Magnetite:
    Implications for Crystal Growth, Proc. Royal Society (London)
    221:385.
Maratea, D., and Blakemore, R.P., 1981, Aquaspirillum magnetotacticum
    sp. nov., a Magnetic Spirillum, Int. J. Syst. Bacteriol. 31:452.
Matsuda, T., Endo, J., Osakube, N., Tonomura, A., and Arli, T., 1983,
    Morphology and Structure of Biogenic Magnetite Particles, Nature
    302:411.
Moench, T.T., and Konetzka, W.A., 1978, A Novel Method for the Isola-
    tion and Study of a Magnetotactic Bacterium, Arch. Microbiol.
    119:203.
Ofer, S., Nowick, I., Bauminger, E.R., Papaefthymiou, G.C., Frankel,
    R.B., and Blakemore, R.P., 1984, Magnetosome Dynamics in
    Magnetotactic Bacteria, Biophys. J. 46:57.
Rogers, H.J., 1983, The Membranes of Bacteria, in "Bacterial Cell
    Structure," edited by Cole, J.A., Knowles, C.J., and Schlessinger,
    D., American Society of Microbiology, Washington, D.C.
Spormann, A.M., and Wolfe, R.S., 1984, Chemotactic, Magnetotactic and
    Tactile Behavior in a Magnetic Spirillum, FEMS Lett. 22:171.

Steifel, E.I., and Watt., G.D., 1979, *Azotobacter* cytochrome b557.5 is a Bacterioferritin, *Nature*, 279:81.

Torres de Araujo, F.F., Pires, M.A., Frankel, R.B., and Bicudo, C.E.M., 1985, Magnetite and Magnetotaxis in Algae, *Biophys. J.*, 50:375.

Towe, K.M., and Moench, T.T., 1981, Electro-optical Characterization of Bacterial Magnetite, *Earth Planet. Sci. Lett.* 52:213.