

# Magnetoglobus, Magnetic Aggregates in Anaerobic Environments

Magnetotactic bacteria live as aggregates, align to magnetic fields, but do not fit the classic definition of being multicellular

Carolina N. Keim, Marcos Farina, and Ulysses Lins

**V**arious species sense and respond to earth's magnetic field, including homing pigeons and migratory birds, fish, sea turtles, and social insects such as honeybees and ants. Among microbes, algae of the genus *Anisonema* and several types of magnetotactic bacteria also sense and react to magnetic fields.

Among these microorganisms, magnetotactic bacteria produce perhaps the best magnetosensors, called magnetosomes. Magnetosomes are intracellular organelles composed of magnetic crystals coated with a bilayer membrane containing specialized proteins and lipids. The magnetosomes attach to protein filaments that arrange the magnetosomes in chains and presumably attach the magnetosome chains to the

structural framework of each cell that they occupy. The magnetosome chain works as a magnetic compass, continuously aligning itself and the microorganism to local magnetic field lines.

Such magnetotactic bacteria move by means of flagella, which are aligned to the magnetosome chain. Because of flagellar propulsion, the alignment of magnetosomes to magnetic fields results in net movement along the magnetic field lines. This behavior is known as magnetotaxis and is the behavioral hallmark of magnetotactic bacteria. The structural hallmark is the presence of magnetosomes and flagella.

All known magnetotactic bacteria are gram-negative and belong to the domain *Bacteria*. However, they are phylogenetically diverse as they are spread in the  $\alpha$ -,  $\delta$ -, and  $\gamma$ -Proteobacteria and in the *Nitrospira* phylum. They are also morphologically diverse, comprising cocci, bacilli, spirilla, vibrios, and multicellular forms.

Multicellular magnetotactic bacteria form spherical assemblies, 2.2 to 12.5  $\mu\text{m}$  in diameter, that are composed of 7–30 gram-negative cells. According to phylogenetic rDNA analysis, multicellular magnetotactic bacteria belong to a single genus containing at least five species in the  $\delta$ -Proteobacteria class in the *Desulfobacteriaceae* family. They have been called many-celled magnetotactic prokaryotes (MMP), multicellular magnetotactic prokaryotes, magnetotactic multicellular aggregates (MMA), or magnetotactic multicellular organisms (MMO). The most extensively studied multicellular magnetotactic bacterium was recently designated *Candidatus Magnetoglobus multicellularis*. We will use the genus name *Magnetoglobus* to refer to

## Summary

- *Magnetoglobus* sp., the multicellular magnetotactic bacteria, are spherical multicellular assemblies that align to magnetic fields, swim in a complex manner, and are found in brackish, marine, or hypersaline environments rich in sulfide.
- Cells in *Magnetoglobus* sp. are polarized and arranged side by side around an acellular internal compartment and have flagella along the outside perimeter of the cells.
- Individual cells die when they leave the multicellular body, and there are no known single-celled *Magnetoglobus* spp.
- *Magnetoglobus* sp. proliferates by dividing into two multicellular bodies, whose cells grow and then divide while the whole body elongates and splits into two new aggregates, which have the same properties as the original.

Carolina N. Keim and Ulysses Lins are Associate Professors at the Institute of Microbiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, and Marcos Farina is a Full Professor at the Institute of Biomedical Sciences, Federal University of Rio de Janeiro.



## Lins Is Attracted to “Romantic” Scientific Questions

Ulysses Lins enjoys visiting the Amazon Forest in northern Brazil, if only to remind himself of the diversity and importance of nature. “All that richness and exploding life everywhere reinforces my belief that we must take good care of our planet and, above all, put some effort into understanding that richness,” he says. “My small contribution is in science.”

Lins, 38, associate professor at the Institute of Microbiology and a professor at the Federal University of Rio de Janeiro, studies magnetotactic bacteria—in particular, the multicellular *Candidatus Magnetoglobus multicellularis*. “All life as we know it evolved under the influence of the small geomagnetic field,” he says. “Many examples of biological responses exist in animals—bees, turtles, and others—mostly for navigation. But bacteria . . . produced tiny magnets—called magnetosomes—within the cytoplasm.

How and why this happened . . . may help us understand how life evolved.”

Lins’ wife, also a scientist, works in biotechnology on energy-related molecules, including ethanol and lipases. “Although this kind of work is very important nowadays, I find myself in the opposite side of science,” he says. “I really enjoy working with pure—and somehow romantic—scientific questions,” although this “sometimes puts me in the difficult position of funding a lab.”

Lins grew up in Rio de Janeiro, the older of two brothers. His mother, a homemaker “taught me how to endure and how to focus on things.” His father, a banker and, later, an insurance executive, “always dreamed of being a medical doctor and taught me how to pursue my dreams. When he heard I was going for biology in college, he both cheered and

was worried about my future. Nowadays he loves to hear about my work—although he confesses that he does not fully understand it.”



As a boy, Lins spent several weeks each summer near the ocean. “As I was a very lousy soccer player, my father gave me a mask and a snorkel to explore the shore,” he says. “I loved to see the small creatures at the sea.” As an adolescent, he ignored natural science and turned to science fiction instead, recalling two books in particular that fascinated him: George Orwell’s *1984* and Ray Bradbury’s *The Martian Chronicles*. “I tried to read Darwin’s *Origin of the Species*, but it was too difficult and detailed at the time,” he says.

the group, and *Candidatus Magnetoglobus multicellularis* to refer to the most extensively studied species.

### ***Magnetoglobus* Exhibits Particular Behaviors in Particular Environments**

When swimming in the oxic conditions of a drop of water under the light microscope, magnetotactic bacteria from the Northern hemisphere swim parallel to the magnetic field, corresponding to a northward migration in the geomagnetic field or a north-seeking behavior. Conversely, magnetotactic bacteria from the Southern hemisphere swim antiparallel to the magnetic field, which means southward migration in the geomagnetic field or a south-seeking behavior. In both hemispheres, the bacteria swim downward as the geomagnetic field inclination is positive and negative in the Northern

and Southern hemispheres, respectively. Near the geomagnetic equator, roughly equal numbers of magnetotactic bacteria swim in each direction because the vertical component of the magnetic field vector approximates zero.

Magnetotactic bacteria are ubiquitous in aquatic environments. They occur mostly in the transition zone between the oxygenated surface waters and the anaerobic layer. This oxygen gradient occurs because the water inside sediments and in the lower part of stratified lakes mixes slowly with overlying water and also because indigenous microorganisms consume oxygen and organic matter as they produce CO<sub>2</sub> and H<sub>2</sub>O. Other chemical gradients, such as Fe<sup>3+</sup>/Fe<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup>, and SO<sub>4</sub><sup>2-</sup>/S<sup>2-</sup> coexist with the oxygen gradient and are also produced by microbial activities. Magnetotactic bacteria use magnetotaxis to find their preferred position in these chemical gradients, swimming

Later, as a university undergraduate—after he returned to studying biology—he read books “written by some of the greatest biology writers of our time—Ernst Mayr, Stephen Jay Gould, and Richard Dawkins, to mention a few,” he says. Lins received his undergraduate degree, master’s, and doctorate at Federal University of Rio de Janeiro, where he teaches today.

As an undergraduate, he took advantage of a government program, called Scientific Initiation, that allowed undergraduate students to develop scientific projects in university labs. “In my first semester at the university, I enrolled in such a program in a large laboratory that developed cell biology studies in microorganisms, in particular those related to Chagas’ disease,” he says. “I loved the microscopy work, but I must confess that the idea of working with medical microbiology was not appealing to me. My first contact

with an electron microscope was very disappointing, but was also decisive.”

Lins unrealistically expected to see cells in three-dimension right away, “like in the biology book illustrations,” he says. Instead, the images were flat, dim, and in poor contrast. However, he sought help from a more experienced researcher. “Raul Machado taught me a lot of things about microscopy and how to beautifully image cells,” Lins recalls. “In a sense, he was an artist as well as a biologist. How he used his big hands to handle tiny pieces of plant tissue remains a mystery to me.”

Lins started then to work with magnetotactic bacteria. “I was very excited with the idea that bacteria could respond to magnetic fields and that we could study them to understand these marvelous microbes and the processes by which they convert ions into highly organized magnets,”

Lins says. In 2000 he began a year at the National Institutes of Health in Bethesda, Md., working in cell biology and learning more about microscopy. When the year ended, he returned to Brazil to start his own lab.

“Some neurological disorders are being associated with the precipitation of iron oxides—magnetite—the same material that magnetotactic bacteria produce,” he says. “Magnetotactic bacteria control the size, shape, and chemical composition of the minerals they produce, and provide an opportunity for mimicking these processes in the production of nanomagnetic materials for industry. The beauty of it, to me, is that no matter how deep we go into these questions, new and more interesting questions will always emerge.”

**Marlene Cimons**

Marlene Cimons is a freelance writer in Bethesda, Md.

forward and backward, responding to the chemical composition in discrete microenvironments within this multilayered habitat.

*Magnetoglobus* are found in brackish, marine, or hypersaline environments worldwide, whose salinities range from about 8 to 75‰; these environments typically are near-neutral pH and are rich in sulfide. Within these environments, *Magnetoglobus* are just below the microaerobic layer and in the upper layers of the anaerobic region, where iron concentrations are highest. This microenvironment occurs mainly in sediments, although it is also found in water columns of stratified lakes and estuaries. As *Magnetoglobus* sp. is phylogenetically affiliated to the group of sulfate-reducing  $\delta$ -Proteobacteria, *Magnetoglobus* sp. might be a heterotroph that uses sulfate or another sulfur compound as its terminal electron acceptor.

*Candidatus Magnetoglobus multicellularis* is

found in Araruama Lagoon, a large hypersaline lagoon in Rio de Janeiro State, Brazil. The microorganism prefers anaerobic conditions at the fourth centimeter below the sediment surface; oxygen is undetectable below 0.3 cm. *Candidatus Magnetoglobus multicellularis* is detected in shallow areas near the border of the lagoon, where salinity varies from 35 to 75‰.

***Magnetoglobus* Aggregates Show Distinctive Motility**

Ensembles of *Magnetoglobus* sp. cells are magnetotactic and, propelled by flagella along the surfaces of such aggregates, swim along magnetic field lines in both directions of magnetic fields. Like other magnetotactic bacteria, in the Northern hemisphere such aggregates swim preferentially toward geomagnetic North,



whereas in the Southern hemisphere they swim in the opposite direction. The velocity of *Magnetoglobus* sp. varies from 30 to 175  $\mu\text{m/s}$ ; *Candidatus Magnetoglobus multicellularis* swim at  $90 \pm 20 \mu\text{m/s}$ .

These aggregates also sometimes exhibit a peculiar backward movement called “ping-pong” or “escape motility” that consists of backward excursions of hundreds of micrometers with decreasing speed, followed by forward movements with increasing velocity. *Magnetoglobus* sp. increases the frequency of these backward excursions when subject to stronger magnetic fields, suggesting that its magnetoreception mechanism is not so simple.

At magnetic fields that are 20 to 30 times earth’s magnetic field, *Candidatus Magnetoglobus multicellularis* usually spin around themselves when they are blocked by a sand grain or at the edge of a drop of water. The escape motility can interrupt this spinning movement, called rotation. When such aggregates are not exposed to external fields and consequently are only under the influence of the earth’s magnetic field, they swim with a constant speed in a looping motion, called walking.

When *Magnetoglobus* sp. moves forward, it travels in an elongated helical and clockwise trajectory (when the aggregate is moving away from the observer). As it moves, the microorganism spins in the same sense of the trajectory, and each turn of the organism coincides with one turn of the trajectory. This pattern differs from that of unicellular bacteria, for which the cell spin follows one sense, while the trajectory spins in the opposite sense.

### Structure of Multicellular Bodies and Cells

*Magnetoglobus* sp. aggregates are spherical, and the outer region of cells along the surface of such aggregates is covered by thin, radially arranged fibers of a glycocalyx. Each cell has about 30 short flagella ( $2.4 \pm 0.5 \mu\text{m}$  in length) clustered in a small region of the cell envelope exclusively along the outer surface of the aggregate. In most individuals, the cells are arranged as a helix.

*Magnetoglobus* cells are arranged side by side forming a single layer, leaving an acellular internal compartment at the center of the sphere. The internal compartment contains small membranous vesicles and a belt probably composed of polysaccharide fibers. Because all cells of such aggregates face this internal compartment, it may be used for

cell-to-cell communication, either through diffusion of soluble chemicals or by vesicle budding and fusion. All cells face the outer environment, too, from which they take up nutrients.

*Magnetoglobus* sp. cells are polarized. The cells that form such aggregates are very close to one another, and the four membranes of two adjacent cells are parallel. The regions of contact between two adjacent cells are flat, and the regions of contact of three or more cells present edges. The region in contact with the outer environment is rounded. To fit the spherical body where all cells face both the outer environment and the internal compartment and are tightly apposed to each other, the cells take a pyramidal shape with the base at the outside and the apex pointing to the center (see Box 1).

Membrane specializations at the edges of the pyramidal cell include regions between cell-to-cell contact and cell-environment interface. Both these membrane specializations and the filaments found in the internal compartment may be responsible for holding the cells together. Cells of *Magnetoglobus* sp. sometimes contain large lipid droplets in the cytoplasm, along with smaller polyhydroxyalkanoate inclusions and polyphosphate bodies. Additionally, amorphous iron-oxygen inclusions are rarely found in the cytoplasm of microorganisms collected in Rodrigo de Freitas Lagoon, a coastal lagoon in Rio de Janeiro city.

In more than 20 years of scientific investigations on *Magnetoglobus* sp., we never observed live unicellular bacteria resembling the cells of a *Magnetoglobus* sp. Spherical aggregates readily disaggregate when held in the light microscope or when treated with distilled water or other disrupting substances such as ethylene glycol tetraacetic acid (EGTA) and azide. Soon after aggregates of cells are disrupted, the individual cells swell, lose their pyramidal shape, and die. However, intact aggregates can remain alive after losing only a few cells, suggesting that maintaining an internal compartment is not essential for keeping cells in the aggregate alive, at least for short periods. In some cases, aggregates lose a string of cells, reinforcing the idea that individual cells within aggregates are highly attached and arranged in a helix.

### Magnetic Organelle Arrangements Are Strictly Controlled

Like other magnetotactic bacteria, *Magnetoglobus* sp. cells contain nanoscaled membrane-

bound magnetic crystals called magnetosomes, whose shape, size, composition, and location are under strict genetic control. *Magnetoglobus* magnetosomes are arranged in chains, and the chains are arranged in plates. A striated structure of unknown composition is frequently found parallel to the magnetosome plates, and may participate in attaching magnetosomes to each other and to the cell fabric. The magnetosome plates are found at the periphery of the spherical *Magnetoglobus* sp. body, parallel to the organism surface.

Unlike unicellular magnetotactic bacteria, which produce magnetite ( $\text{Fe}_3\text{O}_4$ ) magnetosomes, most *Magnetoglobus* species produce iron sulfide magnetosomes, 30–120 nm in length, generally equidimensional, without an obvious geometric shape. Three different iron sulfide minerals are found in the magnetosomes of *Magnetoglobus*: the magnetic mineral greigite ( $\text{Fe}_3\text{S}_4$ ) along with two nonmagnetic minerals, mackinawite (tetragonal FeS) and cubic FeS.

Those nonmagnetic minerals might be precursor phases that convert through solid-state transformations from cubic FeS to mackinawite to greigite. When mackinawite transforms to greigite, half of the iron in the crystal is oxidized. One-fourth of the iron atoms leave the crystalline structure if additional sulfur atoms are not added to the crystal. Indeed, iron and oxygen regions are observed at the periphery of iron sulfide magnetosomes, and they may correspond to the free iron liberated during the mackinawite-greigite transformation.

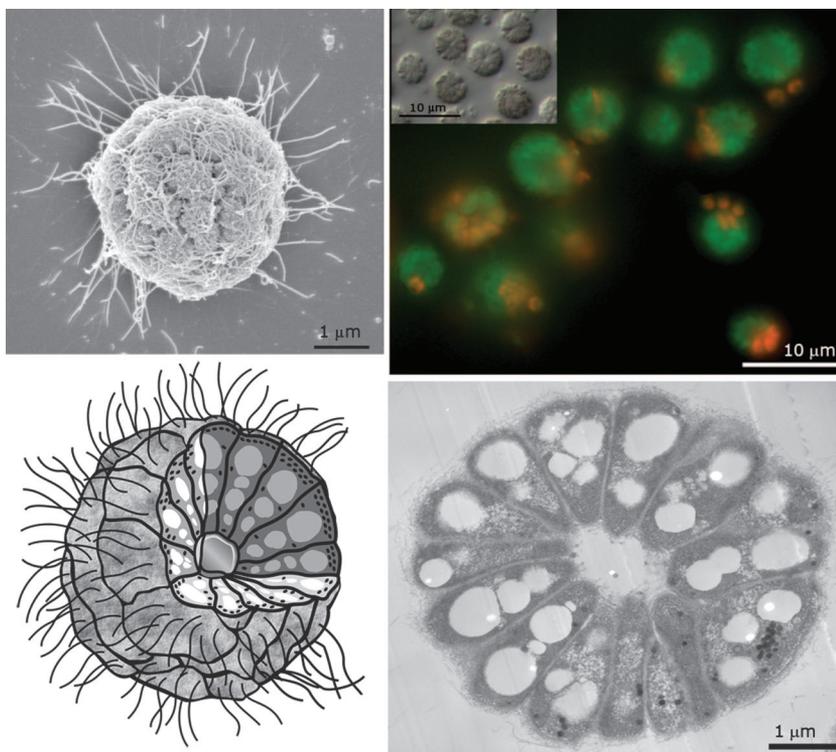
Some *Magnetoglobus* sp. from two coastal lagoons in Brazil also contain bullet-shaped, magnetite ( $\text{Fe}_3\text{O}_4$ ) magnetosomes besides the usual iron sulfides. These magnetite magnetosomes occur in the same chains as iron sulfide crystals. Magnetite is 50% more strongly magnetic than greigite, although greigite is more likely to crystallize at the very low oxygen and relatively high sulfide concentrations where *Magnetoglobus* sp. is usually found. As this type of *Magnetoglobus* sp. that

### The Shape of *Magnetoglobus* Cells

Most unicellular microorganisms cells have cylindrical or spherical shapes. These simple shapes result from the peptidoglycan layer, whose shape is maintained by internal turgor. In *Magnetoglobus* sp., the membrane specializations at the edges of the pyramidal cells and the filaments in the internal compartment appear to play a fundamental role in attaching cells to one another and maintaining the internal compartment of each aggregate of cells. This configuration is unlikely to occur if, say, more than about 14 bubbles were to juxtapose. Such an assembly would accommodate an internal bubble instead of the internal space that occurs in this microorganism. Thus, the topology of *Magnetoglobus* sp. depends on specifically combined physical and biological constraints.

synthesizes magnetite is rarely found, the full ecological and physiological significance of these findings remains unknown.

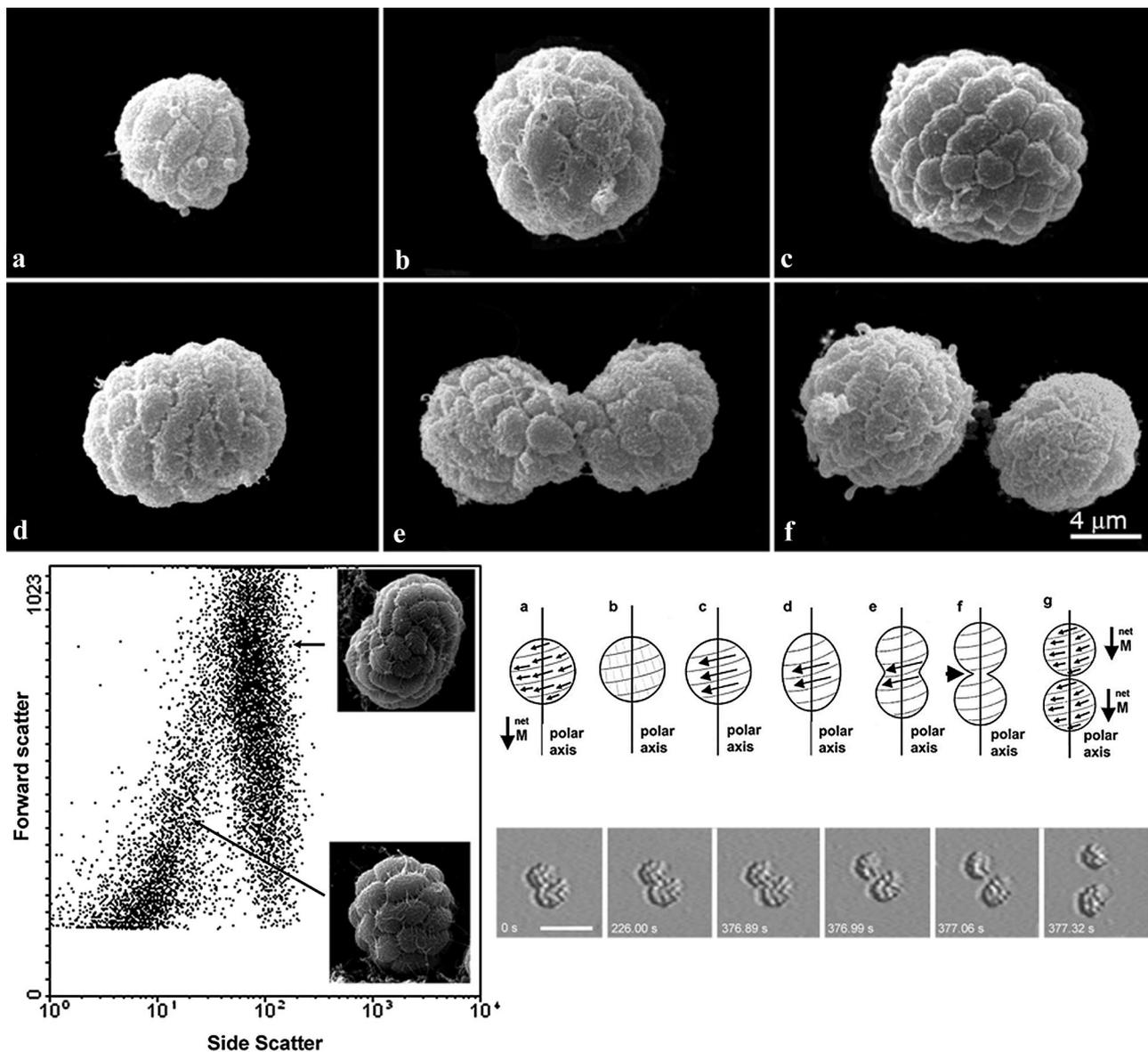
FIGURE 1



Morphology of multicellular magnetotactic bacteria. Top left: Scanning electron microscopy image of *Candidatus Magnetoglobus multicellularis*. Note the numerous flagella on the surface of the microorganism. Top right: Fluorescence microscopy of *Candidatus Magnetoglobus multicellularis* stained with LIVE/DEAD BacLight Bacterial Viability Kit. Cells organized in a multicellular sphere are viable (green), whereas isolated cells are dead (red); inset shows a DIC image of the microorganisms. (Reproduced from Abreu et al. 2007 with permission from Society for General Microbiology.) Bottom left: Scheme of the current interpretation of the structural features of the cells in *Candidatus Magnetoglobus multicellularis*. Bottom right: Transmission electron microscopy image of a microorganism showing the cells arranged around an acellular central compartment. Large lipid inclusion (light areas) and magnetosomes (dark spots) can be seen.



FIGURE 2



Life cycle of *Candidatus Magnetoglobus multicellularis*. Top image: Scanning electron micrographs of selected individuals arranged to illustrate the presumed sequence of the life cycle of the magnetotactic multicellular organisms. Initially (a), the organism is small and spherical; as it grows (b) their cell size enlarges, but not the cell number. Later (c), cells synchronously divide without separating and the organism contains a larger number of smaller cells. In the next step (d), the magnetotactic multicellular organisms become elliptical and then (e) eight-shaped, as two attached organisms. Finally (f), the eight-shaped organism splits into two equal organisms. (Reproduced from Keim et al. 2004 with permission from Blackwell.) Bottom left: Distribution of *Candidatus Magnetoglobus multicellularis* based on forward (size) and side scatter (cell complexity) detection in the flow cytometer. Note the presence of two populations. The scanning electron microscopy images represent these microorganisms in different stages of its life cycle. Bottom left: Cell size (forward scatter) and associated fluorescence of 10,000 *Candidatus Magnetoglobus multicellularis* per sample measured in fluorescence channel-1 (FL1-H) detecting glutaraldehyde fluorescence associated with protein content. (Reproduced from Abreu et al. 2007 with permission from Society for General Microbiology.) Bottom right: Schematic drawings of magnetotactic multicellular organisms. (a) Possible distribution of the magnetic moment in a spherical organism (only the surface facing the observer is represented). The cells are arranged in a spiral, and the magnetic moment of each cell is at a fixed angle to the spiral (an example is given by the small arrows). The net magnetic moment of the organism is aligned to the polar axis (large arrow). (b) The cell division invaginations would be aligned to planes perpendicular to the laps of the spiral (dashed lines). (c-e) Cell movements during organism division. (c) The cells in neighbor laps of the spiral slide in the direction illustrated by the arrows, making the organism to elongate in the direction of the polar axis, as shown in (d). (d) After elongation, only the cells at the middle of the organism continue the movements (arrows), leading to the constriction shown in (e). (e) Only a few cells at the middle continue the movement, until it is formed two spherical bodies linked by a single cell, as illustrated in (f). (f) Now, the terminal cells separate from each other, generating two equivalent organisms similar to the parental one (g). (g) This process is able to generate two equal organisms with the same general cell arrangement and magnetic moment direction as the parental one. (Reproduced from Keim et al. 2007 with permission from Springer, Heidelberg.) The lower image illustrates the sequence of reproduction. (Reproduced from Keim et al. 2004 with permission from Blackwell.)

## Magnetic Properties Sometimes Are Unusual, Even Opposite Those Expected

Rarely, *Magnetoglobus* sp. populations show inverted polarity, by acting south-seeking in the Northern Hemisphere or north-seeking in the Southern Hemisphere. Moreover, *Magnetoglobus* sp. can be demagnetized and remagnetized, meaning that north-seeking microorganisms can be converted into south-seeking microorganisms after a strong pulse of a magnetic field is applied opposite to the direction of the local magnetic field. Sometimes, demagnetized microorganisms fail to align to magnetic fields and swim in random directions.

In experiments involving *Candidatus Magnetoglobus multicellularis*, the magnetic moment of the whole aggregate, which measures the strength of the magnetic field generated by the magnetic crystals, could increase by only about one-fourth of the existing moment after the application of a strong magnetic pulse, meaning that they developed about 80% of the magnetic moment possible for those particular magnetic minerals, chain organization, and cell arrangements they have. This limit implies that natural selection resulted in a high efficiency in magnetic alignment, suggesting that magnetotaxis is of prime importance for the survival of these microorganisms.

*Candidatus Magnetoglobus multicellularis* consists of two populations, one with a magnetic dipole moment of  $1.0 \pm 0.3 \times 10^{-10}$  and the other with a magnetic dipole moment of  $1.8 \pm 0.3 \times 10^{-10}$  emu (electromagnetic unit; 1 emu equals  $0.001\text{A/m}^2$ ). The presence of two distinct populations might signify two stages in the life cycle of this microorganism.

## Life Cycle of Aggregate Cells Leads to Peculiarities

Although magnetically enriched *Candidatus Magnetoglobus multicellularis* from Araruama Lagoon appears to contain a single genome, aggregates fall into two distinct populations of different sizes, based on flow cytometry. These two populations also have distinct magnetic moments. One simple explanation is that these two seemingly distinct populations are merely at different stages of the life cycle, with the larger having just duplicated its DNA and number of magnetosomes.

*Magnetoglobus* sp. grows by increasing cell volume, not cell number. We have observed a few cells of *Candidatus Magnetoglobus multicellularis* with septa, which are radial in relation to the multicellular body. Elliptical and eight-shaped individuals are sometimes observed among the predominant spherical aggregates of *Candidatus Magnetoglobus multicellularis* and other *Magnetoglobus* species. Such observations lead us to speculate that this aggregate proliferates by dividing into two multicellular bodies, without releasing single cells during its life cycle.

More specifically, it appears that spherical aggregates become elliptical and then eight-shaped, with the constriction between the two halves becoming increasingly pronounced until it splits, forming two new spherical aggregates that swim independently.

The spherical multicellular bodies of *Magnetoglobus* sp. grow by increasing the cell volume, with individual cells within the aggregate dividing synchronously and thus generating individual aggregates that contain twice the original cell number. As the aggregate becomes elliptical, a constriction grows in its middle plane, causing it to become eight-shaped and consisting of two attached spherical aggregates. Finally, the connected pair of aggregates separate into two distinct spherical individuals, each with the same magnetic polarity of the original.

The magnetotactic behavior of *Magnetoglobus* sp. implies that the magnetosomes are fixed inside the cells, which are strictly organized in the spherical body, and that this organization must be transmitted to the daughter cells during division. The transmission of the magnetic polarity is epigenetic in other magnetotactic bacteria, and *Magnetoglobus* sp. is not an exception.

The helical arrangement of *Magnetoglobus* sp. cells suggests a mechanism for their epigenetic inheritance of magnetic polarity. If the cells are arranged as a coiled string to form a spherical body, then the net magnetic moment of each cell would be nearly parallel to the sphere surface, and its direction would be at a constant angle to the imaginary line that corresponds to the helix trace. Thus, the sum of the magnetic moments of the cells within an aggregate would be parallel to the axis of the helix, which coincides with the axis of movement. The cell division plane would be perpendicular to the helix path, and the cells along the helix would slide to each other to generate the elliptical and eight-



## Multicellular Microorganisms

Several multicellular forms evolved independently in different lineages in the three domains of life. For instance, animals, fungi, plants, brown algae, and heterocyst-forming cyanobacteria evolved in separate lineages. Considering “midway” examples, including some *Actinomycetes*, *Beggiatoaceae*, *Magnetoglobus* sp., non-heterocyst-forming cyanobacteria, and *Methanosarcina* sp., the multicellular way of life also apparently emerged several times among both bacteria and archaea.

Among these many-celled bacteria and archaea, morphologies are relatively simple, and are limited to linear and branched filaments or spheres. Spheres are common in free-living, aquatic microorganisms, such as *Magnetoglobus* sp. and assemblages of “*Pelochromatium roseum*.” Meanwhile, the filamentous cyanobacteria and *Beggiatoaceae* are found mainly in sediments.

The tendency for aquatic aggregates to form simple spheres and for sediment-dwellers to form filaments can be explained as adaptations to those specific environments. Thus, isotropic, spherical shapes seem suited to water, whereas filaments are better suited for filling spaces between grains in soil and sediment, colonizing large volumes of solid substrates, and covering surfaces. Furthermore, gliding filaments such as certain cyanobacteria and *Beggiatoaceae* can migrate and thus colonize larger regions.

One pressure for becoming multicellular could be to avoid predation by abundant protists specialized in consuming small cells. A multicellular aggregate of even small cells simply might be too large to be ingested. This rationale applied well for filamentous microorganisms, which can become several millimeters long, which is beyond the capacity of predatory protists.

*Magnetoglobus* sp. combines several other characteristics, including high speed and “ping-pong” motility, that help them to avoid predatory protozoa. Further, by constantly maintaining its multicellularity, *Magnetoglobus* sp. avoids a reduction in size, which is one important parameter for protozoa to select their prey. Indeed, protozoa flagellates can consume partially disaggregated *Candidatus Magnetoglobus multicellularis*. However, when in vitro, large ciliates can ingest intact *Candidatus Magnetoglobus multicellularis*, dissolving their magnetosomes in acidic vacuoles and liberating the iron that is recycled to the environment. This iron would otherwise be trapped as crystals in the magnetosomes.

shaped morphologies. To generate an elliptical body, all neighbor cells should slide to each other, elongating the sphere along the helix axis. To become eight-shaped, the cells at the central turns of the helix slide to each other until they constrict into two spheres bound through a single cell-to-cell linkage. During the final division step, these two cells detach, generating two independent aggregates.

During this life cycle, the internal compartment is isolated from the outer environment. The cell arrangement, polarity, morphology, and axis of movement are inherited epigenetically. Because there is no unicellular stage in the *Magnetoglobus* sp. life cycle, this organism is not strictly clonal. However, because it contains only about 20 cells that probably have fixed positions within the ensemble, the descendants

of any single cell could form the ensemble after a mere four to five generations. In practical terms, this small number of generations makes this organism clonal, albeit without having a single-cell stage during its life cycle.

### Does *Magnetoglobus* sp. Satisfy the Concept of Being Multicellular?

Is *Magnetoglobus* sp. a multicellular organism? Although not unicellular, like some other microorganisms, it raises doubts (see Box 2). The concept of multicellular organisms changed significantly during the past three decades. Once based mainly on morphology, that concept now incorporates observations on physiology, cell-to-cell signaling, and behavior. The stricter, classical concept insisted that a multicellular organism include cooperating cells that are specialized for different functions and juxtaposed in a characteristic way. Another definition requires a multicellular organism to have a specific shape and cell organization, and for its cells to lack autonomy and not to compete. Yet another even more recent definition requires only that there be close contact between cells and coordination of at least one key function such as growth, movement, or biochemical activity; the presence of different cell types is a further step towards multicellularity, but is not considered essential.

*Magnetoglobus* sp. fulfills several of these requirements. Each aggregate consists of many cells, which are juxtaposed in a characteristic way. Moreover, none of those cells is autonomous, and they do not compete with each other. There is coordinate growth and movement, which implies a coordination of biochemical activities. However, there is no evidence for cell differentiation and specialization. Thus, *Magnetoglobus* sp. can be considered a multicellular organism according to some definitions. Perhaps it is safer to consider *Magnetoglobus* sp. as being somewhere between unicellular and multicellular.

#### ACKNOWLEDGMENTS

We thank the many students and researchers that contributed to our project. We acknowledge financial support from Brazilian CNPq and FAPERJ (Pronex) programs.

**SUGGESTED READING**

- Abreu, F., J. L. Martins, T. S. Silveira, C. N. Keim, H. G. P. Lins de Barros, F. Gueiros-Filho, and U. Lins. 2007. '*Candidatus Magnetoglobus multicellularis*', a multicellular, magnetotactic prokaryote from a hypersaline environment. *Int. J. Syst. Evol. Microbiol.* 57:1318–1322.
- Abreu, F., K. T. Silva, J. L. Martins, and U. Lins. 2006. Cell viability in magnetotactic multicellular prokaryotes. *Int. Microbiol.* 9:267–272.
- Carrol, S. B. 2001. Chance and necessity: the evolution of morphological complexity and diversity. *Nature* 409:1102–1109.
- Frankel, R. B., D. A. Bazylinski, M. S. Johnson and B. L. Taylor. 1997. Magneto-aerotaxis in marine coccoid bacteria. *Biophys. J.* 73:994–1000.
- Keim, C. N., F. Abreu, U. Lins, H. Lins de Barros, and M. Farina. 2004. Cell organization and ultrastructure of a magnetotactic multicellular organism. *J. Struct. Biol.* 145:254–262.
- Keim, C. N., J. L. Martins, F. Abreu, A. Rosado, M. Farina, H. Lins de Barros, R. Borojevic, and U. Lins. 2004. Multicellular life cycle of magnetotactic prokaryotes. *FEMS Microbiol. Lett.* 240:203–208.
- Keim, C. N., J. L. Martins, H. Lins de Barros, U. Lins, and M. Farina. 2007. Structure, behavior, ecology and diversity of multicellular magnetotactic prokaryotes, p. 103–132. *In* D. Schüler (ed.), *Magnetoreception and magnetosomes in bacteria*, Microbiology Monographs (3). Springer-Verlag, Berlin Heidelberg.
- Lins, U., C. N. Keim, F. F. Evans, M. Farina, and P. R. Buseck. 2007. Magnetite (Fe<sub>3</sub>O<sub>4</sub>) and greigite (Fe<sub>3</sub>S<sub>4</sub>) crystals in magnetotactic multicellular organisms. *Geomicrobiol. J.* 24:43–50.
- Simmons, S. L., and K. J. Edwards. 2007. Unexpected diversity in populations of the many-celled magnetotactic prokaryote. *Environ. Microbiol.* 9:206–215.
- Winklhofer, M., L. G. Abraçado, A. F. Davila, C. N. Keim, and H. G. P. Lins de Barros. 2007. Magnetic optimization in a multicellular magnetotactic organism. *Biophys. J.* 92:661–670.