Magnetite biomineralization and ancient life on Mars
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Certain chemical and mineral features of the Martian meteorite ALH84001 were reported in 1996 to be probable evidence of ancient life on Mars. In spite of new observations and interpretations, the question of ancient life on Mars remains unresolved. Putative biogenic, nanometer magnetite has now become a leading focus in the debate.

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Abbreviations
BCM biologically controlled mineralization
BIM biologically induced mineralization
BSO bacterium-shaped object
PAH polycyclic aromatic hydrocarbon

Introduction
A 2 kg carbonaceous stony meteorite, designated ALH84001, was discovered in a glacial flow in the Allan Hills region of Antarctica in 1984 [1]. It was identified as a Martian meteorite by oxygen isotopic analysis in 1994. The bulk rock matrix, which constitutes ~98% of the mass of ALH84001, crystallized 4.5 billion years ago (4.5 Ga), comparable to the age of lunar rocks. It is the oldest of the 14 known Martian meteorites and the only one from the ancient crust in the southern highlands of Mars. This is the region where evidence for former liquid water was obtained by the Mars Pathfinder mission [2]. Following multiple shock events that produced fractures in the Martian surface [1], ALH84001 was ejected from the surface of Mars by an impact event about 16 million years ago and landed in Antarctica about 13,000 years ago.

In addition to orthopyroxene (a silicate chain mineral common in igneous rocks and present in some stony meteorites), the meteorite contains glassy plagioclase, chromite (Cr, Mg and Fe spinel), and iron pyrite, which together constitute about 1% of the meteorite mass. It also contains lenticular globules of chemically zoned Ca, Mg, and Fe carbonate minerals (~1% of meteorite mass) up to 250 µm in diameter in the rock matrix fractures. These globules formed about 3.9 Ga [3] and were subjected to several shock events after formation but prior to the ejection event [1].

In 1996, McKay et al. [4] reported four features associated with the carbonate globules that together comprised possible evidence for ancient life on Mars: firstly, non-equilibrium distributions of Fe, Mn, Mg, and Ca within the carbonate globules; secondly, polycyclic aromatic hydrocarbons (PAHs) with a mass distribution unlike terrestrial PAHs or those from other meteorites; thirdly, bacterium-shaped objects (BSOs) up to several hundred nanometers long that resemble fossilized terrestrial microorganisms; and lastly, 10–100 nm magnetite (Fe3O4), pyrrhotite (Fe1−xS), and greigite (Fe3S4) crystals. These minerals were cited as evidence because of their similarity to biogenic magnetic minerals in terrestrial magnetotactic bacteria.

The ancient life on Mars hypothesis has been extensively challenged, and alternative non-biological processes have been proposed for each of the four features cited by McKay et al. [4]. In this paper we review the current situation regarding their proposed evidence, focusing on the putative biogenic magnetite crystals.

Evidence for and against ancient Martian life
PAHs and BSOs
Reports of contamination by terrestrial organic materials [5,6] and the similarity of ALH84001 PAHs to non-biological PAHs in carbonaceous chondrites [7,8] make it difficult to positively identify PAHs of non-terrestrial, biogenic origin. On the other hand, ice from the Allan Hills glacier contains no PAHs, other Allan Hills meteorites contain no PAH contaminants, and the PAH distribution in ALH84001 is inconsistent with contamination [9,10]. However, non-biological synthesis of PAHs on Mars is also possible [10]. Similarly, possible sample-preparation artifacts [11,12], terrestrial weathering, and similarity to mineral features produced in crystal-growth experiments and in lunar meteorites [13,14] make the BSOs unconvincing biomarkers. We believe that although sufficient evidence has not been found to exclude a Martian biogenic origin for all the PAHs and BSOs, these features do not provide compelling evidence for the ancient life hypothesis.

Carbonate globules
The age of the carbonates shows that they originated on Mars. In the original hypothesis, McKay et al. [4] proposed that the carbonate globules precipitated from aqueous solutions that infiltrated fractures in the ALH84001 matrix, with subsequent deposition of the carbonate minerals modulated by microorganisms. There has been considerable debate about the origin of these carbonates, however, and evidence for both high-temperature and low-temperature formation has been presented [15,16,18–19,22]. As chemical zoning could also result from nonbiological deposition from aqueous solution, zoning may not be a reliable indicator of biological activity. Complications can also arise from the possible effects of subsequent impacts, including shock melting of the carbonates [17,23,24]. On the other hand, it has been argued that the chemical zoning and oxygen isotope non-equilibrium are evidence against prolonged heating of the carbonates [21].
Nanometer magnetite crystals isolated from ALH84001 carbonates with whisker (1), quasi-rectangular (2), and irregular projected shapes (KL Thomas-Keptra, personal communication).

Nanometer iron sulfides in carbonate globules
Pyrrhotite and greigite crystals occur within two distinct regions of the carbonate rims [4]. Isotopic analysis of sulfur in the pyrrhotite shows no 32S enrichment relative to that in the pyrite in the pyroxene matrix [25]. Because light-isotope enrichment is a hallmark of terrestrial sulfur metabolism, it has been argued [25] that the pyrrhotite is non-biogenic, possibly derived from pyrite. In any case, pyrrhotite has not been confirmed as a biogenic product in terrestrial organisms [26•,27•]. Although greigite is a known product of some marine magnetotactic bacteria [26•,27•], its presence in the ALH84001 carbonates has not been confirmed.

Nanometer magnetite in carbonate globules
The magnetite crystals are also primarily located in the carbonate rims. Transmission electron microscope studies of the crystals in situ and removed from the carbonate matrix have revealed a number of projected shapes [4,28,29,30•,31,32,33••,34••] described as ribbon, whisker, quasi-rectangular, and irregular [33••,34••] (Figure 1). The irregular crystals have aluminum and titanium impurities [33••], some of the ribbons and whiskers have screw dislocations [29,31••], and some of the whiskers are epitaxially associated with carbonate [34••]. These features suggest nonbiological, possibly high-temperature, origins. However, the quasi-rectangular crystals, which constitute about 25% of the total number of magnetite crystals, are reportedly chemically pure, elongated along a [111] axis (see later for an explanation of this nomenclature), and have projected hexagonal shapes when viewed along the elongation axis [32,33••]. It has been suggested that these crystals are virtually identical to magnetite in terrestrial magnetotactic bacteria and, moreover, crystals with these features are not known to be formed in any nonbiological process [33••].

Other magnetite sources
Magnetite from other meteorites
Although no magnetite occurs in lunar rocks, it does occur in many ordinary and carbonaceous chondrites including some Martian meteorites in addition to ALH84001. In some cases magnetite occurs as individual spherules from several micrometers up to 30 µm in diameter, clusters of spheroids, barrel-shaped stacks of discs and other morphologies [35]. Whereas aggregates of magnetite, iron sulfides, and Fe–Ni metal apparently formed at high-temperatures in the Allende meteorite (a stony meteorite that fell in Allende, Mexico), hydrothermal alteration on the parent body is thought to be the source of magnetite in other meteorites [36,37]. Although magnetite occurs in other meteorites, there is no indication that any is biogenic. Moreover, in no case have magnetite crystals like those in ALH84001 been found; however, no other meteorites have been studied as intensively as ALH84001.

Nanometer magnetite from terrestrial sources
Nanometer magnetite occurs in lava flows but usually contains substantial amounts of titanium. It has also been recovered from soils and sediments [38,39], including deep sea sediments. Some of these crystals were identified as biogenic on the basis of their shape and size similarity to known biogenic magnetite in magnetotactic bacteria. In some cases, a biogenic origin was supported by the organization of the magnetite crystals in chains. However, authigenic (nonbiological) nanometer magnetite can also be produced in sediments [38].

Terrestrial biogenic magnetite
Two modes of magnetic formation, biologically induced mineralization (BIM) and biologically controlled mineralization (BCM), are associated with dissimilatory iron-reducing bacteria and magnetotactic bacteria, respectively [38]. Dissimilatory iron-reducing bacteria export ferrous ions into their surroundings and thereby induce the formation of a number of extracellular iron minerals, including magnetite. Although biogenic, these BIM minerals are morphologically indistinguishable from those formed inorganically and are therefore not reliable biomarkers. However, fractionation of iron isotopes in soluble ferrous iron produced in culture by a dissimilatory iron-reducing bacterium has recently been reported [40•]. Whether this fractionation is reflected in magnetite formed by iron-reducing bacteria remains to be
Figure 2

Electron micrographs of magnetosomes in cultured magnetotactic bacteria. (a) Equidimensional (cuboctahedral) crystals in Magnetospirillum magnetotacticum. Small arrows indicate twinned crystals, large arrows indicate clusters of small crystals. (b) Elongated crystals in strain MV-1. (c) Elongated crystals in strain MV-4. Arrows indicate twinned crystals. (d) Elongated crystals with thickness fringes in strain MC-1. Adapted from [43••] with permission.

Figure 3

Idealized magnetite crystal habits based on combinations of {100}, {110} and {111} forms. (a) Equidimensional habit (cuboctahedron). (b–d) Habits with [111] elongation. Adapted from [43••] with permission.
range [38,43]. They are typically organized into one or more chains and contain a permanent magnetic dipole in each cell [45] that functions in a magneto-aerotactic sensory system [46]. Statistical analyses of crystal size distributions in cultured strains are narrow, asymmetric, and have consistent width to length ratios within each strain [43]. Whereas the cultured strains are narrow, asymmetric, and have consistent

Magnetite crystals as biomarkers
The idealized characteristics of BCM magnetite crystals within magnetotactic bacteria may be summarized as follows: enveloping membranes; organization of crystals into chains; consistent habits, commonly with a [111] elongation; consistent width to length ratios; chemical purity; structural perfection (no defects or dislocations); a fraction (~10%) of twinned crystals characterized by the spindel twin law; and an asymmetric size distribution within the single-magnetic-domain size range (~120 nm), skewed to larger sizes.

The most compelling evidence for BCM nanometer magnetite in terrestrial or extraterrestrial materials is the presence of enveloping membranes and organization into chains. The other features by themselves are less compelling because they could conceivably be properties of inorganic magnetite. Impurities, defects or dislocations, inconsistent habits, and lognormal size distribution would exclude BCM magnetite.

If the elongated magnetite crystals in the carbonate rims in ALH84001 have the last six of the characteristics summarized above, they would constitute plausible but not compelling support for an ancient-life hypothesis. Their significance above, they would constitute plausible but not compelling biomarkers in ALH84001. The absence of such support for the ancient-life hypothesis without additional compelling evidence for the extraterrestrial origin of the organic materials in ALH84001 have the last six of the characteristics summarized above, they would constitute plausible but not compelling support for a Martian origin of at least some of the PAHs. Moreover, the PAH concentration in ice from the Allan Hills glacier is too low to account for the concentration within the meteorite. Finally, carbonates do not have extraterrestrial organic compounds found in Martian meteorites, which is consistent with a terrestrial origin, whereas the higher-temperature, carbonatic-rich fraction has a carbon isotopic composition consistent with an extraterrestrial origin.

Conclusions
We believe that it will not be possible to reach a consensus for the ancient-life hypothesis without additional compelling biomarkers in ALH84001. The absence of such additional biomarkers may tend to produce a negative consensus. Resolution might have to await future samples returned from the ancient crust of Mars. In any case, McKay et al. [4] have set a new nanometer standard for investigation of extraterrestrial materials that includes minerals as potential biomarkers.

Update
Two notable abstracts from the 31st Lunar and Planetary Science conference are now available online [48,49]. The first [48] gives evidence that the carbonate assemblages in ALH84001 were formed inorganically; the second [49] suggests that carbonate globules in the meteorite were formed at low temperature.

Useful internet resources
Web sites with general information about Martian meteorites:
http://sn-charon.jsc.nasa.gov/alh84001/sample
http://mars.jpl.nasa.gov/sci
http://cass.jsc.nasa.gov/lp/metereonites/mars_meteorite.html

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest
10. PAHs are depleted in the fusion crust compared to the interior within the meteorite, contrary to what would be expected for contamination in Antarctica. Moreover, the PAH concentration in ice from the Allan Hills glacier is too low to account for the concentration within the meteorite. Finally, carbonates do not preferentially absorb PAHs. The authors argue that these features indicate a Martian origin of at least some of the PAHs.

10. Zolits M, Shook E: Abiotic synthesis of polyisocyanic aromatic hydrocarbons on Mars. J Geophys Res 1999, 104:14035-14049. Thermochemical calculations of condensed PAHs in cooling thermal gases and hydrothermal fluids on ancient Mars suggest that PAHs and aliphatic hydrocarbons could have formed without biogenic intervention. Thus, Martian PAHs in ALH84001 are not necessarily biogenic.


27. Studies of iron sulfide magnetosomes in marine magneticotactic bacteria show growth to be the most abundant mineral. Non-magnetic microspheres (biogenic FeS) and putative cubes: FeS were also found and shown to be precursors to goethite mineralization. Neither pyrrhotite nor nodule were found.


In contrast to dissimilatory iron reduction, the uptake of iron and BCM of magnetite in magnetosomes does not result in fractionation of iron isotopes.


The physiology and biochemistry of magnetite mineralization in magnetotactic bacteria are discussed.


Crystal habits, size distributions and structural features of magnetosome magnetite from five cultured strains of magnetotactic bacteria and synthetic magnetite suggest that size and shape distributions are useful in distinguishing BCM magnetite from non-biogenic magnetite.


Elecron holography was used to visualize the magnetic field lines associated with a chain of magnetosomes within a magnetotactic bacterium.


Carbonate globules with 10–1000 nm magnetite and pyrrhotite grains were synthesized in a multi-step process that included precipitation of chemically zoned carbonates followed by heating to simulate a shock event. This work suggests that the carbonate assemblages in ALH84001 may have formed inorganically.


The effects of thermal demagnetization on the natural remanent magnetization of slices of ALH84001 were studied by scanning SQUID microscopy. The results suggest that the carbonate globules in ALH84001 formed at low temperature and that the meteorite was transferred from Mars to Earth, including ejection from the Martian surface and passage through the Earth atmosphere, without interior heating above 40°C.