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Life on Mars: evaluation of the evidence within Martian meteorites ALH84001, Nakhla, and Shergotty

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Abstract

Analyses both support and are in opposition to the hypothesis that the Martian meteorite ALH84001 contains evidence for possible biogenic activity on Mars. New observations in two additional Martian meteorites, Nakhla (1.3 Ga old) and Shergotty (300–165 Ma old) indicate possible biogenic features. Features in the three Martian meteorites compare favorably with the accepted criteria for terrestrial microfossils and evidence for early life on the Earth. There is strong evidence for the presence of indigenous reduced carbon, biogenic magnetite, and the low-temperature formation of carbonate globules. The morphological similarities between terrestrial microfossils, biofilms, and the features found in the three Martian meteorites are intriguing but have not been conclusively proven. Every investigation must recognize the possibility of terrestrial contamination of the meteorites, whether or not the meteorites are Martian. The search for evidence of ancient life in Martian meteorites has emphasized the difficulties confronting the scientific community with the respect to the positive identification of evidence of past biogenic activity.

1. Introduction

Since the report describing evidence for possible microbial life in Martian meteorite ALH84001 (McKay et al., 1996), an enormous number of studies have been undertaken to refute or corroborate this hypothesis. No single terrestrial rock has been subjected to such an extensive barrage of tests. Furthermore, the questions posed by our hypothesis have highlighted the lack of knowledge and understanding of terrestrial life in unusual environments. The stimulus provided by our paper has been beneficial in forcing the scientific community to address very specific problems in the search for extraterrestrial life with a view to the arrival on Earth of Martian rocks in 2008.

The Viking Mission to Mars in 1976 was the first attempt to search for life in situ on another planet. Each of the two landers carried a camera

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to search for visible signs of life, a gas chromatograph/mass spectrometer to detect reduced carbon compounds, and three biological experiments (a labeled release experiment, a gas exchange experiment, and a metabolic release experiment) to seek evidence of biological activity within the soil samples. Although the concensus among the scientific community is that the results were negative, Levin and Straat (1977) and Levin and Levin (1998) argue that the labeled release experiment did, in fact, detect evidence of life.

Since it was first demonstrated by Bogard and Johnson (1983) that meteorite EETA79001 of the Shergottite–Nakhlite–Chassignite (SNC) class contained trapped Martian atmospheric gases, 16 other meteorites have joined the Martian meteorite group. Seven of them contain trapped Martian atmosphere (Bogard and Garrison, 1998). In addition to the trapped gases, the unique composition of the oxygen isotopes within the silicate minerals of all the SNC meteorites shows they were from a unique oxygen reservoir within our solar system (Clayton and Mayeda, 1983; Romanek et al., 1998; Franchi et al., 1997, 1999).

The lines of evidence which indicate possible biogenic activity in the Martian meteorite ALH84001 (McKay et al., 1996) are: (1) the presence of carbonate globules which had been formed at temperatures favorable for life, (2) the presence of biominerals (magnetites and sulfides) with characteristics nearly identical to those formed by certain bacteria, (3) the presence of indigenous reduced carbon within Martian materials, and (4) the presence in the carbonate globules of features similar in morphology to biological structures. Each of these phenomena could be interpreted as having abiogenic origins but the unique spatial relationships indicated that, collectively, they recorded evidence of past biogenic activity within the meteorite. Both criticism and support have been directed toward this hypothesis (Anders, 1996; Bradley et al., 1996, 1997, 1998; Valley et al., 1997; Kirschvink et al., 1997; Bada et al., 1998; Oró, 1998, 1999; Friedmann et al., 1998; Hoover, 1998; Scott, 1999; Treiman, 1999).

In this paper, we re-evaluate the evidence of our original paper (McKay et al., 1996) in the light of the results of the investigations made subsequent to its publication. We conclude that there is strong evidence for a low-temperature, Martian origin of the carbonate in the meteorite, for the presence of Martian organic carbon, and for a biological origin of a portion of the single domain magnetites in the carbonate globule rims. New morphological structures associated with the carbonates are similar to terrestrial biogenic structures, but the presence of known biological contaminants associated with the fusion crust urges caution in interpretation until the extent of terrestrial contamination has been fully established. We also document the presence of structures in two other Martian meteorites, Nakhla and Shergotty, which are morphologically similar to terrestrial fossil bacteria.

2. Temperature of formation of the carbonate globules

Considering that the evidence for possible Martian biological activity is contained within carbonate globules (Fig. 1) deposited in fractures within ALH84001, the temperature of formation of these globules is critical. Although the upper temperature limit for terrestrial life is not yet known, so far the highest identified temperature for living organisms is 118°C (Stetter, 1996). If the carbonate globules were formed at temperatures very much above this limit, it is not expected that they could have supported evidence for life at least with respect to the currently known temperature constraints on Earth. We (Romanek et al., 1994; McKay et al., 1996) and others (Valley et al., 1997; Warren, 1998; Treiman and Romanek, 1998; McSween and Harvey, 1998) have proposed that these globules formed at low-temperatures by precipitation from an aqueous fluid or that they formed from fluids evaporated from a carbon dioxide-saturated fluid.

Romanek et al. (1994) initially showed that the oxygen and carbon isotopic compositions of the carbonate globules indicated temperatures of formation below 100°C while Harvey and McSween (1996) and Bradley et al. (1996, 1997, 1998) argued that the carbonates formed at temperatures above 600°C. A number of subsequent papers propounded either high (McSween and Harvey, 1998; Scott, 1999) or low (Valley et al., 1997; Warren, 1998; Treiman and Romanek, 1998) temperatures of formations. Warren (1998) reviewed the high-temperature models and concluded that none of them could be supported by the available analytical data. He went on to propose a low-temperature origin such as carbonate precipitation or evaporation in cracked bedrock or from drying Martian lakes. Subsequently, McSween and Harvey (1998) have proposed a similar model with precipitation of the carbonates from Martian salt-rich water (brines) at low-temperatures in agreement with our originally hypothesized formation mechanisms (McKay et al., 1996).

Borg et al. (1998, 1999) reported a formation age for the carbonate globules of 3.94 Ga, determined by Rb–Sr and Pb–Pb measurements. It is believed that, at that time (4.1–3.9 Ga), there was extensive surface water on Mars (Carr, 1996), and possibly even an ocean (Head et al., 1998, 1999). If life ever arose on Mars, it probably would have been during that relatively warm, wet period of time (McKay and Davis, 1991; Westall, 1999). Mars had also been bombarded extensively by then, and the crust would have been extensively fractured, facilitating the movement of ground waters through cracks and fractures surrounding the impact craters. Scott et al. (1998) suggest that carbonates in ALH84001 formed by shock vaporization of pre-existing material followed by re-deposition, so that the carbonates could not preserve evidence of early biogenic activity on Mars. Recent experimental shock studies on carbonate prove its stability to ~ 60 GPa, well in excess of the approx. 40 GPa peak pressure indicated by other shock features in ALH84001 (Bell, 1997; Bell et al., 1998, 1999; Van der Bogert et al., 1999). This work suggests that impact shock was not responsible for producing features such as the carbonate globules, although they may have been disturbed by impact induced shock.

Barrat et al. (1998) showed that small carbonate globules grew within cracks of the Tatahouine meteorite after it fell in Tunisia in 1931. These

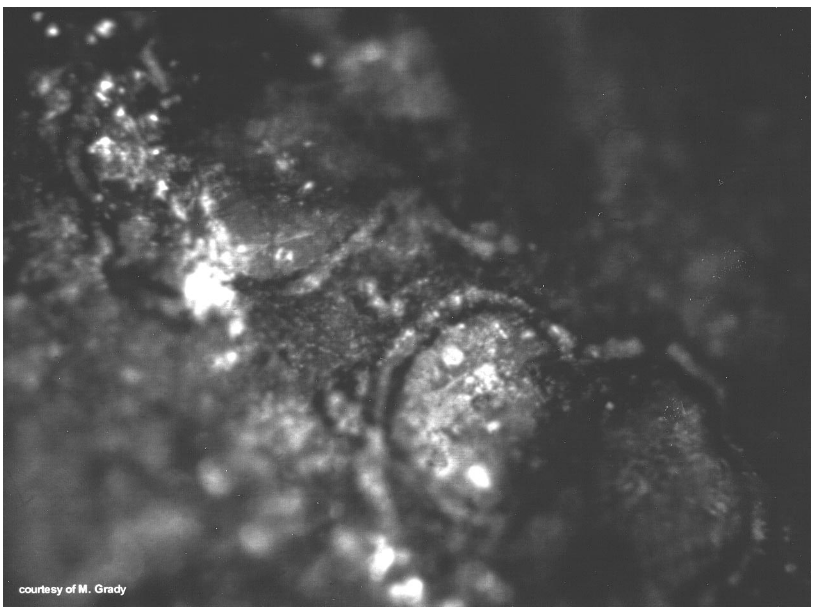

Fig. 1. Optical micrograph of carbonate globules in ALH84001. Carbonate globules are typically 100–300 μm in diameter with a black–white–black banding surrounding the outer edges of the globules. The compositions of the carbonates are typically magnesium- and iron-rich with less than 10 wt. % calcium (Harvey and McSween, 1996; Valley et al., 1997). The black–white–black rims of the carbonates globules contain increased abundances of magnetite (black bands and the white regions are composed of carbonate with only trace quantities of magnetite.

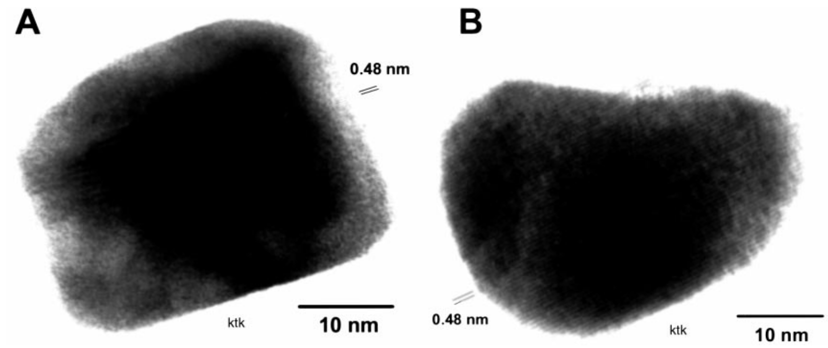

Fig. 2. Transmission electron microscope (TEM) images of magnetite grains found within the rims of ALH84001 carbonate globules. Grain sizes are typically 40–60 nm.

globules are similar to the ones in the ALH84001 meteorite, and they contain small calcite rods and spheres which may be mineral precipitates or may be fossilized bacteria from Earth. Whereas no one disputes that the carbonates in ALH84001 formed on Mars, the Tatahouine meteorite demonstrates that carbonate globules can form in a very arid environment near the ground surface and at relative low-temperatures (within the range of liquid water). This result suggests one possible mechanism for low-temperature formation of carbonates on Mars.

In summary, it is very likely that the carbonate globules have a low-temperature aqueous origin, and that they are relatively undisturbed by subsequent processes. The Tatahouine meteorite provides us with an example of similar carbonates formed in arid environments on Earth.

3. Origin of magnetites in the carbonate globules

We originally proposed that some of the single domain magnetite (Fe_3O_4) grains in the rims of carbonate globules were very similar to magnetites produced by magnetotactic bacteria (McKay et al., 1996; Thomas-Keprta et al., 1997, 1998a). Bradley et al. (1996, 1997, 1998) suggested that the magnetites were formed at high-temperatures either by condensation from a vapor or by shock heating of iron-rich carbonate. However, further studies by Thomas-Keprta et al. (1998a,b), Thomas-Keprta et al., 1999; Thomas-Keprta et al. (2000) have shown that there are actually three populations of magnetite: (1) $\sim 25\%$ of the magnetites are in the single-domain size range, with elongated prism morphologies and pure Fe_3O_4 compositions which are only known to be produced by terrestrial magnetotactic bacteria (Fig. 2); (2) a small percentage ($< 10\%$) of the magnetites are needle-shaped whiskers, which could indeed have a high-temperature origin, possibly related to impact shock and possibly incorporated into the globules as detrital material during precipitation of the carbonate globules; and (3) the majority of the magnetites have irregular and cubic morphologies; in this third group, they are similar to magnetites produced by dissimilatory iron-reducing bacteria and also to abiotically produced magnetites. The presence of all ALH84001 magnetite crystals can be explained by low-temperature biogenic and abiotic mechanisms, as no evidence exists for the presence of high-temperature vapor or shock phases in ALH84001 carbonate globules. In addition to the evidence cited above that the carbonates were not formed at high temperature, Raman spectroscopy of carbonate globules in ALH84001 indicates the absence of CaO and MgO (Bell et al., 1999). Such oxide phases should be found associated with the magnetites if these crystals are high temperature shock products. It is not known if the magnetite crystals in the carbonate globules were formed in situ or were brought in and deposited during globule formation. However, there is absolutely no evidence for the in situ high-temperature formation of any ALH84001 magnetite crystals.

Kirschvink and Vali (1999) have reviewed the characteristics which are expected if magnetite grains have been formed from biogenic processes. They suggest that there are six Darwinian characteristics: (a) crystal size and shape, (b) crystal structural perfection, (c) the presence of magnetite chains, (d) elongation of magnetite crystal structure, (e) anisotropic growth of certain crystal faces, and (f) chemical purity. Thus, Martian magnetites identified by Thomas-Keprta et al., 1999; Thomas-Keprta et al. (2000), and suggested as being biogenic, meet five of the six criteria for magnetites produced by magnetotactic bacteria. To date, we have not observed ALH84001 magnetites in chains but Friedmann et al. (1998) have reported magnetite chains. If Friedmann et al.'s (1998) observations on magnetite chains in ALH84001 are taken into account all six of Kirschvink and Vali (1999)'s criteria are met for biogenic activity.

4. Sulfur isotopes

It has been stated that, on the basis of ion microprobe analysis, the isotopic compositions of sulfides in ALH84001 do not reflect any biologic role in their formation (Shearer and Papike, 1996; Shearer et al., 1996; Greenwood et al., 1997). However, the 40–60 nm-sized particles of greigite (Fe_3S_4) are below the analytical capabilities of the ion microprobe beam (typically 10–20 μm in size). Thus, as noted by Gibson et al. (1997, 1998), the sulfides analyzed by Shearer et al. (1996) and Greenwood et al. (1997) were the relatively large micron-sized pyrites in the meteorite's silicate groundmass, and indeed, they do not show obvious signs of biological processing a change in their isotopic ratios. McKay et al. (1996) did not propose that these larger sulfides were biogenic, and it is unclear whether the large pyrites are associated in any way with the carbonates. Moreover, the majority of bacteria on Earth which assist with sulfide formation may not cause isotopic changes in the sulfur components unless photosynthetic processes are involved (Kaplan, 1983; Schidlowski et al., 1983). Therefore, the ALH84001 sulfur isotope data currently available neither support nor refute the possibility of biogenic activity.

5. The significance of the polycyclic aromatic hydrocarbons (PAHs)

Several critics (i.e. Becker et al., 1997; Oró, 1998) argue that the PAHs we reported in ALH84001 are not diagnostic of life because PAHs form at high-temperatues and also because the PAHs are almost identical to those in the carbonaceous chondrite Murchison (Becker et al., 1999), which is thought to have come from the asteroid belt, not Mars. In addition, Bada et al. (1998) have suggested that the PAHs are probably contamination products from Antarctic ice. In the original paper, McKay et al. (1996) did not claim that the PAHs were directly of biogenic origin; they suggested that the PAHs might be products of the decay and fossilization of bacteria. The PAHs in ALH84001 are not identical to those in Murchison or any other carbonaceous chondrite (Clemett et al., 1998), differing in the presence or absence of some of the attached side chains and in the relative type and abundance of each major compound. Furthermore, Clemett et al. (1998) were able to demonstrate that micrometeorites collected from the Antarctic ice are characterized by their own unique PAH fingerprints, which are specific to each particle. The carbon abundances within micrometeorites have been shown to be variable (Clemett et al., 1993, 1998). Particles range in carbon concentrations from 0.1 to 50 wt% C. The micrometeorites are highly porous and have unusually large surface areas onto which organics within the ice or melt water could have been readily adsorbed. If all the PAHs in these micrometeorites were contaminants from the ice, then all particles would have similar concentrations and types of PAHs.

Clemett et al. (1998) have presented strong evidence that Martian polycyclic aromatic hydrocarbons (PAHs) are present in ALH84001. They showed that the Antarctic environment does not contribute to the introduction of PAHs into ALH84001. As control samples, they measured concentrations of PAHs in numerous ordinary chondrite meteorites that resided longer in the Antarctic ice than ALH84001. The concentrations of PAHs did not increase as a function of exposure or residence time in the Antarctic environment. Clemett et al. (1998) showed that ice from Antarctica did not contain measureable PAHs. As additional evidence for the Martian origin of the PAHs, Clemett et al. (1998) also reanalyzed the PAH profile of ALH84001 from the exterior fusion crust to the interior and found, again, that the abundance always increases from the exterior of the meteorite to the interior (Fig. 3).

In another study of PAHs, Stephan et al. (1998) suggest that the PAHs have a homogenous distribution and are not specifically concentrated in the carbonate globules. They find them in the centers of the igneous mineral grains and apparently depleted in the carbonate globule. These analyses were made on polished thin sections of the meteorite, whereas the analyses of Clemett et al. (1998) were made on freshly broken surfaces. Most polishing procedures use organic solvents or organic diamond paste and require considerable handling in less than clean conditions. Could the thin section making process have introduced PAH contamination? Or could it have smeared out existing PAHs over the polished surface so that they no longer reflect their original location and abundance? It is our opinion (Gibson et al., 1997, 1998) that the results reported by Stephan et al. (1998) represent contamination and do not provide meaningful information about the true state of reduced carbon components within ALH84001.

In summary, the new data of Clemett et al. (1998) show that indigenous reduced carbon components are present within the ALH84001 meteorites. Flynn et al. (1999) have presented data using XANES (X-ray absorption near edge structure) techniques to measure the presence of reduced carbon compounds at a spatial resolution of one micron within individual carbonate globules. They found indigenous reduced organic carbon components in both the ALH84001 and Nakhla meteorites. Thus, for the first time, the presence of reduced carbon compounds have been identified in Martian materials. This accomplishes one of the goals of the Viking experiments some 20-plus years after the mission. The data on their own, however, do not specifically confirm that the reduced carbon is of biologic origin, since PAHs may be prebiotic molecules as indicated by their presence in many other carbonaceous meteorites (Gilmour and Pillinger, 1994).

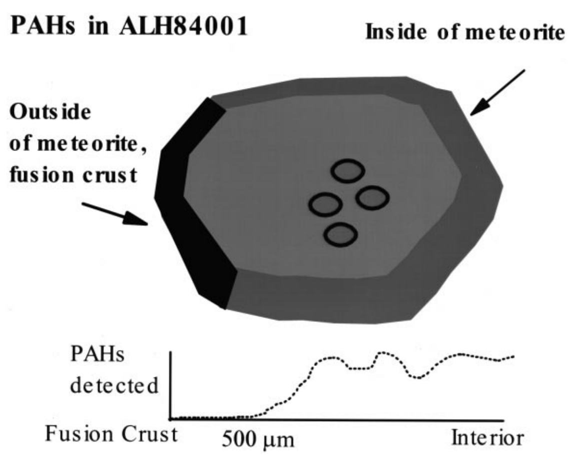

Fig. 3. Schematic trace of PAH concentrations from the rim into the matrix of ALH84001 showing the increase in PAHs from rim to interior as well as enrichment in reduced carbon in the regions of the carbonate globules. Modified from the work of Clemett et al. (1998) and Flynn et al. (1999) who have mapped C–H concentrations within ALH84001.

6. Amino acids

Amino acids were not measured in this meteorite by our research team. Previously, McDonald and Bada (1995) and Bada et al. (1998) demonstrated that the amino acids in certain meteorites are likely due to Antarctic ice contamination. Whereas amino acids are clearly produced by living systems, they are readily soluble in water solutions as opposed to the PAHs which are essentially insoluble in water (Clemett et al., 1998). Therefore, the use of amino acids as a reliable biomarker for Martian meteorites collected in Antarctica is suspect (Bada et al., 1998; Gibson et al., 1998), although they may make reasonable biomarkers for other samples such as materials returned from Mars. In fact, recent studies by Steele et al. (1997, 1999a,b) have documented terrestrial microbial contamination on the fusion crust of the ALH84001 and Nakhla meteorites, which could have contributed to the amino acids measured (but not to the PAHs, which were negligible at the fusion crust).

7. Terrestrial carbon contamination

Carbon-14 measurements such as those of Jull et al. (1998) show that modern-day atmospheric carbon-14 is incorporated into all meteorites which have resided on the Antarctic ice fields. Typically, the carbon-14 is incorporated into secondary weathering products, such as carbonates (not the carbonate globules observed in ALH84001 which are clearly pre-terrestrial). Thus, all components with a carbon-14 signature should be viewed as terrestrial contaminants. Jull et al. (1998) showed that most (at least 80%) of the organic carbon in ALH84001 contains significant modern-day carbon-14 and is therefore terrestrial. This terrestrial carbon may include amino acids and organic material from the air, melt water or even include terrestrial organisms (cf. Steele et al., 1999a,b). However, Jull et al. (1998) also documented one acid resistant component with a carbon isotopic composition of −18‰ (PDB) which did not contain modern-day carbon-14 and which also resisted heating of the meteorite sample to temperatures above 400°C. They interpreted this component to be pre-terrestrial (i.e. Martian) and to originate either from Martian organic carbon (i.e. kerogen or possibly PAHs), or from a very unusual carbonate quite unlike the bulk of the Martian carbonate. This component makes up 15% of the organic carbon in the meteorite and is potentially one of the major discoveries in understanding the nature of carbon within ALH84001.

Grady et al. (1994, 1998) have shown that carbon in the ALH84001 meteorite has a carbon isotopic composition for selected components suggestive of both terrestrial contaminants as well as indigenous Martian carbon phases. The Martian carbon components consist of both magmatic carbon and of carbon from the carbonate globules. Within the globules there may be both carbon associated with the carbonate and an organic carbon phase which has an isotopic carbon composition similar to the carbon-14 free component identified by Jull et al. (1998). Flynn et al. (1997, 1998) have shown that there is an indigenous organic carbon component within carbonate globules which is distributed unevenly throughout the globules.

In summary, although there is evidence of terrestrial carbon and amino acids, the data of Jull et al. (1998), Grady et al. (1994, 1998), and Clemett et al. (1998) have shown that the ALH84001 meteorite contains indigenous Martian organic carbon.

8. Microbial examination of meteorites

Steele et al. (1999a,b),) and Toporski et al. (1999) have used both scanning electron microscopy (SEM) and atomic force microscopy (AFM) techniques to image modern terrestrial microbes on a number of meteorites, including ALH84001, Nakhla, and Murchison. Results show that terrestrial contamination of exposed surfaces by growing microbes is a problem. The microbes attach themselves to the meteorite and use the indigenous carbon in their metabolism. Because of these studies more rigorous methods of sample handling and storage are being investigated. At the present time, the extent of the contamination of the meteorites is being evaluated. Although exterior portions of meteorites may be contaminated with terrestrial microbes, samples from the interior regions of Antarctic and non-Antarctic meteorites are more likely to be free of terrestrial microbial contamination (e.g. Benoit and Taunton, 1997)

9. Microfossil-like structures in ALH84001

McKay et al. (1996) described features 20–100 nm in size that were suggested to be similar to fossil terrestrial nanobacteria (Fig. 4). Criticism of our interpretations centered on the very small size of the features (Schopf, 1999) and the possibility that they could be artifacts (Bradley et al., 1997).

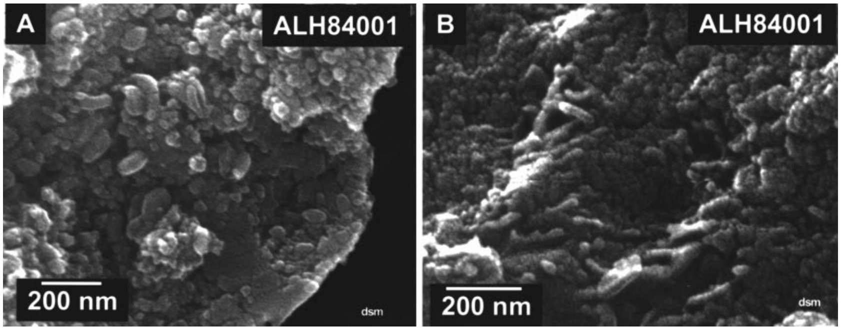

Fig. 4. Bacteriamorph structures in ALH84001 which McKay et al. (1996) suggested might be similar to terrestrial nanobacteria. Criticism of the sizes of these features has been made by Bradley et al. (1997) and Schopf (1999) with regard to the lower size limit of viable organisms.

9.1. The size limit of bacteria

The lower size limit for bacteria is poorly constrained. Most studies of fossil bacteria have been based on light microscope observations of larger types of bacteria (cyanophytes; e.g. Schopf and Walter, 1983). The lower size limit for bacteria is thought to be ~ 100 nm (if in a spherical form, Kirkland et al., 1999, and references therein). However, viable cells have been reported in the size range 80–100 nm from mammalian blood (Kajander and Ciftcioglu, 1998) and sandstones (< 100 nm; Uwins et al., 1998). Biogenic features that are parts of microbes such as lipid micelles (Walde et al., 1994; Morigaki et al., 1997) can form smaller rounded to oval shapes; however it is unclear if these features could become fossilized. A number of studies are in progress to determine the exact nature of oval to spherical < 100 nm sized objects found in other rocks and deposits; are they abiogenic phenomena or actual viable microorganisms or parts of organisms.

Although light microscopy has traditionally been the technique used to search for fossil bacteria, within the last decade electron microscopes have become increasingly utilized (Wuttke, 1983; Monty et al., 1991; Westall, 1994; Martill and Wilby, 1994; Liebig et al., 1996; Westall et al., 1999b). Since most bacteria are < 2 μm in size, the SEM and TEM are by far better suited as instruments of observation. Thomas-Keprta et al. (1998b) used SEM to compare modern bacterial appendages and polymeric secretions similar to some of the features in the ALH84001 carbonates. They determined that, not only were whole bacterial cells mineralized in as little as eight weeks, but parts of the organisms such as flagella were also mineralized. Strong similarities in size and shape were found between some of the biogenic features from the Columbia River Basalt (Stevens and McKinley, 1995) and the possible biogenic structures in ALH84001 (Thomas-Keprta et al., 1998a,b) (Fig. 5). Fossil bacteria > 0.5 μm are known from the last 3.5 Ga of the rock record (Cloud and Hagen, 1965; Wuttke, 1983; Knoll et al., 1988; Monty et al., 1991; Walsh, 1992; Schopf, 1993; Martill and Wilby, 1994; Schopf, 1996; Westall et al., 1999b). These fossils include both carbonaceous permineralized bacteria as well as non-carbonaceous, minerally-replaced bacteria. Folk (1993) and Folk and Lynch (1997) have documented very small oval to spherical structures 20–200 nm in size in a variety of geological environments, and have called them nannobacteria, although their exact nature has not yet been determined (Kirkland et al., 1999). Whereas these features may prove to be inorganic, their presence may be caused by bacteria controlling certain aspects of their environment. Such features may be inorganic, but bacterially mediated, mineral precipitates. Work is underway to determine the differences between fossilized, small bacteria and mineral products (possibly inorganic) produced in the presence of bacteria.

9.2. Artifact production

Bradley et al. (1997) suggested that some of the elongated and spherical structures described by McKay et al. (1996) could be artifacts of heavy gold coatings over mineralogical features. McKay et al. (1997b) were able to demonstrate experimentally that gold coating did not produce these features. However, it is necessary to take into account other kinds of artifact formation. For example, some aqueous corrosion could have created artifacts on mineral surfaces during the 13 000 years the meteorite was on or in the Antarctic ice prior to collection. For example, some elongated structures in ALH84001 resemble emergent plate edges of pyroxene (Bradley et al., 1997) or of clay minerals.

Coccoid fossil bacteria are especially susceptible to confusion with abiogenic spherical structures (Buick, 1991; Westall et al., 1999a,b). Some minerals form spheres similar in morphology to coccoid bacteria, (e.g. silica, pyrite, vesicles). Additional criteria are needed to help distinguish between coccoid fossil bacteria and mineral spheres or organic micelles. We are undertaking an extensive program to document and under-

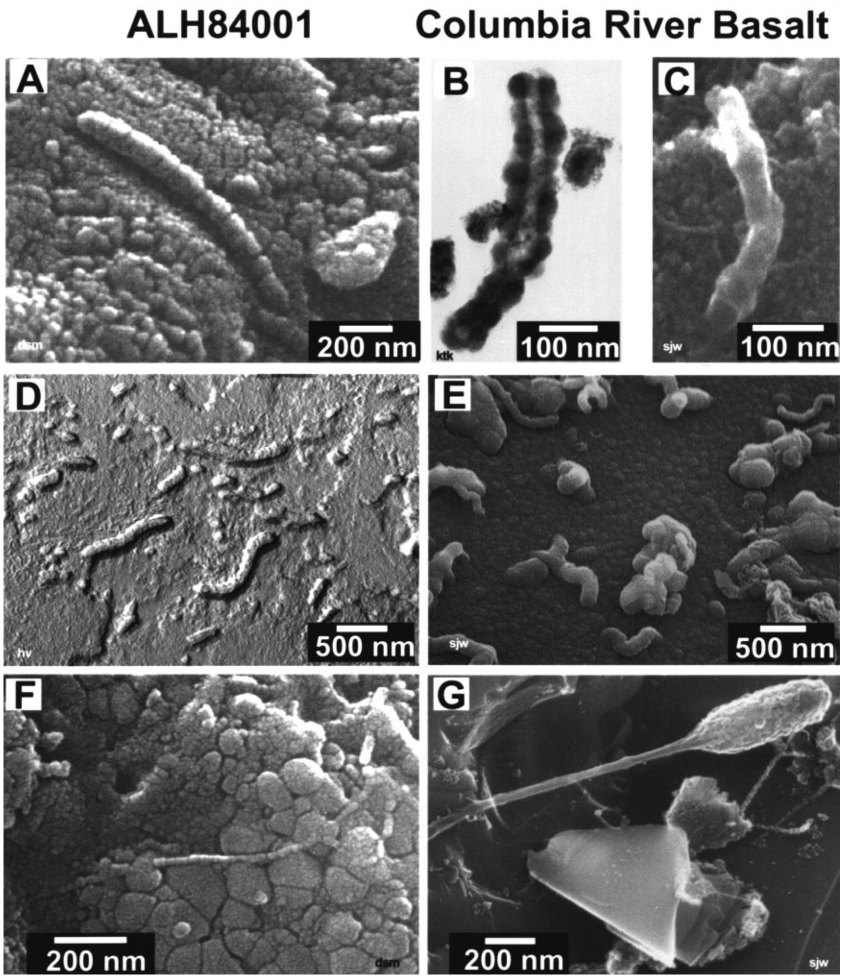

Fig. 5. Biogenic-looking features in ALH84001 (A, D, F) and similar features in the Columbia River Basalts (B, C, E, G; Thomas-Keprta et al., 1998b). The features in ALH84001 are essentially identical to the similar fossilized bacterial features found within the basaltic samples, from which subsurface microorganisms were cultured.

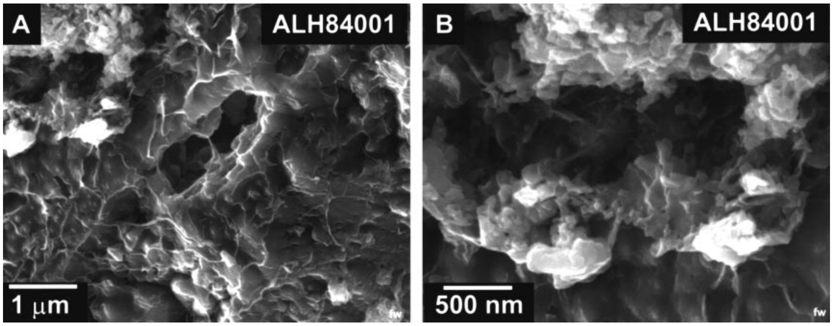

Fig. 6. (A,B) Field emission scanning electron microscopy (FE-SEM) images of possible biofilms within ALH84001. The center of the two images show regions of biofilm-rich areas. Both A and B are similar to biofilms within bacterial molds in ancient terrestrial rocks (Westall et al., 1998; Westall, 1999).

stand a variety of biomarkers, and hope to produce additional criteria for the positive identification of true fossilized bacteria.

10. New possible biogenic structures observed in ALH84001

McKay et al. (1997a) and Westall et al. (1998) described fine films coating the fractures and cracks in ALH84001 and suggested that the films might represent fossilized biofilms (Fig. 6A). Similarly, oval hollows in a carbonate deposit on a fracture surface resemble bacterial molds in terrestrial rocks (Fig. 6B; Westall et al., 1998, 1999b,c; Westall, 1999). Biofilms are shown spanning the fracture in ALH84001 along with coating other mineral substrates. Biofilms are polysaccharide secretions produced by bacterial colonies in order to make their environments more favorable for survival. Whereas these features may be biogenic in origin, the problem of terrestrial contamination and the inorganic production of pits or molds must be addressed.

11. Possible biogenic structures in Nakhla and Shergotty

We have recently begun studying features in the cracks containing pre-terrestrial aqueous alteration products of two additional Martian meteorites, Nakhla and Shergotty. Nakhla was recovered shortly after an observed meteorite fall which occurred in Egypt in 1911. The sample, which was completely covered with fusion crust, was transferred to the British Museum of Natural History shortly after its recovery, limiting the chances for terrestrial contamination. The meteorite is a member of the Nakhlite group of achondrites and is composed mostly of clinopyroxene with minor amounts of feldspar, sulfides and oxides. Nakhla has a crystallization age of 1.3 Ga and contains clay (“iddingsite”)-filled cracks of Martian origin. The clay in other similar Nakhlites (i.e. Lafayette) has been shown to have formed 700 Ma ago (Swindle et al., 1997). Iddingsite-filled cracks near the fusion crust were annealed by the heat of entry into the Earth’s atmosphere (Gooding et al., 1991) proving that the iddingsite is pre-terrestrial. Light microscopy revealed rounded micrometer-sized structures embedded within the iddingsite-filled cracks. In SEM, these features consist of 0.5–2 μm-sized spheres with irregular surfaces (Fig. 7). They occur in distinct cluster-like distributions within the clay. The spheres are sometimes joined together in pairs or triplets. From one triplet, a 20 nm long filament extends from the apex of a terminal sphere (Fig. 8). The rounded and ovoid forms in Nakhla can be subdivided into three groups: those which appear to be on the surface of the iddingsite clay, those which appear to be firmly embedded in the iddingsite, and those which form directly on mineral surfaces. In some cases, the embedded features are covered by a later generation of iddingsite (Fig. 9)

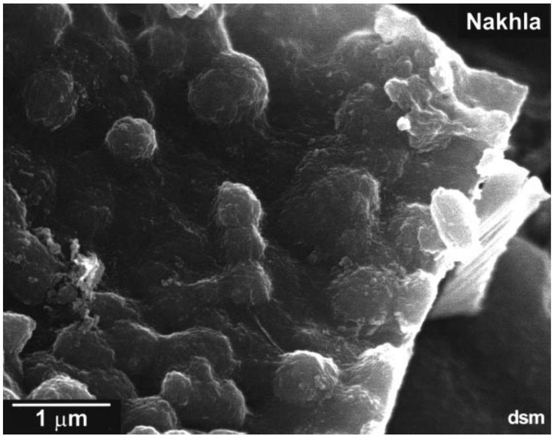

Fig. 7. FE-SEM images of micrometer-sized features embedded in "iddingsite"-filled cracks of the Nakhla meteorite. The "iddingsite" may be approximately 700 Ma old and post-dates the 1.3 Ga formation of the Nakhla meteorite (Swindle et al., 1997). The structures occur in distinct cluster-like regions within the clay.

Features which are formed directly on the primary minerals are usually associated with a covering or halo of clay-mineral plates (Fig. 10). Such features also formed under laboratory conditions in the Columbia River Basalt microcosm when bacteria-containing water was included, but were absent from sterile controls (Thomas-Keprta et

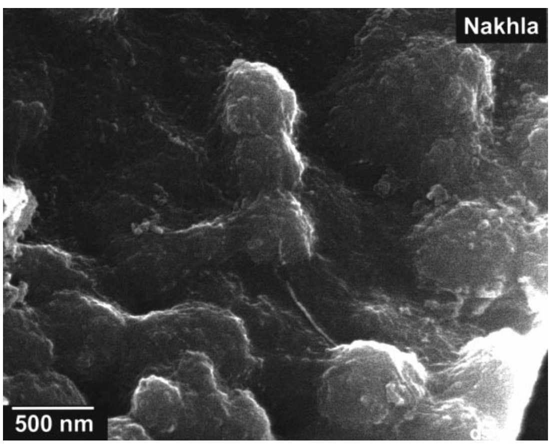

Fig. 8. Close-up (FE-SEM) of a triplet structure within Nakhla with a 20 nm long filament extending from the apex of a terminal sphere in the opposite direction of the triplet. The spheres appear to be coated with biofilm or a later generation of "iddingsite".

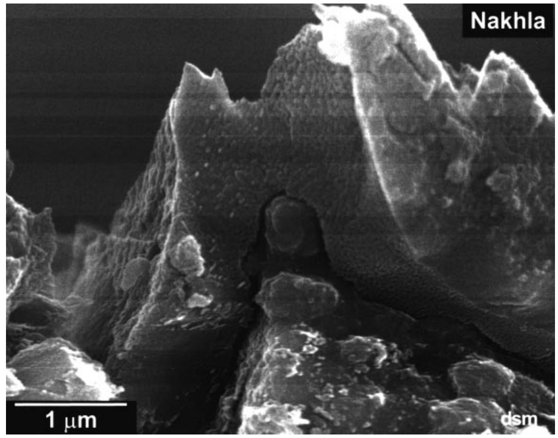

Fig. 9. Spheroidal structures (FE-SEM image) within Nakhla attached to the silicate host; covered by a later generation of Martian "iddingsite", stratigraphically indicating that the sphere is Martian. The "iddingsite" in similar nakhlites have been dated by Swindle et al. (1997) to have formed at 700 Ma, 600 Ma after Nakhla crystallized.

al., 1998b; Fig. 11). EDS analysis of the ovoid features in both Nakhla and the Columbia River basalt shows that they are enriched in Fe compared to the adjacent iddingsite. Replacement of bacterial cells by iron oxide or hydroxide was a common feature in the Columbia River Basalt microcosm samples (Thomas-Keprta et al., 1998b).

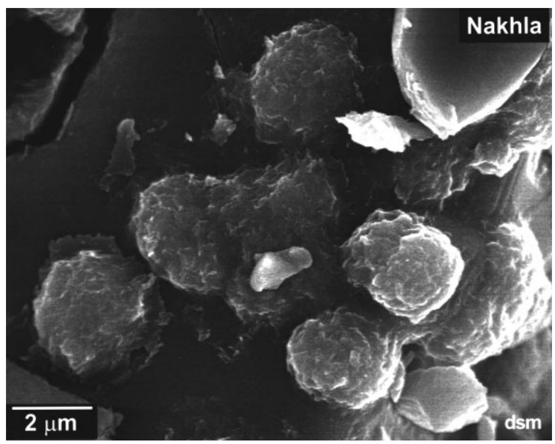

Fig. 10. Spherical structures (FE-SEM image) on Nakhla. They appear to have formed directly on primary silicates and have a covering or halo of "iddingsite" plates.

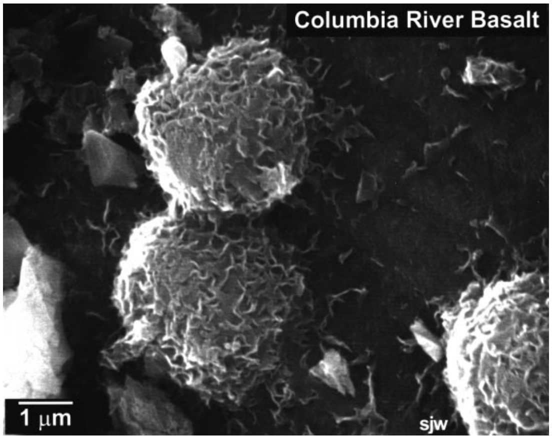

Fig. 11. FE-SEM images of spherical features in the Columbia River Basalt samples. Such features were absent from sterile controls (Thomas-Keprta et al., 1998a,b). Morphology is similar to those in Nakhla (Fig. 10). EDS (energy-dispersive X-ray spectrometry) analysis of the ovoid features in Nakhla show they are enriched in Fe compared to adjacent "iddingsite".

Similar round features with smectite-like clay coating are observed in the meteorite Shergotty (Fig. 12), a meteorite which was an observed fall in India in 1865. The meteorite has a crystallization age of 165–300 Ma. A number of micrometer-sized clay-coated spheres and ovoids occur within selected regions which have undergone preterrestrial aqueous alteration. The numerical age of the alteration has not been determined but it is clearly of Martian origin. Additional chemical and structural analyses of these features in Nakhla and Shergotty are underway, and the confirmation of whether they are Martian or terrestrial must await detailed isotopic data.

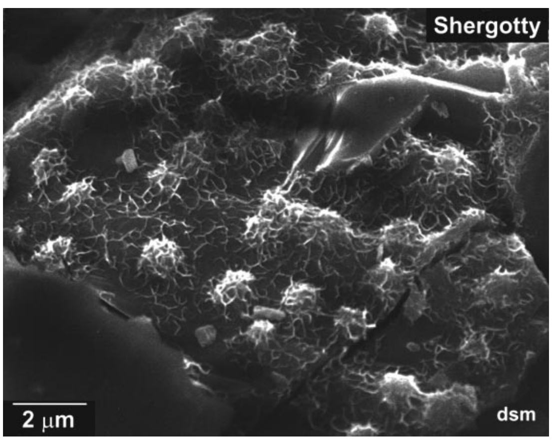

Fig. 12. FE-SEM image of spheroidal features in the Shergotty meteorite with smectite-like clay coatings. These features are similar to those observed in both the Columbia River Basalt samples (Fig. 11) and Nakhla (Fig. 10).

The spheres in Nakhla and Shergotty are similar to the fossils of terrestrial coccoid bacteria (compare Figs. 10–12). However, as previously noted, spherical morphologies alone are not indicative of biogenic activity (Schopf and Walter, 1983; Buick, 1991; Westall et al., 1999a,c). Therefore, the spherical structures in Nakhla and Shergotty are compelling, but not conclusive, evidence for biogenic activity.

12. Criteria for past life: have we met them?

In order to verify that a sample contains evidence of past life or biogenic activity, criteria have been established for terrestrial samples and have been used by the scientific community for many years (Cloud and Morrison, 1979; Schopf and Walter, 1983; Westall, 1999). Lacking independent evidence about the nature of possible past life on Mars, it is appropriate to describe and apply these accepted criteria to the ALH84001 meteorite:

1. Geologic context of the sample must be known; is it compatible with past life?
2. Age of the sample and its stratigraphic location must be known; are they understood well enough to relate possible life to geologic history?
3. Does the sample contain evidence of cellular morphology?
4. Is there evidence for structural remains of colonies or communities within the sample?
5. Is there any evidence of biominerals: do they show chemical or mineral disequilibria?
6. Is there any evidence of stable isotope patterns unique to biological systems?
7. Are any organic biomarkers present?
8. Are any features interpreted to be indicative of biogenicity indigenous to the sample?

12.1. Geologic context

A Martian origin for ALH84001, Nakhla and Shergotty has been shown by their oxygen isotopic compositions and trapped Martian atmospheric gases (Bogard and Garrison, 1998; Franchi et al., 1999). However, the exact Martian provenance for these igneous rocks is unknown. They do contain cracks and porosities which, based on textural microstratigraphy, clearly formed on Mars and could conceivably have harbored water-borne microbial cells and colonies introduced after the rock cooled (on Mars as well as on Earth). Most workers have interpreted the presence of secondary carbonates or globules in cracks of ALH84001 as an indication of relatively low-temperature secondary mineralization by a fluid, probably water (Romanek et al., 1994; Valley et al., 1997; Treiman, 1998; Treiman and Romanek, 1998). The presence of clay minerals detected within the carbonates is additional evidence for low-temperature conditions and for the presence of water (Thomas et al., 1996; Brearley, 1998). Thus, the most widely accepted broad geologic context for ALH84001 is not incompatible with the presence of past life; the carbonate globules likely formed at low-temperatures from aqueous precipitation and their formation is completely compatible with past life, but does not require it.

12.2. Age and history

The isotopic age of ALH84001 is 4.5 Ga, which means that the meteorite is a sample of the early Martian crust. The rock underwent extensive impact shocking around 3.9–4.0 Ga (Treiman, 1998). Evaporation of the fluids percolating through the impact-fractured surface could have resulted in the precipitation of carbonates in cracks and veins (McKay et al., 1996). This carbonate formation occurred at 3.94 Ga (Borg et al., 1998, 1999). At about this time, the planet apparently had abundant water, much greater concentrations of atmospheric gases, and higher surface temperatures (Head et al., 1998, 1999). This corresponds to the time when life first appeared and developed on Earth (Schopf, 1993; McKay and Stoker, 1989). Additional impact and heating events may have occurred later, affecting the detailed structure of ALH84001. The sample resided mostly near, but not on, the Martian surface for several billion years (Treiman, 1998). It was ejected from the surface of Mars 16 million years ago and spent 11 000 years in or on the Antarctic ice sheet (Treiman, 1998).

The Nakhla and Shergotty Martian rocks are much younger than ALH84001. Nakhla has been dated at 1.3 Ga and Shergotty at 300–165 Ma (Bogard and Garrison, 1998), indicating that there was at least intermittent volcanic activity on the surface of the planet. The significance of the secondary mineral-filling in the fractures in these meteorites is that it indicates aqueous alteration occurred after the period when water had disappeared from the surface of Mars. This is believed to have occurred at about 3.5 Ga, but there is much evidence for the presence of liquid water at the planet's surface during intermittent periods since the environmental changes of Mars (Carr, 1996). It is probable that water altered the fractured igneous rocks at the surface during these intervals. Whether life could have survived the changes of the Martian climate and atmosphere is not known. Many types of simple prokaryote terrestrial organisms appear to be extremely robust and can survive in very extreme environments; however, others suggest that it seems unlikely that life could have survived permanently at the surface of Mars (Freidmann and Koriem, 1989; Westall et al., 1999b,c). There are examples of certain terrestrial bacteria that can survive in ice for long periods of time (Abyzov et al., 1998), and therefore it is possible that viable microbes could be frozen in subsurface permafrost and exist on Mars for long periods of time. The episodes of periodic water on the surface of Mars may be related to heating of the permafrost, or possibly to scattered late stage volcanism or hot springs (Carr, 1996). Frozen microbes could have been thawed and brought to the surface where they would have had the chance to metabolize in environments protected from the UV radiation and oxidants. As they are water-borne, they could infiltrate fractures in the surface rocks. Although it is generally believed that microbial life is unlikely to survive at or near the present-day Martian surface because of the UV and radiation environment (Freidmann and Koriem, 1989; Westall et al., 1999b,c), recent work has shown that some microbes are extremely resistant to high levels of radiation (Kajander et al., 1998; Allen, 1999). Furthermore, thin coatings of iron oxides would effectively filter out most of the harmful UV.

12.3. Cellular morphologies

Certain features have been observed in ALH84001, Nakhla and Shergotty that are nearly identical in size and morphology to some microbes on Earth. However, biogenicity of a bacteriomorph structure can rarely be determined by morphology alone. In addition to intact cellular bodies, some structures resembling fossilized appendages of bacteria and biofilms are found on the ALH84001 carbonate globules and in the clay-filled cracks of Nakhla and Shergotty. Although some of the features originally identified in ALH84001 may be weathered mineral structures, some are definitely not (see section on mineral artifacts above). The extent of terrestrial contamination of all meteorites needs to be addressed, and it cannot be determined at present whether the structures observed in Nakhla and Shergotty are actually fossil bacteria. Although ancient Martian life may be primitive, similar to ancient terrestrial microbes, there remains the possibility that Martian cells may not be identical to those on Earth. The presence of a low-Martian gravity field ($0.6 \times$ that of the Earth's) may affect the size and shape of biogenic structures. Clearly, the remarkable similarities of the morphologies and sizes of the features in ALH84001, Nakhla, and Shergotty to terrestrial bacteria and bacteriamorphs are indicative of further investigations.

12.4. Associated biofilms and microbial colonies

In terrestrial environments, biofilms are a product of bacterial polymeric secretion and are, thus, synonymous with the presence of bacteria (Westall and Rince, 1994; Westall et al., 1999a,b). Films similar to terrestrial biofilm coatings occur in ALH84001 and coat the spherical structures in Nakhla. In ALH84001, it has not been conclusively established whether the biofilms are of Martian origin. In Nakhla, the spherical features with possible biofilm coatings are enclosed in Martian iddingsite and it is difficult to conceive of a method of embedding products of terrestrial contamination within the Martian clay. The discrete clusters of rod-shaped or spherical bacteriomorphs in all three meteorites is suggestive of colony formation.

12.5. Biominerals and disequilibria

As discussed above, approx. 25% of the magnetites in the carbonates of ALH84001 exhibit sizes and unusual rectangular prism shapes as well as pure Fe_3O_4 compositions which are characteristic of microbially produced terrestrial magnetites; these crystals match no known non-biologic terrestrial magnetite (Thomas-Keprta et al., 1998a, 1999, 2000). Their formation can be explained by the presence of magnetotactic bacteria on Mars or by invoking an unknown mechanism working on Mars but apparently not on Earth. In defense of the possibility of magnetotactic bacteria on Mars, the presence of an early magnetic field, which would be essential for their existence, has been well-documented (Kirschvink et al., 1997; Acuna et al., 1999). Other irregular magnetite grains could be either biogenic or non-biogenic in origin. Whisker-like magnetites ($< 5\%$ total magnetites in carbonates) described by Bradley et al. (1997, 1998) are quite different in size distribution, shape, and chemical composition and may have had an origin unrelated to the rectangular prisms. Friedmann et al. (1998) have reported chains of magnetite crystals in ALH84001 which they suggest are of biological origin. The nanometer-sized iron sulfides described in our original paper (McKay et al., 1996) are suggestive of chemical disequilibria related to microbial activity. These sulfides coexist with tiny magnetite grains in the carbonate globule rims. The presence of reduced and oxidized Fe-phases in the tens of nanometer size range most likely required biogenic activity because it would be difficult for these phases in this size range to have been produced abiotically.

12.6. Biologic isotopic signatures

Stable isotope patterns have shown the presence of indigenous carbon components which have isotopic signatures of −13 to −18‰ (PDB), that are similar to known biogenic carbon signatures. Additional detailed study of the isotopic signatures is needed to distinguish between indigenous carbon components within ALH84001 and those introduced after its arrival on Earth. Jull et al. (1999) have noted that 80% of the organic carbon components in Nakhla do not contain modern-day carbon-14 and thus appear to be indigenous Martian carbon phases with isotopic compositions in the −15 to −22‰ (PDB) range. Overall, the carbon isotopic signatures of the identifiable non-terrestrial, possibly organic, carbon are compatible with biologic carbon isotopic fractionation when compared with the signatures of abiotic Martian carbonates, but these signatures do not prove that biotic fractionation occurred. Until further carbon isotopic analyses are carried out on a wide variety of Martian materials and in situ on Mars a complete understanding of the different carbon pools with distinct isotope compositions that reside on Mars can only be estimated. At the present time no measurements of sulfur isotopic compositions of possible biogenic sulfide minerals have been made because of the small size of the crystals.

12.7. Organic biomarkers

Possible organic biomarkers are present within ALH84001 in the form of PAHs associated with the rims of the carbonate globules, some of which may be a unique product of bacterial decay. Relatively large concentrations of noncarbonate carbon are distributed inhomogeneously within the carbonate globule rims and interiors (Flynn et al., 1999). The PAH data of Clemett et al. (1998), combined with the recent amino acid data, show that a portion of the detected PAHs are most likely to be indigenous to ALH84001, whereas all the detected amino acids are most likely to be Antarctic contamination. Exhaustive data must be collected before either component can be used as a biomarker for a specific sample. Recent studies of fossilized bacteria in terrestrial rocks have shown that PAHs are, indeed, a decay product of the bacterial systems and may represent a new biomarker. Recent studies by Flynn et al. (1998, 1999) have documented the presence of reduced carbon species within the clay-filled fractures of the Nakhla meteorite. Their investigations have shown that organic components appear to be indigenous to Nakhla. This observation corresponds with the Nakhla carbon-14 measurements of Jull et al. (1999) which indicates 80% of the carbon is not modern-day but Martian carbon.

12.8. Indigenous features

The recent studies of Clemett et al. (1998) have shown conclusively that the PAHs are likely indigenous to ALH84001 and are not terrestrial contaminants. However, other types of organic compounds (e.g. amino acids) are likely to be terrestrial contaminants (Becker et al., 1997, 1999; Bada et al., 1998). Based on the isotopic compositions and textures, the carbonate globules in ALH84001 and their included minerals formed on Mars and are indigenous to the meteorite (Romanek et al., 1994; McKay et al., 1996; Clemett et al., 1998; Flynn et al., 1999). It may be that some of the possible microfossil structures are indigenous, but more work is needed to confirm these results. It is intriguing that, in the study of other Martian meteorites (Nakhla and Shergotty), features have been found which resemble terrestrial biogenic structures. In our 30 years of research, those fossil-like features thought to be biogenic structures, described above in the Martian meteorites, are essentially absent in non-Martian meteorites and the thousands of lunar samples that we have examined with similar techniques. If the preliminary data are substantiated, then the presence of Martian fossilized bacteria would indicate that life may have spanned most of the history of Mars, from 4 Ga (ALH84001) to ∼0.2 Ga (Shergotty) ago. This type of evidence suggests that it is possible that life could be found somewhere on Mars today.

13. Summary

Many, but not all the criteria for demonstrating indisputable evidence for Martian life in ALH84001, Nakhla or Shergotty have been satisfied. The observations are intriguing but two fundamental aspects need to be addressed: (1) the ability to distinguish between nonbiogenic and biogenic bacteriomorphs, and (2) the extent of terrestrial contamination in these Martian meteorites.

In preparation for the return of samples from Mars, many of the problems associated with the search for life in meteorites have been highlighted by studies spanning the last 30 years. It is clear that a detailed study of biosignatures in terrestrial and extraterrestrial samples over the next several years is necessary so that when Martian samples are brought back, the scientific community will be well-prepared to search for evidence of life so that its presence or absence can be determined with confidence. The goal will require an exhaustive database, which does not yet exist, of terrestrial fossils and biomarkers, both recent and ancient. The design of future Mars robotic lander missions as well as the planning for later human exploration of Mars may be heavily influenced by a search for possible evidence for past or current life. A number of Mars missions are planned in the next decade which will search for in situ evidence of life (Mars Express 2003 with the Beagle2 lander), Athena lander 2003, and Mars Sample Return Mission launched in 2008 with Martian samples returned to Earth in 2011. The construction of such a terrestrial biomarker database is an ambitious task that will need the collaboration of many scientific groups.

In conclusion, the studies of ALH84001, Nakhla, and Shergotty provide stimulating and intriguing indications of possible Martian life. Whereas a definitive answer may yet emerge from these meteorites, it may be necessary to await the return of samples from Mars in order to confidently determine if life has ever existed or if it still exists today on Mars. Additional studies on meteorites and varied samples from terrestrial environments, along with experimental studies of fossilization processes are essential if microbial or fossilized microbial life is to be confidently identified in returned Martian samples.

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