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## Research Paper

# Morphological Biosignatures and the Search for Life on Mars

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### ABSTRACT

This report provides a rationale for the advances in instrumentation and understanding needed to assess claims of ancient and extraterrestrial life made on the basis of morphological biosignatures. Morphological biosignatures consist of *bona fide* microbial fossils as well as microbially influenced sedimentary structures. To be recognized as evidence of life, microbial fossils must contain chemical and structural attributes uniquely indicative of microbial cells or cellular or extracellular processes. When combined with various research strategies, high-resolution instruments can reveal such attributes and elucidate how morphological fossils form and become altered, thereby improving the ability to recognize them in the geological record on Earth or other planets. Also, before fossilized microbially influenced sedimentary structures can provide evidence of life, criteria to distinguish their biogenic from non-biogenic attributes must be established. This topic can be advanced by developing process-based models. A database of images and spectroscopic data that distinguish the suite of *bona fide* morphological biosignatures from their abiotic mimics will avoid detection of false-positives for life. The use of high-resolution imaging and spectroscopic instruments, in conjunction with an improved knowledge base of the attributes that demonstrate life, will maximize our ability to recognize and assess the biogenicity of extraterrestrial and ancient terrestrial life. **Key Words:** Biosignatures—Morphological fossils—Microfossils—Stromatolites—Extraterrestrial life—Biogenicity—Paleobiology. *Astrobiology* 3, 351–368.

### INTRODUCTION

ON EARTH, TWO KINDS of morphological fossils have been used to reveal traces of microbial life in the ancient rock record: cellularly preserved microorganisms and laminated micro-

bially influenced sedimentary structures known as stromatolites. In order for morphological fossils to provide definitive evidence for life, they must be characterized by attributes that are uniquely produced by microorganisms and recognizable as such. Microfossils and microbially

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influenced sedimentary structures represent two of the three principal categories of microbial biosignatures. Chemofossils represent the third category. Organic chemofossils include molecular biomarkers that can be assigned to a particular biosynthetic origin, and the biologically fractionated isotope signature encoded during biosynthesis in organic compounds. Inorganic chemofossils include biominerals, their isotopic signatures, and anomalous concentrations or depletions of elements in biominerals and biologically influenced sediments.

Morphological fossils that retain carbonaceous remains of microbial cells (i.e., *bona fide* microfossils) may contain primary biomolecules or diagenetically altered biomolecules known as biomarker compounds (e.g., Summons *et al.*, 2003). Primary and diagenetically altered carbonaceous biomolecules are usually characterized by distinctive carbon isotope signatures due to biological fractionation and preference of the light carbon isotope during metabolism and cell repair (e.g., Des Marais *et al.*, 2003). Minerals associated with morphological fossils may display distinctive morphologies, isotope signatures, chemical compositions, or defect microstructures that can reveal their biological origin (e.g., Banfield *et al.*, 2001). The study of how biologically produced organometalloids could serve as biosignatures is in its infancy.

Morphological fossils can consist entirely of non-organic constituents if, during fossilization, a microbial cell was completely replaced by minerals. However, attempts to decipher the most ancient fossil record on Earth have shown that mineral-replaced morphological fossils cannot be relied upon to provide definitive evidence for life simply because they cannot be distinguished from non-biologically produced pseudofossils. Paleobiologists searching for the earliest signs of life on Earth and astrobiologists searching for evidence of extraterrestrial life face the same challenge—distinguishing microfossils and microbially influenced sedimentary structures from non-biologically produced pseudofossils and stromatolite-like structures.

Mars may prove to be the first extraterrestrial body in our solar system to yield demonstrable evidence for the existence of life beyond Earth. In order for a morphological fossil to be considered positive proof of life on Mars, it must be demonstrated that the object is of biological origin and formed from Mars constituents. These two crite-

ria, demonstrating biogenicity and indigenicity, must be proven regardless of whether the object is found in a martian meteorite or a targeted sample returned to Earth from Mars.

Demonstrating a putative fossil's biogenicity requires that the object display characteristics uniquely attributable to microbial cells or cellular/extracellular microbial processes. The ongoing controversy regarding the claim of microfossils in martian meteorite ALH84001 exemplifies the difficulties encountered in proving biogenicity on the basis of morphological characters that are not unique to microfossils. Indeed, the ALH84001 controversy underscores the need to be able to distinguish the biogenically produced characteristics of morphological microfossils from those produced non-biologically. It is equally important to know how to distinguish between biologically and non-biologically produced stromatolites.

Proving the indigenicity of a morphological fossil requires demonstrating that the rock specimen within which it was found has not been contaminated, on Mars or Earth, by terrestrial microorganisms (e.g., Steele *et al.*, 2003). Contamination can occur very quickly in meteorites upon their arrival. Contamination can also occur at any time during sample collection, analysis, and storage of specimens on Mars, as well as during transport to Earth and in terrestrial laboratories.

Research strategies that improve our understanding of the processes by which all types of morphological fossils are formed, altered, and ultimately destroyed will facilitate a reliable assessment of their biogenicity and indigenicity. Of utmost importance are research strategies that quantify the fundamental processes governing the formation of microbial fossils and microbially influenced sedimentary structures and their abiotic mimics within the range of conditions found in ancient terrestrial and extraterrestrial environments on early Earth and Mars. It is equally important to determine the constraints imposed by physical and chemical processes that alter and degrade ancient microbial biosignatures during diagenesis on Earth, and to establish stringent criteria for demonstrating the biogenicity of morphological fossils over the entire range of observational scales.

A conceptual framework that provides a rationale for the types of advances in instrumentation and research needed to detect and interpret morphological fossils requires an understanding of:

(1) the use of terrestrial-based strategies to locate potential paleobiological repositories on Mars, (2) the limitations of existing criteria for interpreting the biogenicity of fossil evidence, (3) the processes that produce, alter, and ultimately destroy morphological fossils, (4) the need for spatially integrated studies to locate and interpret biosignatures across a wide range of spatial scales, (5) the need for a central database of images and spectroscopic data from biogenic and pseudo-biogenic morphological fossils, and (6) the challenges faced when using morphological fossils as proxies for extraterrestrial life. A select number of examples of the types of high-resolution imaging and spectroscopic methods needed to detect multiple biosignatures from morphological fossils are provided, along with a summary of recommendations for instrument development and research areas that need to be pursued in order to advance the search for extraterrestrial life as well as the search for ancient life on Earth.

### LOCATING POTENTIAL PALEOBIOLOGICAL REPOSITORIES

Terrestrial strategies for locating potential paleobiological repositories on Mars are based upon the strategies used by paleobiologists to study Earth's ancient fossil record of microbial life: Locate rock deposits that accumulated in environments where fossilization of the extensive microbial communities that inhabited them was favored. In a terrestrial context, the most informative assemblages of organically preserved ancient microfossils are found in mid-to-late Precambrian sediments deposited in marine and lacustrine environments. Microbial fossils occur either as three-dimensional cellularly preserved (permineralized) forms embedded in an authigenic mineral matrix of stable mineralogy, or as two-dimensional compressed or flattened forms (acritarchs) preserved in fine-grained, typically clay-rich detrital sediments. Microbial permineralization commonly occurred in ancient shallow evaporative peritidal settings characterized by elevated salinity (e.g., Knoll, 1985). Acritarchs were preserved in ancient deep anaerobic basins, especially where early diagenetic mineralization occurred because of the precipitation of authigenic cements composed of silica, carbonate, phosphate, or clays.

The types of rocks and paleoenvironments that have the highest potential to capture and pre-

serve fossil biosignatures on Mars have recently been reviewed by Farmer and Des Marais (1999). Paleoenvironment types include: (1) mineralizing springs (e.g., sinter-depositing thermal springs in volcanic terrains and tufa-depositing cold springs in alkaline lake settings); (2) evaporite basins (e.g., terminal lake basins, impact crater and volcanic crater paleolakes, and arid shorelines where evaporite deposits, inclusive of carbonates, are formed); (3) mineralizing soils (e.g., surface duracrust and subsoil hard pans that deposit silcretes, calcretes, and ferricretes); (4) subsurface sedimentary systems (e.g., aquifers of voluminous extent that can sustain mineralization over a broad range of temperatures); and (5) permafrost and ground-ice (e.g., frozen soils that preserve microbial biosignatures in ice). In each type of deposit, except perhaps ice-dominated ecosystems that experience repeated freeze-thawing events, morphological fossils could form over a wide range of spatial scales.

The potential for long-term fossil preservation in each type of paleoenvironment on Mars depends upon the stability of the primary mineral assemblage and the amount of weathering and diagenesis the primary deposit has endured over time. Primary aqueous mineral precipitates are metastable; their formation is kinetically, not thermodynamically, favored. Given enough time, such primary mineral assemblages will transform to thermodynamically favored mineral assemblages. The degree to which primary mineral assemblages are affected by weathering and diagenetic alteration depends upon the length of time the deposit is exposed to weathering at the surface (i.e., chemical and mechanical weathering processes) and to fluids that could promote diagenetically induced mineral phase transformations. Since primary aqueous mineral precipitates often entomb or permeate microbial cells and microbially influenced sedimentary structures, subsequent diagenesis could significantly alter primary biosignatures. On Mars, the amount of water and the length of time water might have reacted with primary and secondary minerals in a sedimentary deposit are critical factors that must be considered when estimating the degree to which diagenesis may have altered the deposit.

On Earth, the potential for long-term preservation also depends upon the amount of tectonic recycling of the rocks in which the fossils are preserved. While the amount of tectonic recycling that mineral deposits would have experienced on

Mars is assumed to be negligible, the degree to which any tectonic-scale forces may have altered Mars rocks is not known.

In general, long-term preservation of microbial biosignatures has occurred when fossils and sedimented or precipitated biofabrics and structures were retained in dense, impermeable host rocks composed of stable minerals that resisted chemical weathering, dissolution, or extensive reorganization during diagenetic recrystallization. Favored lithologies for long-term preservation include cherts and phosphorites, rocks that contain silica and phosphate, respectively. Such lithologies have long crustal residence times and, along with carbonates and shales, are the most common host rocks for the Precambrian microfossil record on Earth.

Determining the location of potential paleobiological repositories on Mars requires an understanding of the martian surface in terms of elemental abundances and mineralogy. Progress in understanding martian surface mineralogy has recently been provided by data obtained by the Thermal Emission Spectrometer (TES) experiment carried aboard the Mars Global Surveyor Orbiter (Christensen *et al.*, 2000). TES, which maps at a spatial resolution of 3 km/pixel in the mid-infrared portion of the spectrum (6–14  $\mu\text{m}$ ), detected large deposits of coarse-grained (specular) hematite (iron oxide) at several locations on Mars. This variety of hematite on Earth forms only in the presence of large amounts of water, and typically at elevated (hydrothermal) temperatures (Christensen *et al.*, 2000). The hematite deposits appear to be co-located with geomorphic features previously described as having been formed by the action of liquid water at the surface of Mars. TES has also revealed a basic compositional difference between the southern cratered highlands of Mars (modal composition dominantly basalt) and the northern lowland plains [modal composition more silica-enriched (i.e., basaltic-andesite)] (Bandfield *et al.*, 2000). The significance of this composition difference is unclear since the geological processes responsible for them are unknown.

The search on Mars for common aqueously precipitated mineral assemblages and rock types (e.g., carbonates, cherts, phosphorites, and evaporites) that tend to harbor fossilized microbial assemblages on Earth is still underway. Remote sensing analog studies in Death Valley (Jolliff *et al.*, 2001; Moersch *et al.*, 2001) indicate that detec-

tion of these minerals by TES may not be possible at lower abundances because of the low spatial resolution of the instrument. Thus, future missions will need to consider higher-resolution instruments that can map over a broader range of wavelengths and at spatial resolutions <100 m/pixel. Improved spatial resolution (100 m/pixel) is being obtained with the Thermal Emission Imaging System (THEMIS) on the Mars Odyssey spacecraft, presently orbiting Mars. Full coverage of the planet will be possible at this spatial resolution during the nominal mission. However, the spectral resolution of this mid-infrared mapping spectrometer will be significantly lower than TES, and it is unclear what impact this will have on mineral detection limits at Mars. In 2005, the Mars Reconnaissance Orbiter (MRO) will carry a near-infrared spectrometer (Compact Reconnaissance Imaging Spectrometer for Mars, or CRISM), which will map the surface at resolutions as low as 10 m/pixel. While this hyperspectral instrument will not achieve complete coverage of the martian surface, it will provide high-resolution mapping at targeted sites.

#### LIMITATIONS OF CRITERIA FOR DEMONSTRATING BIOGENICITY: STROMATOLITES AND RELATED MICROBIALLY INFLUENCED BIOFABRICS AND SEDIMENTARY STRUCTURES

Benthic microbial communities are likely to influence the microstructure of deposits that accumulate in their presence, regardless of whether they occur in detrital or mineralizing environments. The key to proving the biogenicity of microbially influenced biofabrics and structures is knowing which of their components resulted from the presence or influence of the organisms and whether these attributes are unique to microbial processes. If the attributes can form abiotically in the absence of microorganisms, then such attributes cannot be used to demonstrate biogenicity.

To provide a framework for assessing the relative roles of different biologic and abiologic processes in stromatolite accretion, we adopt a non-genetic definition of stromatolites first presented in Hofmann (1973): A stromatolite is "an attached, laminated, lithified sedimentary growth structure, accretionary away from a point

or limited surface of initiation" (Semikhatov *et al.*, 1979). This concise definition describes the basic geometric and textural properties of all stromatolites while at the same time allowing for multiple or even indeterminate origins. In this way an objective evaluation can be made of the various processes that may influence stromatolite development. Such an approach can lead to the formulation of specific process models that accurately describe stromatolite accretion dynamics. Process models might routinely predict the relative or even absolute contributions of biological, physical, and chemical effects to stromatolite formation.

The future value of stromatolite research lies in its potential to provide a basis for reconstructing ancient environments and to understand how benthic microbial communities interacted with their environments. This must involve a process-oriented approach to stromatolite morphogenesis in which the correct interpretation of diagenetic and recrystallization textures is as important as understanding microbial diversity and fossilization processes in modern microbial ecosystems. The goal is to build an understanding of stromatolite development that stems from rigorous, quantitative analyses of stromatolite form and lamina texture, including the deconvolution of diagenetic overprints to reveal primary fabrics diagnostic of specific microbial and sedimentary processes. Critical questions regarding the factors responsible for the development of biofabrics, biogenic stromatolite morphologies, and diagenetic textures can then be addressed. For example, over what length and time scales do biological, physical, and chemical processes operate? Do any of these processes—which might be critical at microscopic scales—remain sensitive at larger scales? If not, at what scale does the transition in process response take place? Questions such as these must be answered before we can claim a real understanding of what fundamental properties, such as stromatolite shape, signify.

The most conspicuous feature of stromatolites is their lamination. Individual laminae are the building blocks of stromatolites and therefore make up a time series of progressive, albeit incremental, accretion. The morphology of any stromatolite is a function of how lamina shape, particularly its relief, evolves over time. Topographic anomalies that are reinforced over time give rise to greater relief for successive laminae, and those that are stabilized give rise to greater

inheritance of shape for successive laminae. Those topographic anomalies that are damped over time result in diminished relief.

In addition to lamination, the other distinguishing feature of stromatolites is their shape. A typical stromatolite is made up of numerous successive laminae that stack one on top of the other to form domal, coniform, columnar, or branching columnar structures. Although laminae generally describe convex-upward structures, they can also form concave-upward or discrete conical structures. In general, it is thought that there is a broad but gradational variation in the form of stromatolites encompassing several major morphological motifs (Semikhatov *et al.*, 1979). It has long been observed that stromatolite morphology varies as a function of facies; thus, there is broad agreement that physical environment plays a role in the generation of shape (see papers in Walter, 1976).

At the level of process, however, there is no such guiding consensus, which severely limits our ability to understand either paleoenvironmental or stratigraphic variations in stromatolite form. As Hofmann (1987) stated, ". . . we still have no stromatolite theory, no model that shows which attributes changed in what way through time." Without a viable theory we are always at risk of misinterpreting the genetic significance of growth form.

One can easily list numerous factors that might influence stromatolite development, including light intensity, salinity, nutrient supply, current velocity, sediment grain size distribution, mat community diversity, and degree of mineral saturation, to name a few. In detail, stromatolite growth is dependent on many processes that are complexly interrelated. Not only are the processes mechanistically complex, they also evolve over long time scales that are difficult to reproduce experimentally or monitor in the field. Given these difficulties, our initial goal should be to construct simple process models of complex systems. Even this will be a difficult task, in need of studies that can serve to calibrate important model parameters (e.g., Jorgensen and Des Marais, 1990). In principle, the growth of stromatolites can be described as a simple system that depends on only three fundamental processes: growth and degradation of a microbial mat or biofilm, deposition of sediment, and precipitation of minerals. Interactions among these end-member processes should account for the bulk of stromatolites in the record.

Stromatolite growth depends on the iterative process of upward growth by mats or seafloor crusts alternating with times of sediment deposition. In addition, these processes must be balanced in such a way that sediment does not overwhelm mats or microbial biofilms. On further inspection, an additional but critically important attribute of the iterative process is revealed. The growth of mats tends to produce an irregular, relatively rough surface, whereas the settling of sediment tends to create a relatively smooth surface by filling in the microtopography of the underlying mat. The surface roughness of mats will vary depending on the composition and distribution of the microbial community. Therefore, over many iterations the surface roughness of a growing stromatolite may be enhanced or suppressed, depending on the competitive processes of surface roughening by mats and surface smoothing through sedimentation. Unfortunately, even though it has been recognized in a qualitative sense for decades that microbial mats have variable surface roughness, this attribute has never been quantified. It is recommended that surface roughness be measured in future studies of modern microbial mats, particularly where sedimentation also occurs.

#### *Interface dynamics and stromatolites*

The texture (i.e., preferred orientation) and roughness of any depositional surface or interface is subject to certain force balances and the presence of noise or randomness. A widely applied growth model is represented by the KPZ equation (Kardar *et al.*, 1986), whose relevance to understanding stromatolite growth was recently evaluated (Grotzinger and Rothman, 1996). The validity of the KPZ equation in accounting for the growth of stromatolites was tested and tentatively confirmed by calculating the surface roughness of several stromatolitic laminae, and comparing the obtained scaling exponent (and fractal dimension) with that predicted by the KPZ theory (Grotzinger and Rothman, 1996). Growth of this type may characterize in a quantitative manner the geometry of layering in many Precambrian stromatolites, agates, botryoidal mineral clusters, travertines, and certain types of stromatolites where seafloor precipitation is thought to have been important. For example, stromatolites formed immediately prior to the precipitation of some of the world's largest evaporite de-

posits are characterized by fine, isopachous lamination and internal textures consistent with *in situ* precipitation (Pope and Grotzinger, 1999).

In contrast, for other systems the presence of a diffusing field that reflects pressure, electric potential, temperature, and chemical or nutrient concentrations may lead to a more appropriate growth model for stromatolites since growth is fastest where concentration gradients are steepest. Initial studies of models for diffusion-limited aggregation (DLA) strongly suggest that they are also applicable to understanding the growth of certain stromatolites (Grotzinger and Chan, 1999). Whereas DLA by itself predicts highly branched, dendritic structures, in the presence of incremental sedimentation events a simple DLA model predicts many of the domal, columnar, and branching columnar stromatolite morphologies observed in the geological record. In this model, an episode of upward growth by randomly attaching particles is taken to simulate growth of either mats or crystals, followed by an episode of sediment settling in which the sediment is allowed to settle preferentially in the microdepressions formed in the underlying mat or crystal layer. If thick enough and/or diffusive enough, the sediment may damp all of the initial topography created by the underlying layer. However, if antecedent topography remains, then the next iteration of mat/crystal growth will result in preferential accretion on those topographic highs. The next layer of sediment now has a tougher task to fill depressions, giving rise in the next iteration of mat/crystal growth to an even higher preference for growth on topographical highs. This is a particularly important feature of DLA models, in that small perturbations can be amplified in time to become dominant features of the structure itself. In this manner, no special conditions may be required to generate columns and branching columns in stromatolites—only time and the positive reinforcement of randomly produced protuberances. This type of growth may help account for the diversity of branched columnar stromatolites common in the ancient geologic record.

#### *Biogenic versus abiogenic growth*

By understanding, through the use of simple process models, that stromatolite growth may result from the competitive interaction of upward-growth and surface roughness forced by

microbial mats and dampening of surface relief by sediment settling, it becomes easy to see how the growth of abiotic marine crusts might substitute for mats and create the same end result. The good news is that we may now have a theory that can account for the growth of a remarkable range of stromatolites. The bad news is that this theory predicts that we can no longer accept only morphological descriptions of stromatolites as evidence of their biogenicity. This does not mean that stromatolites may not have grown in the presence of biogenic influences. It means, however, that in many cases morphology may be a non-unique parameter. Biogenicity cannot easily be demonstrated on the basis of relationships observed at the outcrop scale; it is essential to examine lamination textures petrographically and demonstrate the presence of fabrics or textures (primary or secondary) uniquely attributable to the presence of microbial mats or biofilms (Cady and Farmer, 1996; Knoll and Semikhatov, 1998). For many ancient stromatolites such an approach may not be possible because of an indecipherable level of diagenetic recrystallization.

#### *Biosignatures produced by microbial biofilms*

Although we have emphasized the limitations faced when attempting to distinguish the biogenicity of microbially influenced sedimentary structures, there are a number of structures formed by benthic microbial communities that occur as biofilms rather than flat-laminated microbial mat communities. For example, the high-temperature siliceous sinter known as geyserite displays microstructural attributes that result from the presence of benthic microbial biofilms on accretionary surfaces (Cady and Farmer, 1996). Microbial biofilms also occur as cryptoendoliths that form inside minerals such as the biofilm communities that occur at the sediment surface in Antarctic Dry Valleys (Friedmann *et al.*, 1988; Wynn-Williams *et al.*, 1999). If life exists at the surface of Mars it would likely occur as endoliths. The submicroscopic attributes of these types of deposits are clearly influenced by microbial biofilms, yet they may not be detectable by the criteria established for recognizing the biogenicity of stromatolites. The fate of biofilms in natural environments, the fossilization potential of these structures, and how these factors relate to established criteria for demonstrating biogenicity are

priority research topics that have yet to be systematically addressed.

#### LIMITATIONS OF CRITERIA FOR DEMONSTRATING BIOGENICITY: *BONA FIDE* MICROFOSSILS

Recall that any claim of morphological fossils as representing life that once existed beyond Earth must be accompanied by proof that the objects were produced biologically or biosynthetically and that they are not terrestrial contaminants. Of the two methods traditionally used to detect ancient microfossils (e.g., Schopf, 1999), maceration is the easier and faster of the two techniques. Macerations are carried out by dissolving rocks in mineral acids (hydrochloric acid for limestones, hydrofluoric acid for cherts and siltstones). Because of their carbonaceous composition, organic-walled microfossils pass through the technique unscathed. Abundant fossils are concentrated in the resulting sludge-like acid-resistant residue that can be slurried onto a microscope slide for study. Unless specimens are prepared in such a way as to avoid air- and water-borne contaminants, however, this technique is subject to the problems posed by contaminants being identified as false-positives for life.

Petrographic thin section analysis, the other technique traditionally used to detect microfossils in ancient rocks, provides a means to evaluate the indigenicity of purported fossil objects. The possibility of laboratory contamination can be ruled out if the fossils are clearly embedded within the mineral matrix as evidenced in thin sections of the rock within which they are found. Consider, for example, microfossils in cherts. Together with fine-grained clastic sediments, such as siltstones, cherts are one of the most fossiliferous rock types known in the early geologic record. Ancient fossil-bearing cherts are made up of cryptocrystalline interlocking grains of quartz. The grains precipitated initially as opaline silica, taking thousands of years to transform into a full-fledged quartz chert during which time the microorganisms became petrified (technically, "permineralized") and embedded within a solid chunk of rock. The quartz grains that formed inside the cells and surrounded them on all sides developed so slowly that they grew through the cell walls instead of crushing them. As a result,



the petrified fossils are preserved as unflattened, three-dimensional bodies. Except for their quartz-filled interiors and the brownish color of their aged organic matter (kerogen), they bear a striking resemblance to living microorganisms. In petrographic thin sections, only those objects that are entirely entombed in rock can be considered fossil, so it is easy to exclude contaminants that settle onto the surface of a section or are embedded in the resin used to cement the sliver of rock onto the glass thin-section mount.

Optical microscopy of thin sections provides a means to establish that fossil-like objects date from the time a rock formed rather than having been sealed later in cracks and crevices. In this way it is possible to establish that the objects are syngenetic with a primary mineral phase rather than one of secondary or later genesis. Consider, for example, microfossiliferous stromatolitic cherts. When such cherts first form, many contain cavities where gases (often oxygen, carbon dioxide, hydrogen, or methane) given off by the microbial community accumulate in small pockets. Later these cavities can be sealed and filled by a second generation of quartz laid down from seeping groundwater, sometimes tens or hundreds of millions of years after the primary chert precipitated. Microscopic organisms trapped in these cavities and petrified by the second-generation quartz would be true fossils that are younger than the rock unit itself. Fortunately, the various generations of quartz in a chert can be distinguished from each other. Rather than having interlocking grains, the quartz variety that rapidly fills cavities is a type known as chalcedony, which follows the smooth contours of the infilled pocket to form distinctive botryoidal masses. Secondary quartz in cracks or veinlets is also easy to identify since it is angular and its grains are much larger than those first formed.

Because special equipment is needed to prepare thin sections, and their study is exceedingly time-consuming, some workers have focused their hunt for ancient fossils on acid-resistant rock residues. In relatively young (Proterozoic) Precambrian rocks, where the fossil record is well enough known that misidentification of contaminants and fossil-like artifacts can be avoided, this technique is useful, simple, and fast. But to avoid mistakes when examining older (Archean) Precambrian deposits, where the fossil record is not nearly so well known, or, of course, when examining extraterrestrial samples, use of the more rig-

orous thin-section technique is essential. Since criteria by which the biogenicity of *bona fide* microfossils identified by light microscopy have yet to be established and verified at the scale of detection (imaging and spectroscopic) obtained with electron microscopy, we recommend further research of this topic.

Although transmission (TEM) and scanning (SEM) electron microscopy have been used to characterize microfossils previously detected in macerations or thin sections, these techniques have not yet proven reliable for demonstrating the biogenicity of microfossils not identified by other means. In fact, such techniques have revealed a new set of challenges posed by the discovery of submicroscopic objects characterized by microbial morphologies that lack detectable organic components (e.g., McKay *et al.*, 1996; Westall, 1999). In addition to established criteria for assessing microfossil biogenicity (Schopf and Walter, 1983; Buick, 1990; Schopf, 1999), a number of new criteria have been proposed for assessing the biogenicity of submicroscopic-sized microfossil-like objects (Westall, 1999). Regardless of the size of purported morphological fossils the evidence sought for life should be positive—evidence that affirms the biological origin of the features detected (Schopf, 1999). Evidence that is neutral (consistent either with biology or non-biological processes) is by its nature inadequate to establish the existence of past life, and interpretations based on negative reasoning or inference by default are likely to prove erroneous. Such stringency is warranted in the search for life beyond Earth, as well as in the search for the oldest evidence of life on Earth.

#### FORMATION AND ALTERATION OF MICROFOSSILS

Geomicrobiological and mineralogical studies of modern ecosystems recognized as analogs for early Earth or Mars environments, along with laboratory-based experiments designed to simulate microbial fossilization and stromatolite formation, continue to improve our understanding of the processes involved in producing morphological fossils. As discussed by Cady (1998), since microorganisms can occupy nearly every available habitat where water and available carbon and energy sources exist, the number of potential paleobiological sites in which evidence of life

may be preserved has expanded. Indeed, any structural discontinuity in rocks through which mineralizing fluids have passed freely should be searched for morphological fossils, including microfossils and microbially influenced fabrics and structures. Many modern extreme ecosystems and their ancient analog deposits have not been systematically assessed as potential paleobiological repositories (e.g., subsurface rocks, permafrost regions, paleosols, and hydrothermal and epithermal deposits). Because of their high potential to demonstrate life, it is important to understand how microfossils are formed, preserved, and altered. The formation and preservation of microfossils depend upon the intrinsic characteristics of the microorganisms, the chemical and physical characteristics of their environment, and the amount of post-depositional alteration and diagenesis they experience (e.g., Cady, 2001).

#### *Intrinsic characteristics of microorganisms*

The intrinsic characteristics of microorganisms significantly influence whether they become fossilized in either chemically mineralizing environments or in detrital sedimentary environments. Studies to date indicate differences in the cell wall type (e.g., Westall, 1997), the presence of recalcitrant sheaths (e.g., Cady and Farmer, 1996) or other exopolymers (e.g., Allen *et al.*, 2000), and the reactivity of biomolecules within cells (e.g., Fortin *et al.*, 1997) can influence the susceptibility of microorganisms to mineralization. The propensity of some microorganisms to sequester anomalous concentrations of trace metals such as iron, a process that can enhance microbial preservation, has also been shown to depend upon the type, density, and distribution of biomolecules that make up microbial cell walls and various extracellular components (e.g., Ferris *et al.*, 1989; Beveridge *et al.*, 1997). Future studies to determine when and how the composition of cellular and extracellular components changes, and whether those changes alter the susceptibility of a microorganism to fossilization, will provide constraints needed to assess the range of conditions over which trace metal concentrations can be used as biogenic indicators. Furthermore, since the reactivity of cellular surfaces depends upon the microenvironment around the cell rather than the bulk composition of any external fluid, further experiments should concentrate on analyzing fluid geochemistry at the cellular scale.

#### *Extrinsic geochemical characteristics*

As reviewed by Farmer and Des Marais (1999), microbial preservation on Earth occurs in environments where microbial cells are entombed in a fine-grained authigenically precipitated mineral matrix or where they are rapidly buried by fine-grained sediments. These processes protect cellular remains from oxidative degradation.

In detrital sedimentary systems, preservation is enhanced by rapid burial. In addition, fine-grained detrital-rich environments often sustain anoxic conditions that favor early diagenetic mineralization. This can also enhance preservation by reducing permeability and helping to create a closed chemical system that arrests cellular degradation.

In chemically mineralizing sedimentary systems, environments that favor rapid mineral deposition can entomb organisms while they are still alive. This process arrests degradation and enhances the preservation of important aspects of morphology, growth habit, and distribution of organisms within microbial mats and biofilms. Rapid reduction in porosity and permeability is important for cellular preservation in chemically mineralizing systems as well; sustained migration of oxidizing fluids can ultimately remove all traces of cellular remains and promote early diagenetic mineral transformations. It is recommended that the extrinsic geochemical characteristics that affect preservation in modern ecosystems that could have analogs on Mars be systematically studied.

#### *Mineral and biomolecule diagenesis*

Whether microfossils can be detected and reliably identified in the ancient geological record ultimately depends upon their diagenetic history. Diagenesis occurs when an increase in the reactivity of a phase (either due to an increase in temperature or pressure or via water-rock interaction) results in its structural or chemical transformation. Both primary biomolecules and mineral phases can be diagenetically altered. Consideration of the preservation potential of all types of biomolecules suggests that the various classes of lipids, especially glycolipids and lipopolysaccharides, are most resistant to degradation in all types of depositional environments (De Leeuw and Largeau, 1993). The conversion of primary lipids to their diagenetic counterparts involves information loss through structural alteration. However, the original class of lipid can

often be identified even after such diagenetic changes occur (Summons, 1993).

Microfossils are generally preserved in primary kinetically favored mineral assemblages that ultimately recrystallize to thermodynamically stable mineral assemblages. An example of mineral diagenesis that alters chert deposits with time is the transformation of primary opaline silica phases to microcrystalline quartz varieties through intermediary cryptocrystalline opal phases (Hesse, 1989). Recrystallization of primary aqueous mineral matrices often obliterates the fine-scale morphological features of microfossils, which are finer than the minimum grain size of the predominant diagenetic phase (e.g., microquartz by definition has an average grain size of 5–20  $\mu\text{m}$ ). Although recrystallization of primary mineral assemblages is known to occur as a function of time, it is recommended that the details of how such processes alter biosignatures be systematically studied.

#### *Chemical and structural discontinuities*

Regardless of the mechanism by which a microorganism is fossilized or altered during diagenesis, the resultant microfossil will go undetected unless it differs either in composition or in structural organization from the mineral matrix that surrounds it. The most illustrative examples of chemical and structural discontinuities are those between acritarchs (organically preserved cells) or permineralized microorganisms (cellularly preserved cells) and the mineral matrices in which they are preserved. Environmental perturbations can also produce compositional differences between fossilized microbes and their surrounding mineral matrix. The temporal changes that occur in fluids within evaporative environments or in fluid mixing zones can be preserved in the laminated crusts that develop around microbial cells. The sequence of minerals may reveal information about the metabolic character of the microorganism, preserve a cast of a microbial cell, and help reconstruct details about the nature of paleoenvironments. The survival of such features as a function of time and increasing diagenesis is a fundamental research question we recommend pursuing.

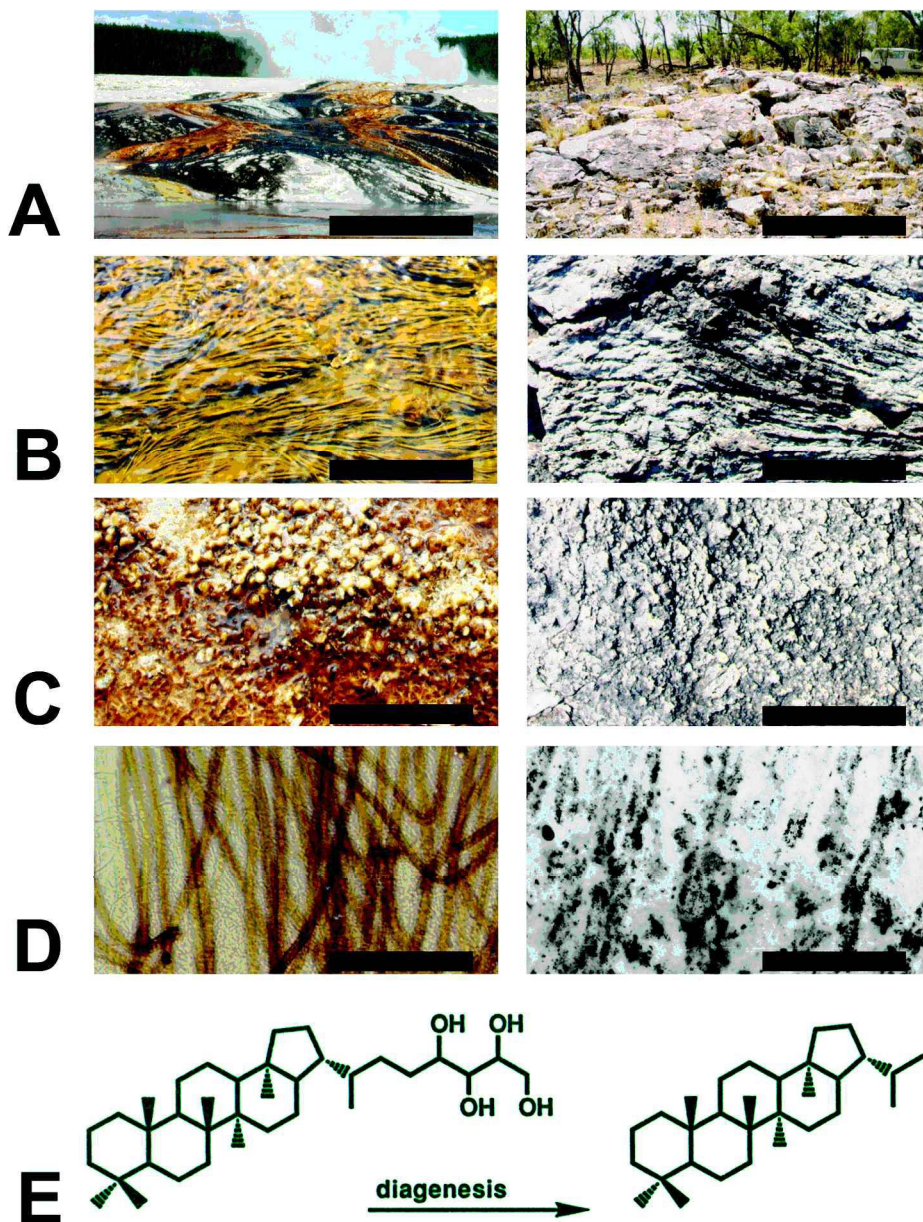
### **SPATIALLY INTEGRATED PALEOBIOLOGICAL STUDIES**

Advances in our understanding of the aforementioned processes, and the ways in which mi-

crobes interact with and influence their environment, must be accompanied by technological advances that will improve our ability to detect biosignatures of life preserved within ancient rocks formed on Earth or beyond. Paleontological interpretations, including those made for extraterrestrial materials, rely upon comparisons between the biology and environmental characteristics of modern and fossilized ecosystems. Morphological fossil remains preserve important aspects of the behavior and distribution of microorganisms, and biomarkers and isotope signatures provide details about their metabolism and functional capacity. Biominerals reflect both the geochemistry of the microbial milieu and the biogeochemical processes by which microorganisms interact with their environment. The structural and geochemical characteristics of sedimentary repositories reflect the paleoenvironments in which microbial populations lived.

A number of factors complicate the use of modern analogs for interpreting the paleobiology and paleoenvironment of ancient paleobiological repositories. The simple shapes and limited size range of microbial cells often preclude diagnostic paleobiological interpretations based entirely on morphology. The progressive taphonomic alteration of both physical and biochemical characteristics of microfossils limits the utility of chemical and mineralogical biosignatures to younger, relatively unaltered rocks. The secular changes observed in biogeochemical cycles (e.g., carbon, sulfur, iron) during Earth's early history indicate that many modern-day ecosystems cannot be regarded as direct homologs of ancient ecosystems. Even with these caveats, many paleobiological studies include parallel geomicrobiological studies of modern ecosystems. Modern analog studies can help constrain and quantify the fundamental biological, physical, and chemical processes that ultimately lead to the formation of a fossil record.

An example of a scale-integrated framework for paleontological investigations based on a comparison of modern and ancient analogs is shown in Fig. 1. The examples illustrate the similarity of attributes at different observation scales of modern hydrothermal spring deposits in Yellowstone National Park (Farmer, 1999) and of ancient siliceous hydrothermal spring deposits in northeast Queensland, Australia (Walter *et al.*, 1998). For each spatial scale, environmental and biological comparisons reflect the lateral facies



**FIG. 1.** Scale-integrated framework for paleontological investigations based on a comparison of modern (Yellowstone National Park) and ancient (northeast Queensland, Australia) hydrothermal spring deposit analogs. The examples illustrate the similarity of attributes at different observation scales. At a regional scale of hundreds of kilometers, the geological context of this continental volcanic terrane provides important constraints for interpreting the major geological environments and facies or depositional trends. **A:** At the scale of a local outcrop of a single hot spring system, meters to kilometers, environmental parameters such as thermal and pH gradients, trends in community distribution, and systematic changes in sinter texture and mineralogy are apparent. **B** and **C:** At the microenvironmental and microfacies scale of the hot spring system, meters to centimeters, distinctive mat community types, surface biofabrics, and major sinter types are evident. **D:** Investigation of the mats at the microscale, centimeters to submillimeters, reveals that each mat type exhibits distinctive mat compositions. Vertical and lateral changes in the distribution of organisms can be related to small-scale changes in environmental characteristics, as well as corresponding microstructural changes in sinter structure characteristics that include lamina shapes and internal fabric. **E:** At the level of the microbial ultrastructure and mineral microstructure, it is possible to obtain nanometer-scale information about the physical conformation and chemical composition of biomolecules.

and vertical temporal changes in the system over different time scales.

Integrating observations over all of the observational scales discussed above is important for understanding the origin of emergent properties of the entire system. Spatially integrated studies also provide important constraints for evaluating hypotheses regarding the nature of the ecosystem. For example, basic mat structures and organizational modes arise from the growth and motility or taxis of the component species. This means that to understand the higher-order mat features requires some knowledge of how the motile species interacts with its environment at the next lower level of organization. A knowledge of motility responses of various taxa in modern ecosystems provides, in turn, a context for testing ideas about the origin of biosedimentary fabrics preserved in ancient deposits. Clearly, an approach that integrates data across spatial scales affords a more robust framework for interpreting paleobiology and paleoenvironments.

In taking an analog approach it is assumed that the laws of physics and chemistry are universally constant, now and in the past. On this basis, the results of investigations of terrestrial analog systems can be extrapolated and applied to other planets, as well as to early Earth, within the constraints of known environmental differences. The practical risks are that (1) we still have a lot to learn about the environments on early Earth and Mars and (2) life on Mars could be entirely unique, possessing unknown properties that cannot be predicted from an Earth-centric model. Nevertheless, even with these uncertainties it is logical to begin with what we know about life based on the one example we have for study and to assume the invariability of natural chemical and physical laws as a framework for defining appropriate strategies for exploration. The alternative is to take a non-Earth-centric approach to the study of microbial biosignatures (Conrad and Nealson, 2001). The essence of analog and non-Earth-centric approaches is the same: search for biosignatures left by microorganisms.

### MORPHOLOGICAL BIOSIGNATURE DATABASE

As discussed in this report, a variety of high-resolution techniques can be used and developed to identify microbial biosignatures. A relatively large

and diverse database of images and spectroscopic data indicative of life will continue to accumulate in the peer-reviewed literature. At the same time, millions of images and spectra from dubiofossils (of unknown origin) and pseudofossils (abiotic mimics) will also continue to accumulate, yet they will rarely appear in publication. On the other hand, those abiotic mimics that have appeared in the literature have often been mistaken, usually because of the lack of a comparative database for recognizing biogenicity in such objects.

Since the same techniques used to identify *bona fide* biosignatures can be used to distinguish them from their abiotic mimics, it would be extremely beneficial to have an easily accessible (i.e., web-based) database of both types of objects. Clearly there is a need for a common database of *bona fide* biosignatures, dubio-biosignatures, and pseudo-biosignatures. An investigator could search the database for similar objects and obtain detailed information about them (e.g., protocol and technique used to obtain the image and spectrum, information about the sample location and collection protocol, whether the object is common in the sample, whether the object has been found in other types of samples, etc.).

Such a database would improve systematically the ability to identify *bona fide* biosignatures and establish efficiently and with a uniform level of quality control the constraints of the various high-resolution techniques. The database would also solve some of the problems posed by restricted (less accessible) sample sets. We therefore recommend that NASA investigate the utility of establishing and maintaining this type of database to avoid detection of false-positives for life.

### MARS: THE CHALLENGES OF USING MORPHOLOGICAL FOSSILS AS PROXIES FOR EXTRATERRESTRIAL LIFE

#### *Lessons learned from ALH84001*

The initial interpretations of the ALH84001 meteorite included nanometer-scale morphological features likened to the remains of nanobacteria (McKay *et al.*, 1996). At the nanometer scale, however, there is a convergence in the morphology of biosynthetic and inorganic forms since both types of structures form in response to similar physical and chemical processes (e.g., diffusion, minimization of surface energy, etc.). This conver-

gence of biosynthetic and inorganic forms at the submicroscopic scale underscores the problems associated with using only morphology as a biogenic indicator. Important lessons learned from the controversy over the origin of the putative nanobacteria discovered in ALH84001 are the lack of specificity of simple nanometer-scale forms and our inability to assess the biogenicity of such features based on morphology alone. We therefore recommend the development of analytical methods that will allow non-destructive, integrated morphological, chemical, and mineralogical analysis at the nanometer scale.

Bradley *et al.* (1998) showed that nanometer-scale structures similar to those found in ALH84001 could be produced artificially when metallic coatings were applied to mineral specimens, a standard procedure used during the preparation of specimens for high-resolution SEM studies of the type reported by McKay *et al.* (1996). However, the conclusions of Bradley *et al.* (1998) were challenged by studies in which the nanometer-scale structures were characterized using atomic force microscopy (Steele *et al.*, 1998). Indeed, studies to examine the range of possible inorganic processes and forms that could explain such features are warranted, and mark a necessary condition for assessing biogenicity.

In addition, nanometer-scale fossilization processes are not well understood. Consequently, the taphonomic framework for assessing the potential biogenicity of nanostructures is poorly defined. The structures reported by McKay *et al.* (1996) could represent microbial preservation by either permineralization or the wholesale mineral replacement of microbial cells. In the former case, the potential to preserve cellular components such as a cell wall would be an important attribute necessary for demonstrating biogenicity. Our present inability to conduct targeted sectioning of specific nanometer-scale features imaged by submicroscopic-scale methods and to map their light element composition precludes any further assessment of the biogenicity of the purported microfossils based on such criteria—clear priorities for instrument development and future technology advances needed to support the study of purported morphological biosignatures.

#### *Planetary protection-related issues*

Regardless of the technique of investigation, it will be necessary to understand how alteration

and sterilization methods affect morphological biosignatures. It appears likely that samples returned from Mars or other planetary bodies will be released from quarantine for scientific study only after they have been certified harmless or sterilized and shown to be devoid of biohazardous components. A number of potential sterilization methods have been proposed, including dry or wet steam heat;  $\gamma$ - or electron beam radiation; alkylating chemicals such as formaldehyde and ethylene oxide; and oxidizing chemicals such as hydrogen peroxide, chlorine dioxide, ozone, paracetic acid, or combinations of one or more of these with plasma. It appears probable that the protocol selected for sterilization will employ dry heat (e.g., 135°C for 24 h, the method used in 1976 to sterilize Viking spacecraft),  $\gamma$ -irradiation (60°C, >1 Mrad dosage), or a combination of both techniques. A new report from the National Research Council's Committee on the Origins and Evolution of Life titled *The Quarantine and Certification of Martian Samples* (2002) updates and extends these recommendations for sterilization. Regardless of the method chosen for sterilization, we recommend that thorough studies be carried out in advance of sample return to determine what effect, if any, the selected protocol may have on the fidelity of morphological biosignatures.

#### EXAMPLES OF INSTRUMENTS CAPABLE OF IMPROVING OUR ABILITY TO ASSESS BIOGENICITY

##### *Time-of-flight secondary ion mass spectrometry (ToF-SIMS) and SEM*

Techniques such as ToF-SIMS, combined with high-resolution field emission gun SEM (FEG-SEM), provide a means to relate morphological features to chemical signatures (Fig. 2). By marking the sample or using tell-tale features on the surface of a sample, it is possible to gain *in situ* surface sensitive data of both positive and negative ions from 0 to 10,000 AMU at very high peak resolutions (1,000–6,000) with a beam spot size that ranges from 50 to 200 nm depending on the instrument. The distribution of any peaks in the spectra can then be correlated with features on the surface of the sample. Although the inclusion of a secondary electron detector in the chamber of a ToF-SIMS instrument can generate SEM images, they generally suffer from low resolution.



It is possible at the present time to overcome this obstacle by imaging the same area analyzed with a ToF-SIMS instrument with high-resolution SEM after ToF-SIMS analysis. This technique has already been successfully applied to fossilized bacterial biofilms (Toporski *et al.*, 2001).

Although the combination of surface mapping and surface analysis using ToF-SIMS with FEG-SEM imaging is extremely promising, this combination of non-destructive techniques is still in its infancy, and we recommend further development be pursued. Specific recommendations are: an improved imaging capacity and mass resolution for the ToF-SIMS, an improved understanding of the mechanisms by which the spectral signatures are produced (a  $\pm 1$  AMU shift sometimes occurs with higher-molecular-weight compounds), an increase in the database of information about various types of microbes (e.g., hopanes have been detected with this instrument), and correlation of chemistry with morphology during sequential stages of preservation.

*Analytical high-resolution TEM (HRTEM), ion microprobe, Raman spectroscopy, and synchrotron x-ray tomography*

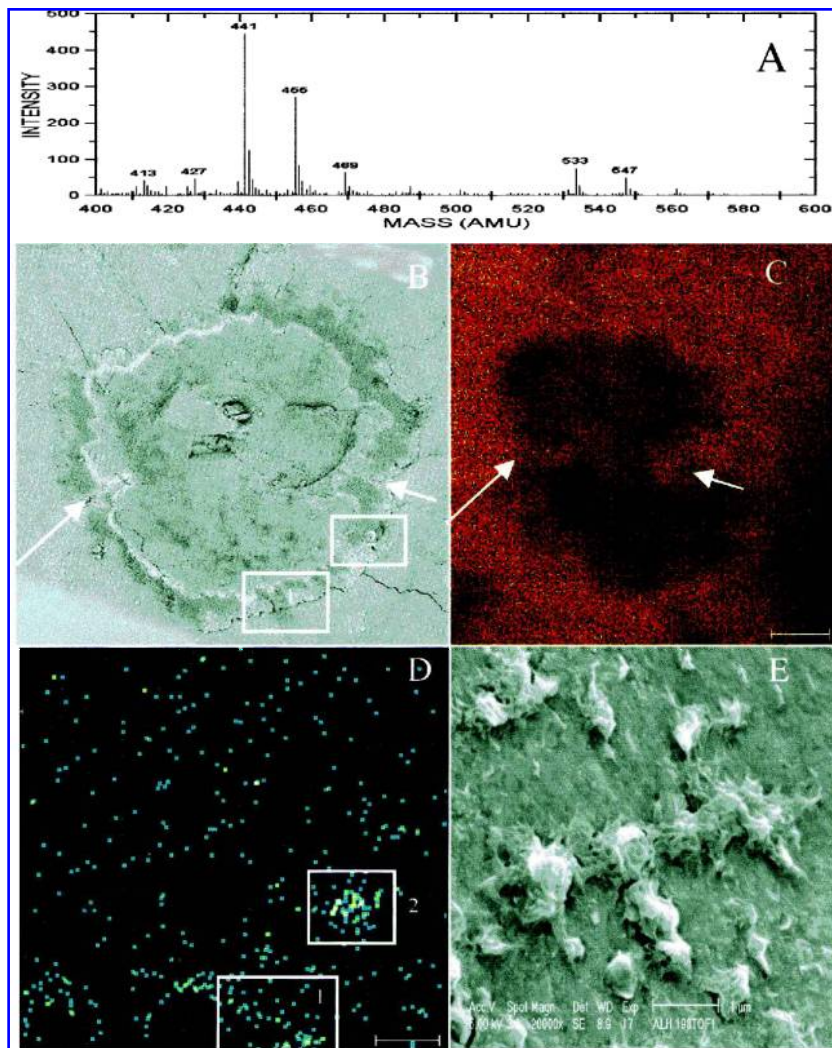
Again, the aim is to correlate chemical molecules of organic remains with morphological features indicative of their cells or cellular remains, and to determine the technological limitations of instruments that have yet to be fully exploited. Other than being evidently "carbonaceous," the chemical composition of Precambrian microscopic fossils is generally not known, information that, if preserved, could provide valuable insight into the original biochemical makeup and physiological capabilities of preserved microorganisms. Additional methods that have yet to be systematically utilized to correlate micromorphology with chemical composition include: (1) analytical HRTEM equipped with high-resolution spectrometers (e.g., an electron energy loss spectrometer); (2) ion microprobe analyses of the carbon (and other elemental) isotopic composition of individual microfossils to reveal aspects of their physiology, a technique that has been used with notable success in an initial pilot study (House *et al.*, 2000); (3) Raman spectroscopy of microscopic fossils to reveal the chemical makeup of their preserved cell walls, a novel technique that has been used to demonstrate the presence of the molecular signatures of disordered and

graphitic carbon in 650-million-year-old microfossils from Kazakhstan and 1,878-million-year-old microfossils from the Gunflint Formation in Canada (McHone *et al.*, 1999); and (4) synchrotron x-ray tomography of rock-embedded microfossils, a new non-intrusive and non-destructive means of fossil detection and chemical characterization that is of special interest for analyses of materials for which only small amounts of samples are available for study, such as those planned to be returned from Mars (Tsapin *et al.*, 2000). As these and related techniques have been shown already to be highly promising for chemical characterization of minute ancient fossils, we recommend major emphasis be placed on their further development and refinement.

*New application for establishing biogenicity: trace element biosignatures*

Many microbes secrete polymers [extracellular polymeric substances (EPS)] outside of their cell. EPS can form either dense structures, such as sheaths or capsules that encapsulate cells, or more diffuse slime matrices that bind together groups of cells. The potential functions of EPS are many, but controlling the chemical microenvironment surrounding cells is among the most important (i.e., Decho, 1990). In microbial communities, organisms exist in a common EPS ("slime") matrix. These materials hold great taphonomic importance because they can control aspects of the chemical microenvironments that promote early diagenetic mineralization, a key factor in microbial fossilization (Farmer, 1999). Cell walls and extracellular exopolymers have many negatively charged surface sites that bind metallic cations and cation complexes. These include anionic carboxyl and phosphoryl groups that are responsible for absorbing and concentrating many of the metallic cations required for growth. EPS is known to bind a wide variety of metals including Pb, Sr, Zn, Cd, Co, Cu, Mn, Mg, Fe, Ag, and Ni (Decho, 1990, and references therein). In addition, cation binding sites can also catalyze the precipitation of initially disordered mineral phases (e.g., Ferris, 1997).

While the property of metal binding and mineralization can promote microbial productivity by providing ready access to high concentrations of micronutrients required for growth, the continued precipitation of minerals can eventually lead to the fossilization and demise of cells. The



**FIG. 2.** A carbonate globule in specimen 198 from ALH84001 (an external chip of the meteorite known to contain terrestrial bacteria) demonstrates the utility of combining a high-resolution FEG-SEM investigation with a ToF-SIMS analysis (Steele *et al.*, 2000). See text for details. **A:** Spectrum in the 400–600 AMU range that contains predominant peaks at 441, 455, 469, 533, and 547 AMU. **B:** FEG-SEM image of the carbonate globule analyzed. **C:** Backscatter SEM image of the same globule. The arrows point to the “waist” of what appears to be two intergrown globules. **D:** ToF-SIMS map of the distribution of peaks between 533 to 561 AMU. Boxes 1 and 2 in B and D represent the areas where the 533 AMU peaks are concentrated. **E:** Remains of bacterial cells surrounded by an organic film; these features were only seen in areas 1 and 2 of B and D. The scale bar in B–D equals 10  $\mu\text{m}$ . The scale bar in E equals 1  $\mu\text{m}$ .

concentration of these metals above background levels presents an interesting possibility for the detection of organisms even after organic materials have been degraded (Farmer, 1999; Conrad and Neelson, 2001). The primary difficulty at present is to identify techniques capable of accurately mapping variations in trace element concentrations relative to suspected microfossil remains in ancient or extraterrestrial materials. The mapping of low-abundance trace metals using standard electron and ion microprobe analy-

sis is difficult and presently subject to many uncertainties because of beam damage and poor counting statistics. However, recent adaptations of analytical HRTEM and synchrotron x-ray tomography (as discussed above) may ultimately provide a useful approach. Used in conjunction with other techniques, trace element mapping could provide an additional criterion for demonstrating the biogenicity in ancient terrestrial and extraterrestrial materials, and we recommend further study of this topic.



## LIMITS OF TECHNOLOGY: A CAUTIONARY NOTE

The inability to detect morphological biosignatures and prove their biogenicity may not be hindered by the lack of technological advances or inadequacies in our knowledge of the processes responsible for their preservation. On the contrary, extraterrestrial objects may lack conclusive evidence of being formed biologically. It is strongly recommended that in addition to funding projects aimed toward optimizing instrumentation to detect morphological biosignatures, that funding be allocated to projects that seek to expand our knowledge base regarding the processes by which objects that mimic morphological biosignatures could form in extinct and past "microbial ecosystems" on Mars.

## SUMMARY OF RECOMMENDATIONS

Our review of the various lines of research regarding morphological biosignatures leads us to recommend the following:

1. Exploit existing high-resolution imaging and spectroscopic techniques for detecting morphological biosignatures, and develop and optimize instruments capable of acquiring multiple biosignatures from the same morphological object in order to demonstrate the object was once a microbial cell or a microbially influenced sedimentary structure.
  - Improve the imaging capacity and mass resolution for the ToF-SIMS and identify the mechanisms that produce ToF-SIMS spectral signatures.
  - Develop and optimize analytical HRTEM, ion microprobe, Raman spectroscopy, synchrotron x-ray tomography, and related techniques.
2. Exploit instruments capable of detecting features that distinguish morphological biosignatures from their abiotic mimics to avoid identifying false-positives for life.
  - Expand the use of analytical HRTEM to study microfossils, pseudofossils, and biogenic and abiogenic sedimentary structures.
  - Develop analytical methods that will allow non-destructive, integrated morphological, chemical, and mineralogical analysis (including trace element anomalies) at the nanometer scale.
3. Pursue research strategies that improve our understanding of the processes by which morphological biosignatures form and become altered to maximize the ability to assess the biogenicity and indigeneity of all types of morphological fossils.
  - Develop growth process models for stromatolites and microbialites that include surface roughness of microbial mats or biofilms, particularly where sedimentation also occurs.
  - Determine the fate of biofilms in modern ecosystems, the fossilization potential of these structures, and how these factors relate to established criteria for demonstrating biogenicity.
  - Identify criteria that can be used for demonstrating the biogenicity of *bona fide* microfossils at the electron microscopy scale.
  - Determine the nature of phenotypic changes in response to environmental perturbations and whether those changes alter the susceptibility of a microorganism to fossilization.
  - Develop the use of techniques that can be used in modern ecosystems and in laboratory experiments to document geomicrobiological processes and fluid geochemistry at the cellular scale.
  - Identify the extrinsic geochemical characteristics that affect preservation in environments recognized as analogs for extant and past Mars.
  - Conduct systematic studies regarding the effects of mineral diagenesis (recrystallization and phase transformations) on taphonomic losses of biosignatures.
  - Integrate data related to biosignature formation, alteration, and destruction across a wide range of spatial scales.
  - Determine what effects the selected sample return sterilization protocol may have on the fidelity of morphological biosignatures.
4. Pursue research strategies (such as those in Recommendation 3) that quantify the fundamental processes that govern the formation of pseudofossils, abiotic stromatolites, and non-biologically influenced sedimentary structures and fabrics that mimic biologically influenced types.

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## ABBREVIATIONS

DLA, diffusion-limited aggregation; EPS, extracellular polymeric substances; FEG-SEM, field emission gun scanning electron microscopy; HRTEM, high-resolution transmission electron microscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TES, Thermal Emission Spectrometer; ToF-SIMS, time of flight secondary ion mass spectrometry.

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