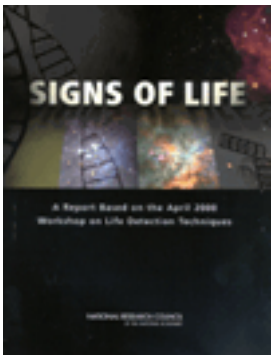


Signs of Life: A Report Based on the April 2000 Workshop on Life Detection Techniques

Committee on the Origins and Evolution of Life, National Research Council

ISBN: 0-309-50944-0, 68 pages, 8.5 x 11, (2002)

Signs of Life

A Report Based on the April 2000 Workshop on Life Detection Techniques

Committee on the Origins and Evolution of Life

Space Studies Board
Division on Engineering and Physical Sciences

Board on Life Sciences
Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS • 500 Fifth Street, N.W. • Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

Support for this project was provided by Contract NASW 96013 between the National Academy of Sciences and the National Aeronautics and Space Administration. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for this project.

International Standard Book Number 0-309-08306-0

Cover design by Penny Margolskee

Copies of this report are available free of charge from:

Space Studies Board
National Research Council
2101 Constitution Avenue, NW
Washington, DC 20418

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Bruce M. Alberts is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Wm. A. Wulf is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Bruce M. Alberts and Dr. Wm. A. Wulf are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

COMMITTEE ON THE ORIGINS AND EVOLUTION OF LIFE

JOHN BAROSS, University of Washington, *Co-chair*
JONATHAN I. LUNINE, University of Arizona, *Co-chair*
LUANN BECKER, University of California, Santa Barbara
SHERWOOD CHANG,* SETI Institute
DAVID W. DEAMER, University of California, Santa Cruz
MARILYN L. FOGEL, Carnegie Institution of Washington
NORMAN R. PACE, University of Colorado
DAVID A. STAHL, University of Washington

Staff

JOAN ESNAYRA, Study Director (Board on Life Sciences)
DAVID H. SMITH, Study Director (Space Studies Board)
CARMELA CHAMBERLAIN, Senior Program Assistant
KIRSTEN ARMSTRONG, Research Associate
BRIAN DEWHURST, Research Associate
ACHEL BHATT, Research Assistant
CRAIG CORNELIUS, Research Assistant

*Term ended in 2000.

Preface

At the close of the 20th century the direct exploration of our cosmic neighborhood had left unanswered one of humanity's oldest questions: Does the universe play host to life on multiple worlds, or is Earth unique in this regard? Both scientific and popular interest in this question have peaked in the last 5 years in response to the discovery of planets beyond our solar system and the controversy over possible signs of biological activity in a meteorite that likely was once a piece of the crust of Mars. NASA has responded to this interest through a partial realignment of its science program in the form of a new interdisciplinary effort called astrobiology. Among astrobiology's goals are the understanding of life's origin(s) and the detection of life, extant or extinct, beyond Earth. To search for life requires that samples of extraterrestrial material be acquired and then analyzed either in situ on a planetary body or in Earth-based laboratories. To fully address the question of earthly life's origin may require going beyond the terrestrial laboratory, where time and sample volumes are limited, to organic-rich environments elsewhere in the solar system where steps toward biology may also have occurred.

Attempts to detect life in material from beyond planet Earth extend back some four decades, including the study of meteorites and the in situ analysis of martian soil by the Viking landers. Although broad physical arguments have been made in support of the notion that life is a natural outcome of the process of cosmic evolution, earthly life remains the singular example. In recent years, the discovery of life in extreme terrestrial environments—namely, ecosystems at extremes of temperature, salinity, and acidity—has bolstered the expectation that extraterrestrial material may hold evidence of past or present life. It has also strengthened the concept, supported by computer calculations and the existence on Earth of meteorites bearing trapped gases of martian atmospheric composition, that life might hitch a ride on impact debris and hence travel in viable form from Mars to Earth or vice versa. This in turn imposes the additional challenge of determining whether life discovered in an extraterrestrial sample had a common or separate origin from that of Earth.

The detection of non-earthly life is a difficult and unsolved problem. Most techniques for detecting contamination of supposedly sterile surfaces are based on the properties of known organisms and on the remarkable uniformity of terrestrial biochemical processes. Yet even small variations in structure, metabolism, or information-encoding mechanisms could yield organisms capable of eluding such techniques. A combination of techniques is therefore required to maximize the chances of finding life, yet in situ packages must be severely limited in mass, power, size, and complexity given the present realities of planetary exploration. The first such package to be deployed on another planet, the Viking Mars landers, produced ambiguous results that failed to establish the presence of life, identified unusual oxidative soil chemistry not anticipated during the experiment design, and left

a lingering controversy over some of the results. Detection schemes in ground-based laboratories are not limited by weight, power, or size and can be heavily reliant on human intervention; yet even such systems may yield ambiguous results. The experience with the ALH84001 meteorite, where the claim of evidence for biological processes remains controversial and unresolved, is an important lesson in the fundamental complexity of identifying the faint traces of extant or extinct life. Perhaps even more difficult, if life or its remains is detected in a sample, will be the determination of whether it is a terrestrial contaminant from Earth, and if so, whether it was delivered by the spacecraft or in the natural process of cross-contamination via asteroidal or cometary impact.

Returned samples could pose a biohazard to life on Earth. The need for elaborate containment facilities, the prospects of sample destruction or alteration prior to release for general study, or even the deferral of sample return until extensive in situ analysis is complete will complicate the use of laboratory facilities to search for life. Conversely, the assurance that spacecraft launched from Earth will not contaminate astrobiologically interesting targets with viable organisms requires that sensitive techniques to assess the efficacy of sterilization processes be applied in the final stages of payload preparation prior to launch.

Given the active interest in the astrobiological exploration of Mars, Europa, Titan, and other targets, it is timely to assess the state of life detection techniques. The Space Studies Board (SSB) charged the Committee on the Origins and Evolution of Life (COEL)—which reports to both the SSB and the Board on Life Sciences—to organize a scientific workshop to explore advances in biology, biotechnology, medicine, and the environmental sciences likely to lead to new approaches to detecting life or its remnants. In response to the charge, the committee held a workshop at the Carnegie Institution's headquarters in Washington, D.C., on April 25-26, 2000. The workshop's goal was to address the following questions:

1. How does one determine if living organisms are on a spacecraft before launch?
2. How does one determine if there are living organisms in a returned sample?
3. How does one determine if living organisms have been present at some earlier epoch and have left fossil remnants behind in a returned sample?
4. How does one determine whether there are living organisms or fossils in samples examined robotically on another solar system body?

COEL was responsible for structuring the workshop, inviting speakers, summarizing the workshop in the form of this report, and drafting appropriate findings based on the presentations and discussions at the workshop and the committee's subsequent deliberations. Organization of the workshop followed from a series of discussions on topics related to life detection held by COEL at the National Academies' Beckman Center on January 27-29, 2000. The deliberations at this organizational meeting resulted in a guest list containing a balanced cross section of the community of scientists interested in life detection. The workshop was designed to review promising detection techniques that are currently available and areas for future research.

To facilitate a logical flow of papers and discussion, the workshop was organized in a fashion somewhat different from the list of questions given above. The workshop program is provided in Appendix B. The workshop opened with an introduction to the history of the search for life and the question of the generality of terrestrial biochemistry. The next session enumerated current understanding of solar system targets for sample return, including meteorites and interplanetary dust particles for which samples are available at present. The final two sessions dealt with techniques for detecting viable (including spore-forming) organisms and fossil remnants of life, respectively. Panel discussions followed the talks in the sessions on detection of extant and extinct life. After the workshop, COEL met on April 27 and again on August 21-23, 2000, to discuss the presentations, deliberate, and formulate its findings.

What is presented in this volume, a report based on a workshop, is but a preliminary step in the effort to develop a sound set of scientific strategies for the detection of life and its remains in samples on or from planetary bodies. To more fully capture the material presented at the workshop, speakers were asked to provide contributed papers derived from their talks. These papers are listed in Appendix C and are reproduced on the CD-ROM that contains the full report. No attempt was made to extensively rewrite or standardize the papers, although

grammatical and stylistic errors were corrected to improve clarity. The papers stand on their own as a detailed record of the workshop presentations.

The main body of the report was written by COEL and was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. The committee thanks the following individuals for their review of this report: Carl Agee (Johnson Space Center), Robert A. Frosch (Harvard University), David Galas (Keck Graduate Institute of Applied Life Sciences), Joseph L. Kirshvink (California Institute of Technology), and Eugene H. Levy (Rice University). Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Mary J. Osborn, University of Connecticut Health Center. Appointed by the National Research Council, she was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

[1]The workshop papers are reproduced on the CD-ROM that contains the full report but are not included in the printed report owing to space limitations.

This book is dedicated to Dr. Gerald A. Soffen (1926-2000),
who led the very first expedition to search for life on Mars and
who fathered the interdisciplinary field of astrobiology.

Executive Summary

INTRODUCTION

A workshop to assess the science and technology of life detection techniques was organized by the Committee on the Origins and Evolution of Life (COEL) of the Board on Life Sciences (BLS) and the Space Studies Board (SSB). Topics discussed in the workshop included the search for extraterrestrial life in situ and in the laboratory, extant life and the signature of extinct life, and determination of the point of origin (terrestrial or not) of detected organisms. Areas not covered or covered only to the extent of their connection to the main topics included mechanisms of terrestrial contamination of other planetary bodies by spacecraft (although techniques to detect such contamination in spacecraft were covered), sterilization of spacecraft components, and quarantine of returned samples. These topics, especially the last, have been considered extensively in recent National Research Council (NRC) reports.

The workshop was designed around a series of four general questions, to be addressed in the papers presented by the participants:

1. How does one determine if living terrestrial organisms are on a spacecraft before launch?
2. How does one determine if there are living organisms in a returned sample?
3. How does one determine if living organisms have been present at some earlier epoch and have left fossil remnants behind in a returned sample?
4. How does one determine whether there are living organisms or fossils in samples examined robotically on another solar system body?

The nature of questions 2 through 4, as formulated, is such that a single, declarative answer to each is not possible given the great uncertainties that remain in our understanding of the possible range of chemistry and morphology that could constitute life. Question 1 can be answered more definitively because of our direct study of terrestrial organisms, but there remains intense debate over the level to which spacecraft sterilization should be achieved for missions to particular solar system bodies. For these reasons, the questions served primarily to frame the scope of the discussions that took place at the workshop and of the contributed papers.

The workshop opened with an introduction to the history and scope of the search for life to date. The next session enumerated current understanding of solar system targets for sample return (including meteorites and

interplanetary dust particles for which samples are available at present). The final two sessions dealt with techniques for detecting viable (including spore-forming) organisms and the signs of past life, respectively. Appendix B of this report gives the workshop agenda. Appendix C lists (and the enclosed CD-ROM contains) the set of papers written by the invited speakers at the workshop. The report itself presents introductory and concluding material written by COEL to relate the papers to the questions to be addressed. To facilitate discussion of the papers and workshop sessions, the introductory material is organized in parallel with the workshop sessions rather than the four questions listed above. The conclusions and recommendations (Chapter 5), however, are grouped in parallel with the questions themselves. The committee emphasizes that this is a workshop report, rather than a detailed strategy study, and so drawing very specific conclusions and recommendations is not appropriate.

CONCLUSIONS AND RECOMMENDATIONS

Detecting Organisms on a Spacecraft Prior to Launch: Preventing Forward Contamination

The most strikingly definitive result coming from the workshop concerns the dramatic improvement in laboratory techniques designed to detect terrestrial organisms, with principal application to spacecraft sterilization and hence forward planetary protection. As new techniques become available, they have to be incorporated into planetary protection protocols because current NASA protocols (based on culturing techniques) could miss up to 99 percent of microorganisms. Also, some techniques may not be suited to distinguishing between viable and nonviable organisms, a key issue in considering forward and back contamination.

Recommendations regarding specific sterilization techniques and levels of sterilization in order to avoid contamination of other planetary bodies were beyond the original purview of the workshop and this report. The main issue regarding sterilization from the point of view of the workshop is the ability to sample, poststerilization, the remaining level of terrestrial microorganisms to ensure that it is below the value required for a particular mission. Because all terrestrial organisms rely on the same basic biochemistry—specifically and most importantly, the RNA and DNA nucleic acid bases—amplification techniques to detect very small remnant levels of contamination are well understood.

The committee recommends that studies of future missions to astrobiologically interesting targets include explicit consideration of the types of sterilization for spacecraft systems, subsystems, and components and that sterilization costs be included in a realistic fashion. The committee recommends that special near-term emphasis be given to the issues of sample selection, spacecraft sample handling, and sample characterization. The committee also encourages further work to refine sterilization approaches to minimize impacts on mission costs and success.

Detection of Living Organisms in a Returned Sample

The committee is strongly encouraged by the multidisciplinary efforts to define the possible range of processes indicative of living organisms. Given the extreme difficulty (or impossibility) of inductively describing all possible living processes based on terrestrial biochemistry, no single approach, or even combination of approaches, will guarantee success with a given sample. Multiple approaches, both chemical (including isotopic and molecular) and microscopic, are key to the successful detection of life in a sample. Because of the rapid improvements in the technology for a variety of techniques, coupled with the realization that return of a sample from Mars (the highest-priority target in life detection) remains a decade away, it would be premature to recommend a particular technique or set of techniques at present.

The committee concludes that a number of very sensitive and specific techniques are available for detecting living or once-living organisms in a returned sample; however, these techniques depend on the organisms' being composed of essentially terrestrial biopolymers. While other techniques exist for detecting a potentially broader suite of nonterrestrial-like (but carbon-based) organisms, their results will not be as definitive. Hence, multiple approaches will be required to establish the presence of life in a definitive fashion, unless such life happens to be essentially terrestrial in nature. There is a pressing need to develop

methods for the detection in single cells of evidence of metabolic activity and of specific macromolecules, including an analysis of their chemical structure and isotopic signature.

The committee recommends that a focused study be done in the near future to address the detection of microorganisms with varying degrees of nonterrestrial biochemistry, and the possible threat that such organisms might pose to terrestrial organisms.

To the extent possible, reasonable efforts (defined through carefully deliberated scientific strategies) should be made to assess the potential for extant life on other planetary surfaces in situ, using robotic missions. Some of the approaches available for the detection of living organisms are available in miniaturized form and are potentially space qualifiable for an in situ life detection mission. The results will markedly increase confidence about the risk factors associated with a given sample that could be returned to Earth for further study and will provide scientific evidence to further justify the expense of a return mission. Since life (or past life) will concentrate in habitats that provide suitable nutrients and chemical or physical conditions, its distribution on any planetary body will be patchy and of varying local abundance. **Appropriate site selection for sample return is critical and will determine the amount of sample required for testing and the need for possible sample concentration.**

Multiple measurements with different techniques will be required to perform triage on a set of field samples at a given landing site, so as to select the most promising samples for in situ or returned life detection. This recommendation holds as well for selection of sites for in situ life detection (extant or extinct), as noted below.

Determination of the Past Presence of Living Organisms in a Sample

The committee concludes that the search strategy for evidence of extinct life must include the identification of suitable landing sites, the selection of the appropriate rock types, and multiple analytical techniques that, in the aggregate, are capable of distinguishing between abiogenic and biogenic signatures. The assessment of extinct biosignatures will likely require a sample-return mission to carry out the sophisticated set of measurements needed to make this determination.

The most vigorous debate at the workshop centered on interpretation of potential signatures of life in samples available today in the laboratory and, in particular, in the SNC meteorite ALH84001, which is generally accepted to have originally been a part of the martian crust. Important disagreements exist within the community over the interpretation of properties of this meteorite in terms of their biological significance, and at least some of the disagreements are the result of a lack of repeat analysis of a particular sample or phase by multiple groups. **The committee recommends that any plans for analysis of returned extraterrestrial samples include a provision for repeat analyses of a subset of the same material, preferably by different teams. The committee encourages early development and testing of appropriate protocols using existing samples of high astrobiological interest (e.g., ALH84001).**

The committee recommends that attention be given to understanding thoroughly the rates and nature of degradation of biosignatures in planetary environments. Theoretical and experimental studies should be supplemented with comparative analysis of putative samples of extraterrestrial biomarkers (e.g., ALH84001), with a specific eye to better understanding the issue of degradation of signatures of past life. Additionally, the identification and development of new and possibly universal biosignature approaches should be an active area of study.

In contrast with the detection of extraterrestrial life, analysis of extraterrestrial organic material is a mature field with a number of important results based on direct analysis of meteorites (including SNCs, which are likely martian) and cosmic dust. Remote sensing analysis of organic molecules in various bodies in the outer solar system and in molecular clouds has provided a foundation for understanding the distribution and abiotic evolution of carbon-bearing material. The absence of detectable organic molecules at the surface of Mars played an important role in the interpretation of the Viking life detection experiments. Detection of simple organic molecules (e.g., methane) has been accomplished for the atmospheres of very cool brown dwarf stars and will play a key role in the protocol for the eventual remote spectroscopic assessment of the habitability of extrasolar planets. Continued

increases in sensitivity and in the diagnostic value of techniques to detect organic molecules in extraterrestrial samples, particularly in situ, will be an important part of the overall effort to assess the existence of past or present life in the solar system. **The committee concludes that it is crucial to continue the development of techniques to detect and analyze in situ organic chemical systems of either biotic or abiotic origin, with the goal of increasing the techniques' sensitivity and diagnostic capability.**

Detection of Life, Extant or Extinct, Examined Robotically on Another Solar System Body

In situ life detection will require commitment to a small subset of available techniques because spacecraft resources will always be constrained, at least for the foreseeable future. Hence, a specific set of hypotheses regarding the samples to be analyzed must be made prior to launch; this intrinsically decreases the likelihood of successfully detecting life, because such hypotheses are invariably based on Earth-centric assumptions. On the other hand, in situ analysis is not subject to the concern of back contamination of Earth; hence sample handling is, in that respect, greatly simplified. Whether and to what extent attempts to detect life in situ will be made prior to return of a sample to Earth is an unresolved issue in NASA's Mars Exploration Program. Potential confusion of the results by terrestrial contaminants is a particular concern for in situ studies, because of the limited number and types of tests that can be done. Accurate knowledge of the prelaunch level of terrestrial contamination and a method of tagging terrestrial organisms would maximize the chances of an interpretable result.

Because of the continuing rapid improvements in technology, it is not appropriate at this time to recommend a specific set of techniques for in situ life detection, but in situ life detection will require commitment to a small set of potential techniques with significant lead time to ensure that they can be space qualified. The committee encourages continued efforts to develop innovative and miniaturizable techniques for in situ life detection. It must be stressed that selecting the combination of techniques for in situ life detection is dependent as well on the physical and chemical characteristics of the sampling site on a particular planetary body.

Appropriate site selection is crucial to maximizing the chances of finding evidence for extant or extinct life in samples either analyzed in situ or collected for return to Earth. While this point seems obvious, the committee notes that over the history of Mars exploration the engineering constraints associated with safe operations usually have conflicted with reaching the most scientifically interesting sites. **For Mars, this means that landing site selection cannot be based primarily on issues of spacecraft safety. Furthermore, proper site selection will require a series of missions including orbital reconnaissance followed by exploration of selected sites by landed vehicles.** An informed and continuing dialogue between scientists engaged in life detection and mission planners is essential if astrobiologically interesting samples from Mars are to be obtained.

1

Detection of Life

INTRODUCTION

In 1976, NASA's two Viking landers made the first attempts to search for life on the martian surface (see Session 1 paper by Soffen). Since then, we have seen the development of new spectroscopy and microscopy techniques for detecting extremely low levels of organic compounds and determining the isotopic signature and chirality of organic molecules. Most of these technologies are not ready for miniaturization and spacecraft delivery but could be applied to samples returned to Earth. Other new technologies including nanotechnology and microsensors are still in the development stage. One of the most dramatic changes since Viking has been the growth in understanding of the nature of life and the concomitant power of analytical tools in the biological sciences. New molecular techniques have helped to identify a huge diversity of new microorganisms not previously detected by culture methods, and to give new insights into the evolutionary history of microorganisms and their importance in the evolution of eukaryotes and biocomplexity. It is clear that a better understanding of the origin of life and the evolution of nucleic acids and proteins will greatly aid in developing strategies and methods to detect biosignatures on other solar system bodies.

WHAT IS LIFE?

The detection of extraterrestrial life first requires an answer to the question, What is life? We make the assumption that if life exists on other planets or moons, it will be carbon based and dependent on liquid water. It will also be self-replicating and capable of evolving (see Session 1 paper by Pace). Carbon is the best element for creating macromolecules; it can form chemical bonds with many other atoms to produce biochemical complexity. This complexity consists of thousands of catalytic and structural proteins and nucleic acids, the informational macromolecules involved in protein synthesis. All life on Earth evolved from a single type of cell, referred to as the last common ancestor, and thus shares the same genetic code and central biochemistry. Consequently, all terrestrial life can be compared via phylogenetic trees based on small-subunit ribosomal RNA sequences. These trees also indicate the importance of lateral gene transfer or intermixing of genomes as mechanisms for creating evolutionary diversity. Organisms that do not fit into the tree of life as currently understood (e.g., being sufficiently different so as to constitute a fourth domain of the phylogenetic tree) almost certainly would be extraterrestrial. However, this does not necessarily imply a separate origin: Such life might have a common origin with Earth life

and subsequently have been transported to Mars by impacts (or vice versa). A converse difficulty, of course, is that extraterrestrial life could be so different from life on Earth that modern methods would fail to detect it.

EARLY STAGES IN THE EVOLUTION OF LIFE

The papers by both Benner and Deamer (see Session 1) underscore the need to better understand the origin of life and the early evolution of biomolecules in order to prepare for the detection of extraterrestrial life. Earth life today is the product of 4 billion years of evolution. An essential property of cellular life is that it uses linear polymers such as nucleic acids and proteins for, respectively, information storage or transfer and catalytic or structural functions. Little is known about the origin of life or the early stages of evolution that resulted in genetic complexity. One hypothesis is that prior to the appearance of life based on nucleic acids and proteins as the fundamental polymers, a simpler form of life may have consisted of a single fundamental biopolymer resembling RNA. This polymer would have the dual functions of catalytic activity and information storage. Whether single- or dual-polymer life would be more common beyond Earth is an open issue (see the paper by Benner).

The early stages of the origin of life presumably included the self-assembly of organic compounds into more complex structures, perhaps encapsulated molecular systems capable of catalyzed polymer synthesis. In the laboratory, lipids and other compounds can assemble into membrane-bound vesicles that are able to encapsulate proteins and nucleic acids. These systems are in a sense models of primitive or "proto-" cells, but at present they lack the capability to host metabolism. As Deamer argues in his paper, such systems incorporate many of the processes defining life and are worthy of continued study to determine just how closely model systems could be made to simulate living cells.

GENERAL CONSIDERATIONS REGARDING THE DETECTION OF EXTRATERRESTRIAL LIFE

Detection of extant carbon-based life can be attempted at the level of simple organic molecules or at the level of more complex macromolecular biopolymers. Highly sensitive methods for the detection of simple biochemical compounds produced by metabolic processes are nonspecific and hence require few assumptions about the nature of the fundamental biopolymers of life. However, as Pace notes, the interpretation of the detection of simple organic compounds is ambiguous, since carbonaceous meteorites contain amino acids and other compounds that might mistakenly be considered indicative of life. Further testing of such molecules to look for properties such as chirality could help resolve the ambiguity, at least for extant life. Signs of extinct life, which degrade with time (e.g., racemization of the chiral amino acids in the case of the above example), present their own difficulties, which are discussed further in Chapter 3. In his paper, Benner sketches a case study of just such a problem—namely, trying to distinguish organics associated with hypothetical martian life against a background of abiotic organics delivered by meteorites.

Given that life on Earth has at its core polymers that replicate and provide structure and function, a more specific approach to the detection of life is to look for linear ionic polymers. Both Pace and Benner argue that such polymers would be a nearly unambiguous signature of extant life, since there are no known examples of the abiotic production of linear ionic polymers with the complexity of DNA or RNA and proteins. The problem is that techniques that aim to amplify small amounts of genetic material require some a priori knowledge of the nucleic acid sequences. Thus, as Pace cautions in his paper (see Session 1), molecular probes based on terrestrial gene sequences may not detect extraterrestrial life unless it is very closely related to life on Earth. Molecular probes such as the polymerase chain reaction do, however, provide exquisitely sensitive tests for the presence of terrestrial organisms and hence are useful in testing the level of sterilization of spacecraft prior to launch.

An alternative is to try to detect single biopolymers. In his paper, Benner sketches an approach based on the property that such polymers should have regularly spaced positive or negative charges. Single macromolecules, such as nucleic acids and proteins, can be detected by various so-called nanotechnologies under development. One of these—nanopore detection—is highlighted by Deamer and described in detail in the paper by Meller and Branton (see Session 3). Because such technologies are in their infancy, their utility in the search for extraterrestrial

life can be gauged only after extensive development. However, nanotechnologies constitute a very active area of research, and more than one novel approach to single molecule detection is under development.[1]

SEARCHING FOR EVIDENCE OF LIFE BEYOND THE SOLAR SYSTEM

While most of the efforts to search for extraterrestrial life are currently focused on either sample return or in situ experiments on Mars, Europa, and Titan (see Chapter 2), there is growing interest in the possibility of detecting habitable planets around nearby stars. One of the strategies for detecting habitable planets is to obtain low- to moderate-resolution thermal-infrared spectra of their atmospheres (see the Session 1 paper by Kasting).

A spectroscopic examination of Earth's atmosphere would reveal the presence of CO_2, H_2O, and O_3 (ozone), the last as a proxy for the spectroscopically inactive O_2. The analyses would also show that atmospheric O_2 is orders of magnitude out of thermodynamic equilibrium with reduced gases such as CH_4 and N_2O. Extreme disequilibrium in these gases may signify the presence of living organisms and therefore could be useful for detecting life on extrasolar planets.

The presence of ozone in the atmosphere of an extrasolar planet is a particularly interesting bioindicator since it could signify the presence of O_2 at concentrations indicative of photosynthesis. However, it is conceivable that there are planets inhabited only by anaerobic microorganisms or that O_2 produced by photosynthesis is titrated in situ by reduced metals such as iron. The geologic record tells us that Earth's atmosphere had a very low O_2 content during the first 2 billion years. Thus, for almost half of its history, Earth would have appeared lifeless by the "ozone criterion," even though it supported life. Furthermore, a planet in the process of rapidly losing its water by atmospheric escape, as Venus might have early in its history, would show a strong signature of ozone even though life might not be present.

Methane could also be a bioindicator, particularly if found in high concentrations. Anaerobic microorganisms produce most of the methane on Earth. These methanogens are strict anaerobes and are believed to be evolutionarily ancient. There are caveats for using methane as a bioindicator. It is produced by abiotic sources as well, and very little is known about the compositions and chemistry of early Earth-type anoxic atmospheres.

Another technique that could be used to detect evidence of extraterrestrial life outside the solar system is radio astronomy. With this method, many organic molecules have already been detected and their abundances determined in interstellar and circumstellar gas. The bases of identification are the unique rotational spectra of these chemical compounds. With the continuing improvement in detector sensitivity, more complicated species, including isotopic and isomeric variances, may be detected in the interstellar medium that could be bioindicators.

Deeper understanding of the evolution of the planets in our own solar system, particularly Mars and Venus, will provide some ground truth on the possible evolutionary paths that planets may take away from habitability, and the consequent spectroscopic signatures.

REFERENCE

1. H. Craighead, "Separation and Analysis of DNA in Nanofluidic Systems," AAAS Annual Meeting, San Francisco, 2001.

2

Sample Return

INTRODUCTION

This chapter, along with the corresponding workshop session, deals with the bodies in our solar system that are thought to be attractive targets for life detection and outlines current or planned missions to sample or reconnoiter those bodies. It is here also that the question of public concerns about sample return, and National Aeronautics and Space Administration (NASA) responses to those concerns, are addressed. The principal public concern is whether samples that are returned from another planet might harbor unknown organisms that could pose a threat to life on Earth. Most scientists would agree that the risks are exceedingly small, yet no one can truthfully say that there is no risk. There are also concerns, ethical and scientific, associated with the possible introduction of terrestrial life to other habitable planetary objects. The introduction of terrestrial microorganisms could compromise life detection and possibly disrupt a native biology or prebiology, and is thus a fundamental consideration for mission design. Any sample return mission must therefore explicitly address both forward and back contamination, but all missions to targets that potentially harbor life must deal with the forward contamination issue. Unfortunately in the case of Mars, spacecraft of varying degrees of sterility have already impacted or landed on the surface. Europa's surface has not yet been reached, but the question of contamination already is affecting the design of the next mission to that object. The technology of spacecraft bioburden assessment is addressed in the contribution by Nealson (see Session 2) as well as in the technique papers presented in Session 3.

METEORITES AND COMETARY DEBRIS

It is useful to begin this section with a perspective on actual and perceived risk. Although we normally do not think of it as "sample return," meteoritic infall has continuously delivered vast amounts of extraterrestrial material to Earth's surface throughout the 3.5 billion years of biological history, with estimates in the range of tens of tons per day (see the Session 2 paper by Brownlee). Typical extraterrestrial infall ranges in size from microscopic dust particles to ordinary meteorites and can be considered to represent a random sample return from asteroids and comets. On a time scale of centuries, much larger objects reach Earth, such as the Tunguska bolide that exploded in the atmosphere over Siberia in 1908. On a time scale of millions of years, kilometer-sized objects such as the one that formed the Chicxulub crater 65 million years ago (the physical effects of that impact altered the course of terrestrial evolution) have struck Earth. Despite this steady rain of material ranging from dust to asteroid-sized

objects over Earth's history, there is no evidence that organisms that might have been contained within such objects have engendered extinctions.

Might comets and asteroids be carriers of extraterrestrial life? It is highly unlikely that life could have begun and been sustained in the interior environment of a comet, where liquid water exists only for transient periods when the comet is at or near perihelion. Likewise, with the exception of a very early period in the solar system's history when liquid water might have existed on the parent bodies of today's asteroids and comets, the environments of meteorites and asteroids are also likely to be sterile because of a lack of liquid water. The strong association between life and the presence of liquid water is based on the known nature of cells and the absence of living organisms in places where liquid water (including interstitial liquid layers) is absent. For example, even after 4 billion years of evolution, no community adapted to grow in that environment has been found that can live in the relatively mild cold of the antarctic high desert regions in the absence of liquid water. These considerations can be used to argue that sample return of dust particles, comets, and asteroids poses a relatively low risk, similar to the known negligible risk of lunar sample returns.

As described by Brownlee, the Stardust spacecraft will, in 2004, collect dust from the coma of comet Wild-2 as the spacecraft passes just 150 km from the comet's nucleus. Together with previous collections of dust along the spacecraft's interplanetary trajectory, the sample container will be parachuted into the Utah desert in 2006. Acquisition of this material will represent the first collection of extraterrestrial samples by spacecraft since the U.S. and Soviet lunar missions of the 1960s and 1970s. In addition to the arguments presented above, the material collected by Stardust is thought to carry very negligible risk of contamination, since it will be collected by impact with an aerogel material and hence will be heated to sterilizing temperatures of 10^4 °C. The aerogel collection material melts around the dust, forming a protective glass layer.[1]

From a scientific point of view, the material collected by Stardust will be of very high scientific value given its documentable origin in a cometary coma. Comparison of the composition of this material with that of interplanetary dust particles (IDPs) that rain down naturally into our atmosphere will deepen our understanding of the origin of IDPs. The laboratory analysis of cometary material will place in context decades of remote sensing observations as well as the in situ analyses of the coma of comet Halley. Given that comets are leftover planetesimals from orbits at and beyond Jupiter, analysis of their dusty debris is of astrobiological interest in tracing the source regions of the organics from which life on Earth began some 4 billion years ago.

MARS

Sample return from Mars requires more serious consideration in regard to both contamination issues and appropriate site selection in the search for life (see the Session 2 paper by Nealson). Mars is a central focus of solar system exploration, because of the increasing evidence that liquid water was present and stable early in the planet's history. The discovery of relatively recent surface features that could be due to liquid water makes Mars even more interesting. Although the recent outflows would be extremely important sites for sampling purposes, they are on steep slopes and, for this reason, will not be initial targets due to limitations of existing Mars rover technology. More likely sites are in relatively flat terrain near what may be ancient seas that could have deposited sedimentary layers. Based on what we know of microbial populations on early Earth, such sediments might contain microfossils and other biosignatures of bacteria that either originated on or were delivered to Mars by impacts. There is even some prospect of extant microbial life on Mars in deep deposits of liquid water produced by geothermal activity beneath the surface. For this reason, of all sample return missions, those to Mars have the highest risk of back contamination, and until the problems are better understood, space agencies should plan on highly secure procedures to limit potential risks. However, such concerns should be tempered by the fact that more than a dozen SNC meteorites are actual samples of the martian crust delivered to Earth over millions of years as a result of crater-forming impacts on Mars. The best known of these—ALH84001—has been carefully studied by numerous investigators. There is no evidence that it contains viable life forms, irrespective of whether it bears evidence of extinct life (for the latter topic, see the papers in Session 3 by Kirschvink and in Session 4 by D. McKay).

EUROPA

Europa, one of the four Galilean satellites of Jupiter, is approximately the size of Earth's Moon and is tidally heated by Jupiter (though to a lesser extent than the innermost Galilean moon, Io). Images of Europa's surface taken by NASA's Galileo spacecraft from 1995 through 2000 have revealed extraordinary vistas of a planet-spanning network of cracks in an otherwise smooth surface. Other evidence, most notably the Galileo magnetometer's mapping of an induced magnetic signature, is indicative of subsurface liquid water. A probable explanation for the cracks is that they represent breaks in a global ice layer overlying a liquid water ocean. The solid crust could be tens of kilometers or tens of meters thick, and no tighter constraint is available from the Galileo spacecraft. Such a europan ocean would be the only known example of globally extensive liquid water other than the oceans of Earth. It will require the Europa Orbiter mission, as currently planned by NASA, to demonstrate the existence of an ocean and identify promising sites for sampling oceanic material.

Given liquid water and a source of biogenic compounds, it is conceivable that microbial life could originate and flourish in deep europan seas (see the Session 2 paper by Chyba). Hydrothermal systems may also exist on Europa, similar to those on Earth that today support abundant and diverse communities of macro- and microbiota. The risk levels of sample returns from Europa are relatively high, and such samples will have to undergo some form of quarantine until stringent testing shows that no living organisms are present. The committee notes that there is a risk of forward contamination of Europa in the seeding of the subsurface global ocean with terrestrial microorganisms, a factor that is already an important consideration in mission design of the Europa Orbiter. The optimal orbit for that mission is polar, which allows complete imaging and radar coverage as well as geodetic mapping that will determine the time-varying shape of the moon and hence sense the presence of a subsurface ocean. However, because of the complex gravitational influences of both Europa and Jupiter on the spacecraft, such an orbit would lead to impact of the orbiter on the surface, whereas lower inclination orbits are more stable. Likewise, the desire to orbit very close to Europa must be measured against the consequences for the stability of the orbit. Extensive analysis of these and other risks connected with Europa exploration has been presented in a previous report of the Space Studies Board.[2]

TITAN

Titan, the largest of Saturn's moons, is an extremely cold environment, with a surface temperature of 95 K (about –200 °C). It seems highly improbable that life could have originated under these conditions or that any living organism could survive. Instead, the aim of in situ studies or a sample return mission from Titan is to learn more about the remarkable organic chemistry that occurs in this moon's upper atmosphere and on its surface (see the Session 2 paper by C. McKay).

Atmospheric photochemical reactions lead to the synthesis of complex organic compounds called tholins that form the orange haze visible in spacecraft images of Titan. Tholins are solid polymers composed of the biogenic elements—carbon, hydrogen, oxygen, and nitrogen—and may provide clues to the kinds of organic synthetic reactions that produced complex organics during the origin of life on Earth. Tholins are themselves just one product of the photochemistry ongoing in the stratosphere of Titan. Much of the methane and nitrogen chemistry terminates with the condensation of lighter hydrocarbons and nitriles. The condensed aerosols, including tholins, fall to the surface. Some of these—ethane and other saturated hydrocarbons—would exist as liquids at the surface temperature of 95 K. Other photochemical products would be stable as solids, so that the surface of Titan may be a melange of liquid and solid hydrocarbons resting on a primarily water ice crust. Remote-sensing data taken from Earth suggest a complex and variegated surface.

The Cassini-Huygens mission will arrive at Saturn and deploy a probe into Titan's atmosphere in 2004. It will conduct a comprehensive survey, based on which decisions can be made as to what kinds of future missions (if any) should be mounted to Titan (e.g., sample return versus in situ analyses). Laboratory simulations are of demonstrated utility to the study of organic synthetic mechanisms and hence to replicating much of what is made in the atmosphere. The next step beyond Cassini-Huygens would likely be in situ analyses of the poorly understood surface. Sample return ought to be contingent on whether there are any products of organic chemistry

present in the atmosphere or on the surface of Titan that cannot be simulated readily in the laboratory or analyzed satisfactorily in situ. For the high-altitude organic haze, the answer appears to be no, but this conclusion might not hold for organic phases that have undergone longer-term chemistry on the surface. The risk associated with sample returns from Titan should be small, since organic compounds found on the surface exist under cryogenic conditions (95 K), and organics in the upper atmosphere are processed by free-radical chemistry that breaks down biopolymers. In any event, the extreme distance of Titan from Earth will limit organic analysis on the latter to in situ experiments for the foreseeable future.

PLANETARY PROTECTION

There are practical and societal reasons for ensuring planetary protection for all interplanetary missions (see the Session 2 paper by Rummel). Although the probability that an extraterrestrial life form could be pathogenic to humans, or even viable at all in the terrestrial environment, is very low, it cannot be shown to be zero. During the past few years it has become well established that microorganisms inhabit environments thought at one time to be too extreme to harbor life. Some of these environments are associated with geothermal or hydrothermal systems and thus exhibit high temperatures, high concentrations of heavy metals and volatiles, and acidic pHs. Other inhabited environments include desert rocks and deep subsurface basaltic aquifers. These findings have expanded the number of extreme environments on Earth and other solar system bodies that might harbor organisms.

One of the recommendations of a previous National Research Council (NRC) report for handling Mars samples returned to Earth is that they should be contained and treated as though potentially hazardous until proven otherwise.[3] This recommendation poses problems in both sample containment and the kinds of analyses to be used for life detection. What criteria will be used to declare whether a returned sample is hazardous or not? A recent NRC report attempts to define these criteria and outlines necessary containment procedures.[4]

Important issues related to forward contamination include compromising the search for life and the possibility that Earth life could grow elsewhere, a possibility of particular concern for missions to Europa.[5] The essential issue in forward contamination is ensuring that the landing vehicle is free of contamination by Earth microorganisms. For back contamination, the important issues are identifying biosignatures for extraterrestrial life and developing methods for detecting these biosignatures at low levels (see Chapter 3).

REFERENCES

1. Space Studies Board, National Research Council, *Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies,* National Academy Press, Washington, D.C., 1998.

2. Space Studies Board, National Research Council, *Preventing the Forward Contamination of Europa*, National Academy Press, Washington, D.C., 2000.

3. Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997.

4. Space Studies Board, National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002.

5. Space Studies Board, National Research Council, *Preventing the Forward Contamination of Europa*, National Academy Press, Washington, D.C., 2000.

3

Detecting Extant Life

INTRODUCTION

After the Viking and Apollo missions of a quarter-century ago, biologists largely turned away from developing research programs designed specifically to look for life on another planetary body. Only in the last 6 years, with more insight from reconnaissance missions and the controversy over the martian meteorite ALH84001, has interest in extraterrestrial life detection—and sterilization of spacecraft—been renewed. This rising interest comes, in a sense, on the heels of a revolution in the field of microbiology, extraordinary progress in understanding the geobiological history of Earth, and detection of life in extreme environments. In the past decade alone, more than 1,500 new species of microorganisms have been discovered and genetically sequenced. In the next decade, it can be expected that phylogenetic trees will be redrawn and restructured and that genomics—the listing of an organism's entire genetic code—will become economical for all molecular microbial geologists to undertake. What remains to complete the picture regarding terrestrial life is to understand what microorganisms are doing, where and when they are doing it, and how these organisms and their metabolisms have evolved within Earth's environment over geologic time.

The detection of extremely low levels of microorganisms after spacecraft sterilization involves increasing refinement of laboratory techniques designed to detect known types of terrestrial organisms. It also requires research into the possibilities and consequences of failure to detect unknown or poorly known microorganisms that exist within Earth's environment. The detection of extant life on samples returned from another planet, or analyzed in situ, is a much less well-defined venture. It requires a set of assumptions about the fundamental nature of life that might exist on another planet. It also requires selection of techniques that will work well in difficult quarantine laboratory environments or in miniaturized and automated form on an extraterrestrial body.

This chapter summarizes the workshop session focused on in situ life detection. Whereas previous chapters and corresponding workshop sessions address more general considerations regarding what life might be like beyond Earth and what targets are most promising, this chapter and the associated workshop papers are technique based. The committee attempted, within the confines of a reasonable length to the workshop, to cover a broad spectrum of types of techniques and to characterize each technique in adequate depth to indicate its capabilities and limitations. For those reasons, the papers are not comprehensive in terms of addressing all possible techniques. The techniques selected include those that are very specific and powerful in their detection capability but are hard to generalize beyond terrestrial organisms because of their specificity (e.g., polymerase chain reaction

[PCR]). They also include more general approaches that could be useful in characterizing a broad range of life forms very different from those of Earth, but for which the interpretation could be ambiguous (e.g., time-of-flight mass spectroscopy). In this sense some of the papers could arguably have been included in the session on extinct life (e.g., see the Session 3 paper by Kirschvink on magnetic biomineralization), and indeed a corresponding paper on iron biomarkers is given in Session 4 (see the paper by Anbar). These papers have been split between the two different sessions, along with the papers on microbe-mineral interactions, to emphasize the utility of such techniques in the search for either extant or extinct life. The distinction between extant and extinct life detection becomes arbitrary for the most general techniques that rely on mineral biomarkers or other morphological characterizations.

Most of the participants who presented techniques suitable for assessing the degree of sterilization also emphasized their application to extraterrestrial life detection. In large measure, this reflected the emphasis of the workshop on the search for life elsewhere. To correct this imbalance the committee prepared Table 5.2 (see Chapter 5), which lists techniques discussed at the workshop and gives their sensitivity (typically, when applied in terrestrial laboratories rather than extraterrestrial environments), limitations, and some indication of development needed. Finally, the committee's narratives in this chapter and in Chapter 4 do not attempt a detailed discussion of each technique presented in the sessions. In particular, it was not the purview of the committee to rank in some way the applicability of the techniques. The workshop discussions and the session papers represent a snapshot of the kinds of techniques and their capabilities available at a time when interest in finding life beyond Earth has reached a new crescendo.

BIOMOLECULES AND MOLECULAR TECHNIQUES

New technologies originating in the biomedical community will be available for use in detecting extant life on other planets. Testing these methods on Earth, where many biological communities can be studied with sophisticated laboratory setups, is crucial. The ambiguities associated with metabolic tests for extant life, demonstrated in the prolonged (some would say continuing) controversy over the Viking lander results, demand a broader suite of sensitive detection approaches. The evolution of molecular techniques is toward much higher specificity, in the sense that one can look at all the major components of cellular structures. At issue, of course, is how to generalize from the characteristics of these structures to those that might differ from the terrestrial. Imaging techniques, although less reliant on specific biochemical assumptions, are more subject to misinterpretation.

To apply molecular techniques to the search for life elsewhere requires deciding how geocentric one must be. Must one look for a different set of biochemical molecules, perhaps based on compounds that are structured differently from those of terrestrial life? The Session 3 papers by Stahl; Meller and Branton; and Ruvkun, Finney, Gilbert, and Church present approaches that will work for life forms that use the basic biopolymers found in all terrestrial life—specifically, RNA and DNA. Stahl focuses on the use of molecular methods coupled with microscopic techniques, which provides a powerful combination of sensitivity and generality. The paper by Meller and Branton and that by Ruvkun, Finney, Gilbert, and Church offer two techniques capable of single-molecule detection that are miniaturizable for in situ deployment: nanopore technology and robotic PCR detection. An alternative imaging approach, described in the Session 3 paper by Jacobsen, allows one to image hydrated organic samples in an organism, thus potentially obtaining the structure of the ribosomes in the organism, and simultaneous structural and compositional data.

Highly sensitive techniques for measuring a variety of organic molecules, such as proteins, complex lipids, and carbohydrates, are active areas of investigation. Although there are simple monomeric organic molecules that occur both in terrestrial biology and in abiotic organic phases of meteorites, the macromolecular nature of biologically produced versus abiotically produced molecules should be diagnostic. Cotter describes (see Session 3) improvements in time-of-flight (TOF) mass spectrometry, for which significant technological advances have been made. TOF mass spectrometers can now be miniaturized to allow in situ analysis of biomolecules on an extraterrestrial surface. The versatility and sensitivity of this technique are high. Protein sequencing has been demonstrated in terrestrial laboratories using this technique.

More general biomolecular approaches to the search for extant extraterrestrial life involve looking for biomolecules or biological structures that do not exist on Earth. In his Session 1 paper, Benner argues the case for looking for biological systems that do not rely on our dual-biopolymer world of DNA and RNA as information carriers and proteins as structural or catalytic molecules. However, it is unclear how amplification of non-DNA or RNA information-carrying molecules could be accomplished or, alternatively, whether single-molecule techniques (e.g., involving nanopores or other nanotechnologies) can be sensitive to and diagnostic of a broad range of extraterrestrial biomolecules. As discussed in Chapter 1, analysis of smaller organic molecules (e.g., amino acids) can be broader but also far more subject to ambiguity in the interpretation of a biological or abiotic origin. Time-of-flight mass spectroscopy will be useful in analyzing whatever molecules are encountered in the mass range of sensitivity, but again interpretation in terms of a biological origin remains problematic.

OTHER TECHNIQUES DISCUSSED DURING THE WORKSHOP

An even broader approach eschews looking for biomolecules and instead looks for mineralogical traces of organisms through geochemical structures that have been altered by biological processes. Electron microscopy at the organism-mineral interface would be pursued on returned samples or, after an aggressive technology development, on candidate rocks at the landing site. More terrestrial data are required to fully understand how living organisms affect the geochemistry and morphology of mineralogic substrates.

As they explain in their Session 3 paper, Barker and Banfield have characterized the microbe-mineral environment, and potential mineralogical biosignatures, in lithobiontic microbial communities. From these observations, they developed a model intended to be predictive of mineralogical and textural properties that could be indicative of biological processes. This work not only provides an important context for examining signatures of organisms in extraterrestrial samples, but also leads to specific protocols for sample preparation that avoid altering or destroying the evidence. These considerations for morphological detection of extant organisms should also be compared with the approaches to morphological characterization of extinct organisms discussed in Session 4 (see the papers by Cady, Moldowan, and D. McKay).

Some terrestrial organisms produce their own minerals with uniquely biological properties. For example, the crystal structure and other structural or chemical aspects of biologically produced magnetite crystals can be diagnostic, as described in the paper by Kirschvink. He argues that a detailed and systematic comparative analysis of biogenic magnetite from terrestrial bacteria leads to the conclusion that the magnetite crystals in the SNC meteorite ALH84001, generally accepted to have come from Mars, are the signatures of martian biology. The conclusion itself is clearly controversial and led to a very vigorous workshop discussion. Kirschvink's conclusions highlighted an important point coming out of the workshop debate—the need for multiple techniques and multiple groups to analyze precisely the same portions of and phases in retrieved samples.

Stable isotopic measurements are yet another approach to discerning the presence of life (or previous life) in a sample. Stable isotopic measurements of organic and inorganic compounds have been widely used on Earth for selecting critical biological reactions in complex ecosystems or determining when in Earth's history important biological mechanisms evolved. Fogel argues (see her paper in Session 3) that protein-chip technology provides a new, potentially miniaturizable approach to the isotopic detection of candidate biomaterials.

SUMMARY OF METHODS FOR DETECTING EXTANT LIFE

Given the presentations at the workshop and the committee's subsequent deliberations, there appear to be a variety of techniques currently available for the detection of viable microorganisms and, if organisms are present, for determining their biomass, growth rates, and metabolic and enzymatic activities. Most of these methods have been applied to both liquid and solid samples and thus could be useful in detecting viable cells from spacecraft and martian samples. Some of the imaging systems, including flow cytometry, measure total numbers of cells in a liquid medium. While the molecular and some of the biochemical methods are designed for detection of terrestrial organisms, other methods, including microscopy coupled with the use of macromolecule-specific fluorescent dyes, uptake of radio-labeled organic and inorganic compounds, microcalorimetry, and stable isotope analyses,

should be useful in detecting viable life from extraterrestrial environments that might have different biochemical characteristics. The most effective approach for detecting extant life is to use multiple techniques that can give some indication of growth and activity of specific taxonomic or physiological groups of microorganisms.

Measuring Biomass

The biomass, an index of the number of cells in a sample, is measured either directly by using imaging technologies or cultivation to quantitatively enumerate the cells in a sample or indirectly by using a biochemical proxy for the number of cells. The best biochemical methods can be used to approximate the number of cells in a sample and are very useful in detecting low numbers of "free-living" cells or cells attached to solid substrates. The current limitation for enumerating microorganisms in sediments by epifluorescence microscopy is approximately 100,000 cells per cubic centimeter.[1] Lower numbers of cells can be detected using fatty acids as a proxy for biomass. In some cases it is advantageous to dislodge microbes from particles, sediment grains, and rocks in order to obtain quantitative biomass results. Microbes are dislodged from solid material by grinding the samples with a mortar and pestle and/or by using detergents and mild sonication.[2,3] Other methods are available for isolating a single cell from a sample. These involve the use of micromanipulators and lasers to direct single viable cells into capillary tubes for subsequent culturing in defined media or for single-cell PCR analyses.[4,5] So far, three methods have been applied to liquid samples, and there is no method reported for the removal of a single cell that may be attached to a solid substrate. **There is a pressing need to develop methods for the detection in single cells of evidence of metabolic activity and of specific macromolecules, including an analysis of their chemical structure and isotopic signature.**

Direct Measurements of Biomass

The following methods involve direct imaging of intact cells or their cultivation on specific media. The direct techniques include observations of general morphological characteristics of intact cells, identification and quantification of phylogenetic groups of microorganisms, and specific physiological biomarkers. Fluorescent dyes—specific for different macromolecules such as proteins, lipids, and nucleic acids—are routinely used to identify organisms in natural samples using epifluorescence microscopy. These techniques include the following:

- *Light microscopy*. This technique is generally limited to samples with more than than 10^5 organisms per milliliter or gram of sample. It is generally used in conjunction with various stains (Gram stain, lipid and protein stains, and so on) or to observe motility of viable cells.[6] (Technique not discussed during the workshop.)
- *Epifluorescence microscopy*. Acridine orange, 4,6-diamidino-2-phenylindole (DAPI), and other dyes can be used for enumerating organisms with DNA, RNA, and protein. This technique can determine the presence of microorganisms on surfaces of solid substrates, in soil, on rocks, and in biofilms. It also allows for the concentration of low levels of microorganisms from liquid samples by filtration. It can be used to enumerate bacteriophages and other viruses (from 30- to more than 50-nm diameter).[7–9] (Technique not discussed during the workshop.)
- *Autofluorescence with epifluorescence microscopy*. This technique can be used for enumerating organisms with autofluorescing compounds such as flavins (methanogens) and chlorophyll.[10,11] (Technique not discussed during the workshop.)
- *Image-analyzed fluorescence microscopy*. This technique gives the numbers, size, and distribution of microorganisms.[12] (Technique not discussed during the workshop.)
- *Electron microscopy*. Scanning electron microscopy (SEM) with electron diffraction (EDX) can be used to identify microorganisms on surfaces, along with elemental analyses of organisms and the surrounding environment. Environmental SEM allows observation of samples without the use of fixatives. Transmission electron microscopy (TEM) is most important when using ultrathin sections of microorganisms because internal membranes, cell walls, and ribosomes can be readily observed. These techniques can be combined

with methods that remove microbes from solid materials such as soils and rocks, where they can be concentrated, fixed, and prepared for thin sections.[13] (Technique not discussed during the workshop.)

- *Immunofluorescence (FA).* This involves specific antibodies for specific physiological groups of bacteria such as nitrifiers, methanogens, and methylotrophs, or specific protein antigens such as RuBPCase and nitrate reductase. It can also be adapted for the general detection of proteins or cellular membranes from any organism.[14–16] (See workshop presentation by Fogel in Session 3.)
- *Coulter counter.* Use of this device has limited application with natural populations of bacteria, although new laser models might be used to measure organisms that fluoresce or that can be stained to fluoresce. It is useful for determining growth rates of pure cultures of bacteria and phytoplankton and for measuring protozoan grazing rates of bacteria. It requires large volumes of water.[17] (Technique not discussed during the workshop.)
- *RNA or DNA probes.* These are used to identify specific species of bacteria or genes using epifluorescence microscopy (fluorescent in situ hybridization; FISH), dot blots, and quantitative PCR. General 16S rRNA fluorescent probes of unique sequences can differentiate eubacteria, eukaryotes, and archaea. Additional probes are also available for detecting specific taxonomic groups of microbes. The FISH technique is dependent on cells having sufficient amounts of rRNA and hence a full complement of ribosomes.[18] (See workshop presentation by Stahl in Session 3.)
- *Quantitative PCR.* This technique quantifies microbial communities at cluster to species levels; it could be developed into a useful procedure to enumerate specific groups of organisms that are not amenable to FISH because of low amounts of rRNA.[19,20] (Technique not discussed during the workshop.)
- *Analytical flow cytometry.* This is a technique for rapidly characterizing, quantifying, and sorting particles based on simultaneous, multiple measurements of cellular light scatter and fluorescence. Recent experiments couple flow cytometry with fluorescence and fluorescent antibodies or fluorescently labeled DNA or RNA probes. Large volumes of water are required.[21–23] (Technique not discussed during the workshop.)
- *Plate counts and "most probable number" (MPN).* These methods are based on determining the number of viable bacteria capable of growing on specific media. They are useful for isolating specific physiological groups of bacteria or for estimating the numbers of coliforms and other bacteria important to public health. Most environmental microorganisms have not been cultured, so culturing methods have limited application in characterizing the diversity and biomass of microbial communities from most environments.[24,25] (Technique not discussed during the workshop.)
- *Laser-scanning confocal microscopy (LSCM).* This approach enables two- and three-dimensional images of environmental samples to be obtained without fixation.[26,27] (Technique not discussed during the workshop.)

Indirect Measurements of Biomass

All organisms have specific biochemical characteristics that serve as biosignatures. Some of these characteristics can also be used to estimate the number of cells present in a sample. Lipids are the most versatile of these biosignatures in that they can be used to estimate the number of cells in a sample, provide taxonomic information, and indicate the physiological condition of the microbial community (starved, stressed, or dormant). All of the indirect measurements can be used to detect very low levels of cells and have the added feature that these compounds can be extracted and concentrated from solid samples. These techniques include the following:

- *Adenosine triphosphate (ATP).* This is a useful indicator of biomass, although the ATP content of cells can vary depending on their metabolic state. The procedures are easy and do not require expensive equipment. Actively growing cells have a total carbon to carbon in ATP ratio of 1:273.[28,29] (See the paper by Soffen in Session 1.)
- *DNA and RNA.* These have limited applications as biomass indicators and depend very much on the size and metabolic state of cells. Moreover, the RNA content of cells can vary greatly in different species. The genome size of different phylogenetic groups of microorganisms can vary by a factor of 3. Quantitative

procedures are tedious, particularly if the sample contains low levels of microorganisms.[30,31] (Technique not discussed during the workshop.)

- *Muramic acid.* This specific biosignature for bacteria is best applied when measuring the biomass of attached bacteria or bacteria associated with animal guts and other environments having a complex multikingdom flora. Quantitative correlation between muramic acid and bacterial biomass depends on the absence of significant numbers of Gram-positive bacteria and a good determination of the average size (volume) of the bacteria in the population. The quantitative procedures for muramic acid assay are tedious.[32,33] (Technique not discussed during the workshop.)
- *Lipopolysaccharide.* The Limulus assay is specific for Gram-negative bacteria and very sensitive (detects <10 cells per milliliter). The method has wide applications in medical microbiology, particularly for detecting bacteria in urinary tract infection. Too much contaminating lipopolysaccharide in aquatic environments prevents this method from being practical.[34] (Technique not discussed during the workshop.)
- *Pigments.* Chlorophyll, phycocyanin, and other pigments are generally measured using fluorescent imaging systems.[35] (Technique not discussed during the workshop.)
- *Lipids.* Ester- and ether-linked fatty acids are used for differentiating eubacteria from archaebacteria. Also, unusual lipids, such as long-chain polyunsaturated fatty acids, esoteric branched chains, and so forth, can be diagnostic for specific groups of bacteria and their metabolic state. Some investigators have made the assumption that lipids represent some fixed percentage of cell carbon (usually 1 percent of total cell carbon) so as to use lipids as biomass indicators. A fatty acid database is available for many bacteria, and fatty acid profiles are used to identify specific groups of bacteria, particularly those having public health significance.[36,37] (Technique not discussed during the workshop.)
- *Other biochemical parameters.* Esters, specific enzymes, and combinations of compounds can also be used as indirect indicators of biomass. (Technique not discussed during the workshop.)

Determining Growth Rates

The ultimate test of the viability of cells is their ability to grow and divide. All of the methods described below can be applied to natural populations of microorganisms. The direct methods rely on microscopic observations of dividing cells and measurements of increasing numbers of cells in growth chambers or on glass slides in situ. Radio-labeled substrates are used to make indirect measurements of growth rates based on the rate of DNA, RNA, and protein synthesis. These methods are very sensitive and measure growth rates in environmental samples containing low number of cells. Recently, the combination of molecular methods with microautoradiography has proven useful in estimating the growth rates of specific taxonomic groups of microorganisms. At present, there are no methods for measuring the growth rates of microbial communities or specific taxonomic groups of microorganisms in situ.

Direct Measurements of Growth Rates

The direct methods for estimating microbial growth rates are not particularly quantitative but can provide some information about the viability of a community and its potential for growth. Laboratory culture methods rarely provide the conditions necessary for growth of all the different taxonomic groups of microorganisms within a community. These techniques include the following:

- *Frequency of dividing cells.* This involves correlation of the number of dividing cells in a natural population of bacteria with growth rate, diffusion plates with synchronous cultures, time experiments that prevent separation of dividing cells, and so forth. Evidence of cell division (chains of cells, fruiting bodies, budding cells) is a first-order observation for the presence of viable cells in a sample.[38,39] (Technique not discussed during the workshop.)
- *Microautoradiography.* This technique is used mostly to differentiate active from nonactive cells but has

been used to estimate the rate of division of bacteria in situ, and the activity of specific physiological groups.[40,41] (Technique not discussed during the workshop.)

- *Laboratory culture methods.* These include chemostats, growth chambers, growth on filters and glass slides, and the formation of microcolonies on solid substrates. These methods have been useful in obtaining growth rates for microbes and microbial communities from oligotrophic environments. Other culture methods, such as the ability to grow in liquid nutrient and solid nutrient media, tissue culture, or animal hosts, are best suited for microorganisms that have complex nutrient requirements or are animal pathogens. Culturing methods including inoculation of samples into suitable hosts should be applied to martian samples for detection of viable microorganisms including animal pathogens.[42–44] (See the paper by Soffen in Session 1.)

Indirect Measurements of Growth Rates

Most of these methods are widely used for measuring growth rates of heterotrophic and chemoautotrophic organisms, and organisms in aquatic environments. Some of these methods have been adapted for estimating microbial growth rates in sediments, biofilms, and detrital particles. The use of tritiated organic substrates allows for the measurement of uptake rates by low numbers of microorganisms ($<10^4$ cells per milliliter) or with microbial communities having slow growth rates (less than one doubling per day). These techniques include the following:

- *[^{3}H]-Thymidine and/or[^{3}H]-adenine uptake.* This method is used to determine the rates of DNA and RNA synthesis and to correlate them with growth rates. It is used primarily for measuring growth rates of heterotrophic bacteria and is limited to microorganisms that are capable of assimilating thymidine or adenine. Thymidine uptake is very specific for heterotrophic bacteria, and most of the thymidine is incorporated into DNA. Adenine is assimilated by some phototrophic bacteria and eukaryotes; adenine is incorporated into both DNA and RNA. RNA content has been shown to vary considerably in bacteria from aquatic environments and thus is not useful for growth rate determinations. These methods can measure growth rates in samples with relatively low numbers of microorganisms (10^3 to 10^4 per milliliter).[45–48] (Technique not discussed during the workshop.)
- *[^{14}C]- and [^{3}H]-amino acid and [^{14}C]-CO_2 uptake.* This approach measures the rate of incorporation into macromolecules and calculates a micro rate of increase in proteins, nucleic acids, lipids, and so on. The rate of [^{3}H]-leucine incorporation into proteins as a proxy for growth rates has been shown to correlate well with growth rates determined by uptake of [^{3}H]-thymidine into DNA.[49,50] (Technique not discussed during the workshop.)
- *Radio-phosphorus uptake.* This approach measures [^{32}P]-phosphate incorporation into phospholipids and nucleic acids.[51] (Technique not discussed during the workshop.)
- *DNA measurements.* These involve quantitative extraction of DNA followed by measurement of DNA stained with DAPI (or related dyes) using a fluorometer. They are used in light and dark bottle in situ experiments to measure the growth rate of photosynthetic and chemoautotrophic organisms.[52] (Technique not discussed during the workshop.)

Measuring Metabolic and Enzymatic Activity

Many methods have been developed that estimate the rates of specific metabolic reactions in microbial communities from environmental samples or identify the metabolic potential of these communities. Since most of these methods require manipulation of the environmental sample, such as the addition of radio-labeled carbon or energy sources or substrates for specific enzymes, it is very important to re-create as many of the in situ conditions as possible during the incubation period. Other methods, including the use of microelectrodes and microcalorimetry, can be performed in situ. Some of the molecular methods currently available or in the developmental stages allow for determination of specific metabolic activities associated with specific taxonomic groups of

microorganisms along with identification of the specific genes being transcribed in situ by microbial communities. The combination of molecular methods and stable isotope analyses of membrane lipids has proven to be extremely useful for identifying the taxonomic groups of microorganisms involved in specific metabolic activities such as anaerobic methane oxidation in marine sediments.[53]

Metabolic Activity

The identification of specific metabolic activity in environmental samples is particularly useful in sediments and biofilms that have steep spatial gradients of specific metabolic groups of microorganisms or in environments that are dominated by chemoautotrophic microorganisms. These activity measurements can be divided into those that measure rates of activity and those that identify metabolic groups involved in tranformation of various inorganic nutrients (e.g., those involved in nitrogen, sulfur, and metal cycles). Microelectrodes and other sensors are used in situ to measure various chemical species, temperature, pH, and E_h in submicron gradients of sediments and biofilms and may have some utility in searching for evidence of active life on Mars. Relevant techniques include the following:

- *Heterotrophic potential*. ^{14}C- and ^{3}H-labeled organic compounds are used in different concentrations along with application of Michaelis-Menten kinetic analyses. Uptake by natural populations is assumed to follow first-order kinetics. This technique is particularly useful with sediment or soil samples in which the rate of $[^{14}C]$-CO_2 evolution can indicate respiration rates of microbial communities.[54–56] (Technique not discussed during the workshop.)
- *Radio-carbon uptake*. This technique measures $[^{14}C]$-CO_2 uptake by photosynthetic and chemoautotrophic bacteria.[57] (See the paper by Soffen in Session 1.)
- *Isotopes for measuring specific metabolic activities*. The uptake, oxidation, and reduction of a variety of ^{14}C- and ^{3}H-labeled carbon and hydrogen sources, isotopes of metals (^{54}Mn), phosphorus (^{32}P), and sulfur (^{35}S) are measured. These methods are particularly useful for measuring rates of methane production and consumption, CO production and consumption, hydrogen oxidation, and incorporation of phosphorus into nucleic acids and sulfur into S-amino acids.[58–62] (See the paper by Soffen in Session 1.)
- *^{35}S-labeled energy sources*. Uptake is used to measure the activity of sulfur-oxidizing and sulfur-reducing bacteria. This technique is very useful in submarine hydrothermal vent and salt marsh environments.[63,64] (Technique not discussed during the workshop.)
- *Respiratory activity*. The respiratory-activity method is based on the reduction of dyes, including cyanoditolyl tetrazolium chloride (CTC).[65–67] (Technique not discussed during the workshop.)
- *O_2, N_2, and sulfur uptake and consumption*. This involves in situ measurements with microelectrode probes and respirometer experiments. Microelectrodes can be used to detect micromolar levels of O_2, nitrogen, and sulfur compounds (NO_2^-, N_2O, H_2S) through micrometer E_h and pH gradients in sediments, biofilms, and rocks.[68] (Technique not discussed during the workshop.)
- *Microcalorimetry*. Since all biological processes are accompanied by heat production, it follows that all heat evolved during metabolism and growth is equal to the change in enthalpy. Enthalpy changes can be measured by microcalorimetry. This method has been used to detect evidence of active life in sediments and soils.[69,70] (Technique not discussed during the workshop.)
- *Electron transport system*. This approach measures the reduction of dyes by natural populations of bacteria.[71] (Technique not discussed during the workshop.)
- *Microbial fractionation of stable isotopes of nitrogen, sulfur, and carbon*. These analyses are particularly useful for nitrogen- and sulfur-cycle reactions and the production and consumption of methane. They can be used to follow carbon, nitrogen, and sulfur through food chains. It is assumed that extraterrestrial life would also fractionate these elements.[72–74] (Technique not discussed during the workshop.)

Enzymatic Activity

The activity of most enzymes can be measured in environmental samples if the sample contains sufficient levels of active enzymes. The methods that have been developed in microbial ecology focus on enzymes that indicate specific metabolic activity such as nitrogen cycle reactions and the rate of degradation of macromolecular organic compounds (e.g., proteins and carbohydrates) requiring enzymatic hydrolysis into soluble compounds that can be transported into cells. The new methods that utilize soluble fluorogenic compounds as a proxy for macromolecules are very sensitive and can detect low levels of extracellular hydrolases in environmental samples. These techniques include the following:

- *Nitrogen-cycle reactions.* These can be used in a number of different ways, including (1) acetylene reduction as a measure of nitrogen fixation, (2) acetylene block method for measuring denitrification, and (3) ^{15}N uptake as a measure of both dissimilatory and assimilatory reactions.[75,76] (Technique not discussed during the workshop.)
- *Calvin-Bensen cycle enzymes.* These indicate phototrophy and/or chemolithotrophy and can be coupled with fluorescent antibody methods.[77] (Technique not discussed during the workshop.)
- *Exoenzyme activity.* This is used frequently in sediments and biofilm samples as an indication of potential microbial heterotrophic activity. A number of new enzyme assays appear to be very specific and are currently being widely applied to studies of marine sediments. Probably the most important breakthrough is in the use of soluble fluorescent artificial substrates that allow measurement of rates of macromolecule degradation in situ (cellulose, lignin, chitin, protein, lipids, and organic phosphorus and sulfur compounds).[78–81] (Technique not discussed during the workshop.)

Molecular Methods

At the present time, molecular methods are being developed to determine some specific genes that are being transcribed in situ, and new methods are being developed for determining the genes being transcribed by microbial communities in situ. These techniques include the following:

- *mRNA analyses.* Specific mRNA synthesized is indicative of specific activity expressed (e.g., mRNA for DNA polymerase indicative of cell replication, nif-mRNA indicative of nitrogen fixation). (See the paper by Stahl in Session 3.)
- *Downstream sequencing.* Sequencing downstream from 16S rRNA genes is used to help infer some physiological and metabolic potential and to clone and express unknown genes in *Escherichia coli* or other hosts. (Technique not discussed during the workshop.)
- *Gene sequencing.* This technique is used to amplify specific functional genes from natural populations, sequence, and construct trees. It works well if there is a large enough sequence database to infer specific phylogenetic groups of organisms.[82] (Technique not discussed during the workshop.)
- *Environmental genomics.* This technique is under development and will elucidate the metabolic potential of organisms within an ecosystem and the genes being transcribed in situ. (See the paper by Stahl in Session 3.)

Metabolic State

The metabolic state can indicate whether in situ microbial communities or pure cultures of bacteria are actively growing, slowly growing, or not growing. Methods for assessing metabolic state can also indicate whether organisms are showing evidence of stress and other physiological states that are under genetic control and may or may not involve a dense population of cells (quorum sensing). These can be estimated by a variety of methods, including the following:

- *Energy charge*. The ratio of ATP to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) can be used to determine whether natural populations of microbes are active or dormant.[83,84] (Technique not discussed during the workshop.)
- *Ability of bacteria to divide*. This approach exploits the use of antibiotics such as nalidixic acid, which allows bacteria to continue to grow but not to divide.[85,86] (Technique not discussed during the workshop.)
- *Cyclic AMP and lactones*. These serve as an index of primary or secondary metabolism or an indication of quorum sensing.[87,88] (Technique not discussed during the workshop.)
- *Bacterial size*. Does small size indicate starvation or the presence of active oligotrophic microbial communities? The lower size limit for oligotrophic aquatic microorganisms is approximately 200 to 400 nm in diameter).[89] (Technique not discussed during the workshop.)

SUMMARY CONSIDERATIONS

A combination of sensitive techniques is required to provide sample selection, compositional and structural determination at the molecular level, and identification of biogenic structures on the microscopic (but supramolecular) scale. Mass spectroscopy is among the most robust and sensitive of molecular techniques and is likely to be on any life detection package. It has to be coupled to other molecular techniques to ensure that identification of specific compounds is possible from the sample mix. However, mass spectroscopy by itself is powerful for sample *selection*, in no small measure because it can be readily miniaturized. Imaging techniques, although potentially more powerful because of their generality, yield results that may not be definitive and could lead to prolonged debate regarding the biogenicity of particular structures. Reliance on the analogy to terrestrial organisms emphasizes the need to broaden imaging studies to the vastly larger ensemble of hitherto unstudied organisms.

REFERENCES

1. M. Summit, A. Peacock, D. Ringelberg, D.C. White, and J.A. Baross, "Phospholipid Fatty Acid-Derived Microbial Biomass and Community Dynamics in Hot, Hydrothermally Influenced Sediments from Middle Valley, Juan de Fuca Ridge," in R.A. Zierenberg, Y. Fouquet, D.J. Miller, and W.R. Normark (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results* 169:1-19, 2000.

2. M. Summit, A. Peacock, D. Ringelberg, D.C. White, and J.A. Baross, "Phospholipid Fatty Acid-Derived Microbial Biomass and Community Dynamics in Hot, Hydrothermally Influenced Sediments from Middle Valley, Juan de Fuca Ridge," in R.A. Zierenberg, Y. Fouquet, D.J. Miller, and W.R. Normark (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results* 169:1-19, 2000.

3. M.I. Velji and L.J. Albright, "Improved Sample Preparation for Enumeration of Aggregated Aquatic Substrate Bacteria," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 139-142.

4. J. Fröhlich and H. König, "New Techniques for Isolation of Single Prokaryotic Cells," *FEMS Microbiol. Rev*. 24:567-572, 2000.

5. R. Huber, H. Huber, and K.O. Stetter, "Towards the Ecology of Hyperthermophiles: Biotopes, New Isolation Strategies and Novel Metabolic Properties," *FEMS Microbiol. Rev*. 24:615-623, 2000.

6. R.G.E. Murray and C.F. Robinow, "Light Microscopy," in P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (eds.), *Methods for General and Molecular Bacteriology*, American Society for Microbiology Press, Washington, D.C., 1994, pp. 7-20.

7. B.C. Crump, C.A Simenstad, and J.A. Baross, "Particle-Attached Bacteria Dominate the Columbia River Estuary," *Aquat. Microb. Ecol.* 14:7-18, 1998.

8. J.E. Hobbie, R.J. Daley, and S. Jasper, "Use of Nucleopore Filters for Counting Bacteria by Fluorescence Microscopy," *Appl. Environ. Microbiol*. 33:1225-1228, 1977.

9. K.G. Porter and Y.S. Feig, "The Use of DAPI for Identifying and Counting Aquatic Microflora," *Limnol. Oceanogr*. 25:943-948, 1980.

10. H.J. Doddema and G.D. Vogels, "Improved Identification of Methanogenic Bacteria by Fluorescence Microscopy," *Appl. Environ. Microbiol*. 36:752-754, 1978.

11. E.A. MacIsaac and J.G. Stockner, "Enumeration of Phototrophic Picoplankton by Autofluorescence Microscopy," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 187-197.

12. R. Psenner, "Determination of Size and Morphology of Aquatic Bacteria by Automated Image Analysis," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 339-345.

13. T.J. Beveridge, T.J. Popkin, and R.M. Cole, "Electron Microscopy," in P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (eds.), *Methods for General and Molecular Bacteriology*, American Society for Microbiology Press, Washington, D.C., 1994, pp. 42-71.

14. L. Campbell, "Immunofluorescence Method for the Detection and Characterization of Marine Bacteria," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 295-300.

15. M.V. Orellana and M.J. Perry, "Optimization of an Immunofluorescent Assay of the Internal Enzyme Ribulose-1,5-Bisphosphate Carboxylase (Rubisco) in Single Phytoplankton Cells," *J. Phycol*. 31:785-794, 1995.

16. B.B. Ward, "Immunology in Biological Oceanography and Marine Ecology," *Oceanography* 3:30-35, 1990.

17. D.K. Button, F. Schut, P. Quang, R. Martin, and B.R. Robertson, "Viability and Isolation of Marine Bacteria by Dilution Culture: Theory, Procedures, and Initial Results," *Appl. Environ. Microbiol.* 59:881-891, 1993.

18. R. Amann and W. Ludwig, "Ribosomal RNA-Targeted Nucleic Acid Probes for Studies in Microbial Ecology," *FEMS Microbiol. Rev.* 24:555-565, 2000.

19. M.T. Suzuki, L.T. Taylor, and E.F. DeLong, "Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations Via 5′-Nuclease Assays," *Appl. Environ. Microbiol.* 66:4605-4614, 2000.

20. K. Takai and K. Horikoshi, "Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes," *Appl. Environ. Microbiol*. 66:5066-5072, 2000.

21. G. Boeck, "Current Status of Flow Cytometry in Cell and Molecular Biology," *International Review of Cytology—A Survey of Cell Biology* 204:239-298, 2001.

22. D.K. Button and B.R. Robertson, "Determination of DNA Content of Aquatic Bacteria by Flow Cytometry," *Appl. Environ. Microbiol*. 67:1636-1645, 2001.

23. A. Shalapyonok, R.J. Olson, and L.S. Shalapyonok, "Arabian Sea Phytoplankton During Southwest and Northwest Monsoons 1995: Composition, Size Structure and Biomass from Individual Cell Properties Measured by Flow Cytometry," *Deep-Sea Research Part II, Topical Studies in Oceanography* 48:1231-1261, 2001.

24. H. Davey and D. Kell, "Flow Cytometry and Cell Sorting of Heterogeneous Microbial Populations: The Importance of Single-Cell Analyses," *Microbiol. Rev*. 60:641-696, 1996.

25. P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (eds.), *Methods for General and Molecular Bacteriology,* American Society for Microbiology Press, Washington, D.C., 1994.

26. T.A. Norton, R.C. Thompson, J. Pope, C.J. Veltkamp, B. Banks, C.V. Howard, and S.J. Hawkins, "Using Confocal Laser Scanning Microscopy, Scanning Electron Microscopy and Phase Contrast Light Microscopy to Examine Marine Biofilms," *Aquat. Microb. Ecol*. 16:199-204, 1998.

27. T. Wilson, *Confocal Microscopy*, Academic Press, London, 1990.

28. D.M. Karl, "Cellular Nucleotide Measurements and Applications in Microbial Ecology," *Microbiol. Rev*. 44:739-796, 1980.

29. D.M. Karl, "Total Microbial Biomass Estimation Derived from the Measurement of Particulate Adenosine-5′-Triphosphate," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology,* Lewis Publishers, Boca Raton, Fla., 1993, pp. 359-368.

30. D.M. Karl, "Total Microbial Biomass Estimation Derived from the Measurement of Particulate Adenosine-5′-Triphosphate," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology,* Lewis Publishers, Boca Raton, Fla., 1993, pp. 359-368.

31. R.M. Atlas, "Extraction of DNA from Soils and Sediments," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 261-266.

32. J.D. King and D.C. White, "Muramic Acid as a Measure of Microbial Biomass in Estuarine and Marine Samples," *Appl. Environ. Microbiol*. 33:777-783, 1977.

33. D.J.W. Moriarty, "Bacterial Biomass and Productivity in Sediments, Stromatolites and Water of Hamelin Pool, Shark Bay, Wash.," *Geomicrobiol. J*. 3:121-133, 1983.

34. S.W. Watson and J.E. Hobbie, "Measurement of Bacterial Biomass as Lipopolysaccharide," in J.W. Costerton and R.R. Colwell (eds.), *Native Aquatic Bacteria: Enumeration, Activity and Ecology,* American Society for Testing and Materials, Baltimore, Md., 1979, pp. 82-88.

35. R. Iturriaga and S.L. Bower, "Microphotometric Analysis of the Spectral Absorption and Fluorescence of Individual Phytoplankton Cells and Detrital Matter," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 377-385.

36. F.C. Dobbs and R.H. Findlay, "Analysis of Microbial Lipids to Determine Biomass and Detect the Response of Sedimentary Microorganisms to Disturbance," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 347-358.

37. M. Summit, A. Peacock, D. Ringelberg, D.C. White, and J.A. Baross, "Phospholipid Fatty Acid-Derived Microbial Biomass and Community Dynamics in Hot, Hydrothermally Influenced Sediments from Middle Valley, Juan de Fuca Ridge," in R.A. Zierenberg, Y. Fouquet, D.J. Miller, and W.R. Normark (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results* 169:1-19, 2000.

38. M. Fabiano and R. Danovaro, "Enzymatic Activity, Bacterial Distribution, and Organic Matter Composition in Sediments of the Ross Sea (Antarctica)," *Appl. Environ. Microbiol*. 64:3838-3845, 1998.

39. N.D. Sherry and A.M. Wood, "Phycoerythrin-Containing Picocyanobacteria in the Arabian Sea in February 1995: diel patterns, spatial variability, and growth rates," *Deep-Sea Research Part II, Tropical Studies in Oceanography* 48:1263-1283, 2001.

40. N.D. Gray, R. Howarth, R.W. Pickup, J.G. Jones, and J.M. Head, "Use of Combined Microautoradiography and Fluorescence In Situ Hybridization to Determine Carbon Metabolism in Mixed Natural Communities of Uncultured Bacteria from the Genus *Achromatium*," *Appl. Environ. Microbiol*. 66:4518-4522, 2000.

41. C.C. Ouverney and J.A. Fuhrman, "Combined Microautoradiography-16S rRNA Probe Technique for Determination of Radioisotopic Uptake by Specific Cell Types In Situ," *Appl. Environ. Microbiol.* 65:1746-1752, 1999.

42. A. Bianchi and L. Giullano, "Enumeration of Viable Bacteria in the Marine Pelagic Environment," *Appl. Environ. Microbiol.* 62:174-177, 1996.

43. D.K. Button, F. Schut, P. Quang, R. Martin, and B.R. Robertson, "Viability and Isolation of Marine Bacteria by Dilution Culture: Theory, Procedures, and Initial Results," *Appl. Environ. Microbiol.* 59:881-891, 1993.

44. P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (eds.), *Methods for General and Molecular Bacteriology,* American Society for Microbiology Press, Washington, D.C., 1994.

45. R.T. Bell, "Estimating Production of Heterotrophic Bacterioplankton Via Incorporation of Tritiated Thymidine," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 495-512.

46. G. Chin-Lao, "Bacterial Secondary Productivity," in C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter (eds.), *Manual of Environmental Microbiology*, American Society for Microbiology Press, Washington, D.C., 1997, pp. 263-271.

47. B.C. Crump, C.A, Simenstad, and J.A. Baross, "Particle-Attached Bacteria Dominate the Columbia River Estuary," *Aquat. Microb. Ecol.* 14:7-18, 1998.

48. S.E. Findlay, "Thymidine Incorporation into DNA as an Estimate of Sediment Bacterial Production," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 505-508.

49. D.L. Kirchman, "Leucine Incorporation as a Measure of Biomass Production by Heterotrophic Bacteria," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 509-512.

50. P. LaRock and J.-H. Hyun, "Bacterial Growth Rates Measured by Pulse Labeling," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 537-546.

51. J.W. Ammerman, "Microbial Cycling of Inorganic and Organic Phosphorus in the Water Column," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 649-660.

52. D.K. Button and B.R. Robertson, "Determination of DNA Content of Aquatic Bacteria by Flow Cytometry," *Appl. Environ. Microbiol.* 67:1636-1645, 2001.

53. L.-U. Hinrichs, J.M. Hayes, S.P. Sylva, P.G. Brewer, and E.F. DeLong, "Methane-Consuming Archaebacteria in Marine Sediments," *Nature* 398:802-805, 1999.

54. J.W. Deming, "^{14}C-Tracer Method for Measuring Microbial Activity in Deep-Sea Sediments," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 405-414.

55. L.B. Jugnia, M. Richardot, D. Debroas, T. Sime-Ngando, and J. Dévaux, "Variations in the Number of Active Bacteria in the Euphotic Zone of a Recently Flooded Reservoir," *Aquat. Microb. Ecol.* 22:251-259, 2000.

56. J.D. King and D.C. White, "Muramic Acid as a Measure of Microbial Biomass in Estuarine and Marine Samples," *Appl. Environ. Microbiol.* 33:777-783, 1977.

57. G.R. DiTullio, "Incorporation of $^{14}CO_2$ into Protein as an Estimate of Phytoplankton N-Assimilation and Relative Growth Rate," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 573-578.

58. R.L. Cuhel, "Sulfate Assimilation by Aquatic Microorganisms," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 611-619.

59. M.A. DeAngelis, M.D. Lilley, E.J. Olson, and J.A. Baross, "Methane Oxidation in Deep-Sea Hydrothermal Plumes of the Endeavour Segment of the Juan de Fuca Ridge," *Deep-Sea Research* 40:1169-1186, 1993.

60. J.P. Cowen, G.J. Massoth, and E.T. Baker, "Bacterial Scavenging of Mn and Fe in a Mid to Far-Field Hydrothermal Particle Plume," *Nature* 322:169-171, 1986.

61. G.M. King, "Applications of ^{14}C and ^{3}H Radiotracers for Analysis of Benthic Organic Matter Transformation," in C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter (eds.), *Manual of Environmental Microbiology*, American Society for Microbiology Press, Washington, D.C., 1997, pp. 317-323.

62. K.H. Nealson and D. Saffarini, "Iron and Manganese in Anaerobic Respiration: Environmental Significance, Physiology, and Regulation," *Annu. Rev. Microbiol.* 48:311-343, 1994.

63. R.L. Cuhel, "Sulfate Assimilation by Aquatic Microorganisms," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 611-619.

64. B.B. Jørgensen, M.F. Isaksen, and H.W. Jannasch, "Bacterial Sulfate Reduction Above 100°C in Deep-Sea Hydrothermal Vent Sediments," *Science* 258:1756-1757, 1992.

65. T. Posch, J. Pernthaler, A. Alfreider, and R. Psenner, "Cell-Specific Respiratory Activity of Aquatic Bacteria Studied with the Tetrazolium Reduction Method, Cyto-Clear Slides, and Image Analysis," *Appl. Environ. Microbiol.* 63:867-873, 1997.

66. B.H. Pyle, S.C. Broadway, and G.A. McFeters, "Factors Affecting the Determination of Respiratory Activity on the Basis of Cyanoditolyl Tetrazolium Chloride Reduction with Membrane Filtration," *Appl. Environ. Microbiol.* 61:4304-4309, 1995.

67. B.F. Sherr, P. del Giorgia, and E.B. Sgerr, "Estimating Abundance and Single-Cell Characteristics of Actively Respiring Bacteria Via the Redox Dye CTC," *Aquat. Microb. Ecol.* 18:117-131, 1999.

68. N.P. Revsbeck and B.B. Jørgensen, "Microelectrodes: Their Use in Microbial Ecology," *Adv. Microbiol. Ecol.* 9:293-352, 1986.

69. G.P. Privalov and P.L. Privalov, "Problems and Properties in Microcalorimetry of Biological Macromolecules," *Energetics of Biological Macromolecules* 323:31-62, 2000.

70. L. Yu, Y.G. Hu, R.S. Lon, H.L. Zhang, Z.D. Nan, and F.H. Li, "The Effects of Environmental Conditions on the Growth of Petroleum Microbes by Microcalorimetry," *Thermochimica Acta* 359:95-101, 2000.

71. T.T. Packard, "Measurement of Electron Transport Activity of Microplankton," in H.W. Jannasch and P.J. LeB. Williams (eds.), *Advances in Aquatic Microbiology*, Vol. 3, Academic Press, New York, 1985, pp. 208-281.

72. M.L. Fogel and L.A. Cifuentes, "Isotope Fractionation During Primary Production," in S.A. Macko and M.H. Engel (eds.), *Organic Geochemistry, Principles and Applications*, Plenum Press, New York, 1993, pp. 73-100.

73. B.M. Jakosky, "Martian Stable Isotopes: Volatile Evolution, Climate Change and Exobiological Implications," *Origin of Life and Evolution of the Biosphere* 29:47-57, 1999.

74. P. Stapp, G.A. Polls, and F.S. Pinero, "Stable Isotopes Reveal Marine and El Niño Effects on Island Food Webs," *Nature* 401:467-469, 1999.

75. H. Bothe, G. Jost, M. Schloter, B.B. Ward, and K.-P. Witzel, "Molecular Analysis of Ammonia and Denitrification in Natural Environments," *FEMS Microbiol. Rev.* 24:673-690, 2000.

76. D.G. Capone, "The Marine Microbial Nitrogen Cycle," in D.L. Kirchman (ed.), *Microbial Ecology of the Oceans*, John Wiley & Sons, New York, 2000, pp. 455-493.

77. M.V. Orellana and M.J. Perry, "Optimization of an Immunofluorescent Assay of the Internal Enzyme Ribulose-1,5-Bisphosphate Carboxylase (Rubisco) in Single Phytoplankton Cells," *J. Phycol.* 31:785-794, 1995.

78. B.C. Crump and J.A. Baross, "Particle-Attached Bacteria and Heterotrophic Plankton Associated with the Columbia River Estuarine Turbidity Maxima," *Mar. Ecol. Prog. Ser.* 138:265-273, 1996.

79. H.-G. Hoppe, "Use of Fluorogenic Model Substrates for Extracellular Enzyme Activity (EEA) Measurement of Bacteria," in P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publishers, Boca Raton, Fla., 1993, pp. 423-431.

80. M. Unanue, B. Ayo, M. Agis, D. Slezak, G.J. Herndl, and J. Iriberri, "Ectoenzymatic Activity and Uptake of Monomers in Marine Bacterioplankton Described by a Biphasic Kinetic Model," *Microbial Ecology* 37:36-48, 1999.

81. Y.-A. Vetter and J.W. Deming, "Extracellular Enzyme Activity in the Arctic Northeast Water Polynya," *Mar. Ecol. Prog. Ser.* 114:23-34, 1994.

82. R. Amann and W. Ludwig, "Ribosomal RNA-Targeted Nucleic Acid Probes for Studies in Microbial Ecology," *FEMS Microbiol. Rev.* 24:555-565, 2000.

83. S.L. Walker, T.F. Brocklehurst, and J.W.T. Wimpenny, "Adenylates and Adenylate-Energy Charge in Submerged and Planktonic Cultures of *Salmonella enteritides* and *Salmonella typhimurium*," *Int. J. Food Microbiol.* 44:107-113, 1998.

84. W.J. Wiebe and K. Bancroft, "Use of the Adenylate Energy Charge Ratio to Measure the Growth State of Natural Microbial Communities," *Proc. Natl. Acad. Sci. (U.S.A.)* 72:2112-2115, 1975.

85. E.R. Pecke and R.R. Colwell, "Application of a Direct Microscopic Method for Enumeration of Substrate-Responsive Marine Bacteria," *Can. J. Microbiol.* 27:1071-1075, 1981.

86. P.S. Tabor and R.A. Neihof, "Direct Determination of Activities for Microorganisms of Chesapeake Bay Population," *Appl. Environ. Microbiol.* 48:1012-1019, 1984.

87. J.W. Ammerman and F. Azam, "Characterization of Cyclic AMP Transport by Marine Bacteria," *Appl. Environ. Microbiol.* 53:2963-2966, 1987.

88. R. Bachofen and A. Schenk, "Quorum Sensing Autoinducers: Do They Play a Role in Natural Microbial Habitats?" *Microbiol. Res.* 153:61-63, 1998.

89. Space Studies Board, National Research Council, *Size Limits of Very Small Microorganisms, Proceedings of a Workshop,* National Academy Press, Washington, D.C., 1997.

4

Detecting Extinct Life

INTRODUCTION

Experience gained in the decades-long search for evidence of ever more ancient past life on Earth suggests three major stages in the detection of extinct life on other planets:

1. Identification of specific sites of likely fossil preservation associated with the past presence of liquid water;
2. Selection of fossiliferous rocks for study in those locations; and
3. Actual analyses of the rocks themselves for structural, molecular, or isotopic evidence of past life.

On other planets, the choice of sites is based on geological and compositional observations made from orbit, the rocks are selected using chemical measurements made by landers or rovers, and the analyses can be made either in situ or on samples returned to Earth.

Although it appears unlikely that evidence of fossil life can be obtained by orbital observations of an interesting site, the local environment may have preserved the ecological imprint of biological activity. Ecological signatures, possibly subtle and requiring broader spatial coverage for detection with rovers or networks of instruments, may be reflected among local rocks in chemical or mineralogical compositions or in gradients of these properties, as described in the paper by Fogel (see Session 3). On the other hand, depending on spatial resolution, future orbital observations ought to be able to detect mineral formations, analogous to carbonates on Earth, that are either directly or indirectly the result of biological activity. Research on the nature and identification of such signatures is essential. Definitive evidence of fossil life, however, will require more direct and detailed observations of appropriately selected rocks.

Rock selection serves two purposes:

1. To demonstrate that the sample formed in a sedimentary environment, and
2. To show that the sample contains organic matter.

Although the absence of organic matter in a sedimentary rock would not preclude a biological imprint, a rock containing organic matter would offer more lines of evidence to follow in establishing the presence of past life. The detection of organic matter can be accomplished by many techniques differing in sensitivity, molecular

structural information, requirements for sample preparation, and degree of reliability in distinguishing biogenic materials from those produced by nonbiological processes.

METHODS FOR DETECTING FOSSILS

Evidence of extinct life can be sought in detail at various levels ranging from macroscopic stromatolitic structures to microfossils to the intramolecular distribution of carbon isotopes in organic compounds. Depending on their depositional environment, fossilization mechanism, and diagenetic history, both macro- and microscale biogenic structures, including biofilms, can be preserved with varying amounts of their original organic contents. In all cases, especially in the absence of organic matter, the field context of the sample site and the texture and fabric of the structures are critical in determining biogenicity. Since the latter also undergo degradation over time, laboratory and field studies of both fossilization and subsequent diagenesis processes are needed to determine the time scales over which biogenic signatures are lost or preserved under various environmental conditions (see the paper by Cady in Session 4).

The paper by D. McKay, also in Session 4, describes a variety of electron beam techniques. Scanning and transmission electron microscopy combined with optical microscopy provide powerful tools for characterization of putative fossil structures, often in three dimensions, with respect to their location within the mineral matrix, morphology, texture, and size. The energy-dispersive x-ray analyzer (EDXA) and electron energy-loss spectrometer (EELS) attachments and electron microprobes (EMs) provide essential chemical and mineralogical measurements that may support a biogenic origin. Similarly, ion-beam techniques such as time-of-flight–secondary ion mass spectrometry (TOF-SIMS) afford high-spatial-resolution imaging of organic matter and even stable isotopic measurements of specific structures or locations within structures.

No one of these techniques, however, can unambiguously address the question of biogenicity. In light of the continuing controversy surrounding martian meteorite ALH84001, it remains unclear whether the use of an array of these methods can provide unequivocal proof of biological structures (see the paper by Kirschvink in Session 3). The absence of organic remains within the structures makes the problem even more difficult. Highly relevant in this context are studies aimed at determining what biological morphologies, fabrics, and features cannot be produced by inorganic processes.

MOLECULAR AND ISOTOPIC METHODS FOR DETECTING EXTINCT LIFE

Developments in the chemistry of natural products and organic geochemistry over the past decades have yielded molecular structural, isotopic, and stereochemical attributes that are common features in compounds of biological origin. These properties have been instrumental in establishing the antiquity of life on Earth and in tracking the early evolution of biological innovations in the geological record. The use of these traits for discerning extraterrestrial life hinges on how common they are to all biochemistries, earthly and alien. Studies of the organic chemistry of meteorites and laboratory simulations of planetary chemistry provide criteria for characterizing products of nonbiological processes. In the latter case, the value of abiogenic criteria depends on their absence in all biochemistries. Application of both sets of criteria to the organic matter in extraterrestrial samples holds the promise of distinguishing biological from nonbiological materials, as Becker's paper argues (see Session 4).

A lesson of evolution on Earth is that a small number of universal enzymatic processes govern biosynthesis at the cellular level in all life. To fulfill requirements for structure and function, these processes impose distinctive patterns of restricted variation in molecular structure on the building blocks of membranes, proteins, and nucleic acids, the major components of living systems. Moldowan describes these patterns in his paper in Session 4. For membrane lipids, enzymatic pathways preferentially synthesize a small number of specific carbon chain isomers over a broad distribution of chain lengths. Repeating isopentenyl or acetyl subunits are a structural motif, chirality occurs at quaternary (or tertiary-substituted) carbon sites, and—where branched isomers occur—the branching exhibits positional preference. In peptides, only 20 amino acids among the multitude of possible structures are commonly employed and because of their biosynthetic pathway, all are levorotatory α-amino acids with an α-hydrogen. Enantiopurity, characteristic of virtually all quaternary carbon centers in biology, represents a

stereochemical feature that provides compelling evidence of life (see the Session 4 paper by Bada). In the nucleic acids, only four bases are commonly used, and—with two sugars—sugar-phosphate backbones form linear polyionic species. Signatures expected to be common to all biochemistries, even very "primitive" ones, are the pattern of restricted stereochemistry and structural variation and the limited number of compound classes employed. What will not necessarily be common are the molecular identities of the various building blocks and polymers. In his paper in Session 4, Anbar argues that isotopic ratios of multiple elements beyond carbon, such as iron, could provide supporting and well-preserved indicators of past biological activities.

In contrast to biosynthetic pathways, abiotic syntheses, as manifest in the organic chemistry of meteorites, yield distinctly different arrays of molecular structure across many compound classes. Within each class—for example, amino acids—the abundances of isomers decrease smoothly as carbon number increases, with branched isomers often exceeding linear structures. At low carbon numbers, complete molecular diversity prevails and all possible structural isomers occur. Similar results are produced in simulations of prebiotic planetary chemistry. Small enantiomeric excesses occur among meteoritic amino acids, however, which suggests that chirality must be used cautiously to distinguish biogenic compounds from those of nonbiological origin. How widespread enantiomeric asymmetry may be among other classes of meteoritic compounds remains to be determined (see the Session 4 paper by Chang).

Once deposited in a sedimentary environment, biopolymers and biomembranes undergo degradation relatively rapidly over geologic time scales. On Earth, the molecular biomarkers with greatest longevity are hydrocarbons derived from decomposition and rearrangement of biolipids, some even with retention of chiral quaternary centers. The dominant component of ancient sediments, however, is the insoluble macromolecular "kerogen." Martian analogues, if they exist, are likely to persist in environments protected from atmospheric oxidants. Asteroidal debris has been delivered to the surface of Mars (and other solar system bodies) over time, and some of that meteoritic organic matter may also have survived. Given a cooler and less tectonically active surface such as that of Mars, the persistence of other classes of compounds including amino acids is possible, regardless of their origins.

The determination of molecular structures in a mixture of compounds that is required to assess biogenicity can be carried out by existing methods such as combined gas chromatography-mass spectrometry (GC-MS) or capillary electrophoresis alone or in combination with MS. The use of chiral chromatographic substrates adds a stereochemical dimension to these analyses. The sample preparation required may range from simple sublimation to more complex combinations of solvent extractions, filtrations, and derivatizations. Although these analyses pose no unusual challenges for samples returned to Earth, carrying them out remotely on Mars requires development of new robust miniaturized instruments with low power requirements. These capabilities appear feasible with emerging technologies, and Bada describes one such approach in his paper.

PROMISING LITHOLOGICAL ENVIRONMENTS FOR THE SEARCH FOR EXTINCT LIFE

As elements in the development of a search strategy for signs of extinct life on Mars and beyond, many of the life detection approaches presented in this chapter were guided by studies of the earliest life signs (e.g., fossils) on Earth. In the early terrestrial record, this would have been microbial life—the most robust form of living organisms even on present-day Earth. However, as we have learned from our own fossil record, identifying extinct organisms and their biosignatures requires careful mapping to identify the appropriate environments and a suite of measurements capable of distinguishing evidence for biological processes on several levels (e.g., morphological, isotopic, and metabolic).

The search for signs of ancient life on Earth indicates that the most suitable locations for the accumulation and preservation of biosignatures are deposits associated with ancient or modern ground or surface water environments or sedimentary rocks usually characterized as fine grained with aqueously derived mineral assemblages. The most promising sedimentary lithologies for preserving signs of extinct life are cherts, carbonates, phosphates, and clays that are usually laid down in aqueous environments (e.g., oceans, lakes, thermal springs, and evaporite deposits). Such biosignatures include microfossils, macroscopic textures (e.g., stromatolites), chemical fossils, and isotopic fractionation patterns. All of these types of evidence will be needed to determine definitively whether life ever

evolved beyond our own planet. Careful consideration of the type of lander, the suite of instruments needed for sample selection, and the appropriate size sample must self-evidently be part of a life detection strategy. In his Session 4 paper, Huntress describes a focused program at NASA's Jet Propulsion Laboratory that seeks to identify the most promising approaches to detecting extinct (or extant) life through in situ measurements.

Although sedimentary rocks are arguably the most promising locations for preserving biosignatures, the fact that some terrestrial life forms exist on or in igneous rocks must also be recognized. Thus, hydrothermal systems associated with volcanic regions on Mars may be or may have been sites for life and, as noted above, must be on the list of potentially interesting sites for sampling.

5

Conclusions and Recommendations

INTRODUCTION

Convened by the Committee on the Origins and Evolution of Life, the Workshop on Life Detection Techniques presented an opportunity for a multidisciplinary group of experts ranging from laboratory biologists to planetary scientists to consider the current state of detection technologies and sampling strategies. Detection of extant and extinct extraterrestrial life, in situ and in the laboratory, was discussed, as were assays of terrestrial microorganisms for the purpose of gauging spacecraft cleanliness. The committee's goal was not to select a particular technique or techniques for use in detection in each case, but rather to identify key issues that must be addressed in the course of developing sample-return and in situ detection missions. Table 5.1 provides a list of biosignatures and their applicability to detecting evidence of extinct or extant life or to detecting terrestrial biological contaminants on spacecraft or in martian samples. These techniques would also have some applicability to the detection of novel life that may have a biochemistry somewhat different from that of terrestrial life. Most of these biosignatures, however, are based on the assumption that putative extraterrestrial life would be carbon based, would have structures that could be recognized as evidence for life, and would utilize available energy sources that are known to support terrestrial life. While Table 5.1 lists biosignatures indicative of extant and potentially viable life, it does not cover all of the methods available that could provide evidence for the growth, metabolic activity, and physiological potential of viable organisms.

Table 5.2 provides an index of the biosignature-measuring techniques presented at the workshop, the properties they measure, their sensitivity, their limitations, and the need for future developments. The table includes techniques that were not covered in the workshop presentations, but whose inclusion here in summary form provides a comprehensive basis for comparing the approaches.

DETERMINING IF LIVING ORGANISMS ARE ON A SPACECRAFT BEFORE LAUNCH

The most strikingly definitive result evident from the workshop is the dramatic improvement in laboratory techniques designed to detect terrestrial organisms, with principal application to spacecraft sterilization and, hence, planetary protection issues relating to forward contamination.

Recommendations regarding specific sterilization techniques and levels of sterilization to avoid contamination of other planetary bodies go beyond the original purview of the workshop and this report. From the point of view

TABLE 5.1 Biosignatures: Specificity for Life Detection, and Applicability to Detecting Extant and Extinct Life and Terrestrial Contamination of Spacecraft

Signatures of Life	Application for Life Detection	Specificity	Fossil and Nonterrestrial Life Detection
Morphology (Micro- and Macroscopic)			
Shape; size; replication structures (buds, chains of cells, septa, fruiting bodies, and spores); some biominerals; macrostructures such as biofilms and stromatolite-like structures	Detect extant terrestrial life, fossils, indication of active cells; application to spacecraft contamination.	Shape and size are not definitive (terrestrial life is >100 nm in diameter); replication structures are definitive indicators of life; can identify eukaryotes; biofilms and stromatolite-like structures could be definitive.	Replication structures can be definitive; size, shape, and numbers of identical morphotypes such as are seen in biofilms or laminated structures observed in stromatolites may or may not be definitive for life, and additional chemical and isotopic analyses are necessary.
Organic Chemistry and Biochemistry			
Cell walls (variety of biopolymers) Membranes (fatty acids) Nucleic acids (DNA, RNA) Proteins Hydrocarbons, steroids, hopanes Amino acids Organic metal and phosphate compounds Porphyrins, flavins, etc. Carbohydrates	Nucleic acids with same genetic code as terrestrial life would likely indicate terrestrial contamination; steroids generally indicate eukaryotes; hopanes found in cyanobacteria; chirality and presence of the 20 key amino acids associated with terrestrial life indicate terrestrial and spacecraft contamination.	Bacteria, archaea, and eukaryotes have specific cell-wall chemical structures; chirality, enantiomeric excess, and repeating structural units such as C_5, C_6 (sugars), C_2 (polymethylenic lipids), C_5 (polyisoprenoids), α-substitution of protein amino acids, and *L*-amino acids and *D*-sugars are canonical for terrestrial life. Nucleic acids, proteins, and phosphates and organic-phosphate compounds could be indicative of recent or extant life.	Hydrocarbons, steroids, and hopanes have been observed in the fossil record; other macromolecules (nucleic acids, proteins, and carbohydrates) are extremely labile. Nothing is known about the long-term stability of cell-wall polymers of archaea and their chemical transformations during fossilization.
Inorganic Chemistry			
Iron minerals (e.g., magnetite) Sulfur compounds Carbonates Silicates Other biologically important metals (e.g., Cu, Mo, Ni, W, etc.) Nitrogen compounds Phosphorus compounds Ratios of biologically important elements (CHONPS) Disequilibrium in biologically important oxidation-reduction couples	Best application is as additional information in conjunction with microscopic, isotopic, and organic chemical analyses for fossils and possibly for detecting presence of living extraterrestrial organisms; probably not applicable for detecting spacecraft contamination.	C, N, and S can be highly specific for terrestrial life in conjunction with stable isotope or organic analyses. Some bacteria form iron compounds with highly specific structures (e.g., magnetosomes and the ferruginous ribbons formed by the bacterium *Gallionella* spp). Other microbes deposit silicates and carbonates and elemental sulfur as metabolites.	Some crystal structures of magnetite are thought to be produced only by organisms. C, S, and N isotopes are essential additional targets of analyses for inferring past life. Oxygen isotope ratios associated with phosphates may be indicative of life. Heterogeneous distribution of biologically important minerals (Cu, Mo, Ni, W) and disequilibrium in the chemistry of rock samples could be supporting evidence for past life.

Isotopic Analyses			
Carbon, nitrogen, sulfur, oxygen, and possibly heavy metals	Best application is to confirm extant and fossil life where there may not be evidence of intact cells; new methods also allow stable isotopic analyses of individual organic molecules and iron. Probably not applicable to detecting spacecraft contamination.	Stable C, N, and S isotopes can be definitive indicators of different metabolisms. Best used to detect CO_2 reduction by photosynthesis, chemosynthesis, methanogenesis, and sulfate reduction.	Vital analyses to help confirm biogenic origin of minerals or cell-like structures observed microscopically on rock or soil samples. Used to understand the nature of carbon, nitrogen, and sulfur cycles in ancient environments.
Environmental Measurements			
Global atmosphere measurements (spectral identification of volatiles such as ozone, hydrogen, methane, oxygen, water)	Identify metabolic indicators of extant life, potential for habitability, and sites of high concentrations of volatiles and water; visual indication of vegetation or other indications of life.	Other measurements necessary to confirm presence of living organisms and ecosystems.	Not applicable for detecting extinct life unless there is some visual indication of past vegetation such as stromatolites.
Macroscopic life forms (imaging systems)			

TABLE 5.2 Techniques Used to Measure Biosignatures

Biosignature and Technique	Measurement for Life Detection	Sensitivity	Limitations and Developments Needed	Workshop Paper by
Global				
Spectroscopy (TPF)	Spectral lines in planetary atmospheres of extrasolar planets	Oxygen: 1% of Earth atmosphere; other gases to be determined	Imaging interferometry needs technical development	Kasting
Macroscopic imaging systems (e.g., Galileo's Solid State Imager)	Morphology of macroscopic life and ecosystems	Less than ~10-m spatial resolution	Solar system objects only	Soffen
Morphological				
Light microscopy	Structure; evidence for viability (motility, biofilms); noninvasive	0.2-μm spatial resolution	Morphology only; no chemistry	—
Electron microscopy (environmental SEM [ESEM], SEM, EDX)	High-resolution morphology and chemical composition (ESEM is noninvasive)	1-10 nm, 0.2 keV, 1% relative abundance	Requires contamination-free microscopes	Barker and Banfield
Electron microscopy (TEM, SAED, EELS)	Structure, redox state, mineralogy; invasive	1 nm, 0.2 keV, 1% relative abundance	Invasive sample preparation	—
X-ray microscopy	Electronic state of molecules	Nanometer resolution	Requires sectioning	Jacobsen
Fluorescence microscopy	Structure; detect very small entities, macromolecules (nucleic acids and proteins); identify organisms (^{16}S and ^{18}S rRNA) and possibly viability (number of ribosomes; mRNA)	Can be used to enumerate viruses (30-50 nm)	Better preparation methods needed with rock and soil samples; at present time need to dislodge organisms for FISH	Stahl, D. McKay, Jacobsen, Cady
CT scan-XAFS imaging	Internal structure; noninvasive	Millimeters to centimeters	Higher spatial resolution needed	—
Mineralogical				
SEM-EDX	Structure and abundance of elements	1-10 nm, 0.2 keV, 1% relative abundance	Reduce diameter of EDX beam (<200 nm)	Barker and Banfield, Kirschvink
Ion and electron microprobes	Chemical and isotopic composition	Single organic molecules	—	D. McKay
Light microscopy, optical broadband spectroscopy	Chemical composition; noninvasive	0.2-μm spatial resolution	Limitations in spatial resolution	Jacobsen, Cady, D. McKay

Infrared (IR) spectroscopy	Structure and composition	1-μm spatial resolution	Improve signal-to-noise ratio	—
XRD-XRF	X-ray structure; minerals and elements; noninvasive	200-μm spatial resolution	Develop higher spatial resolution	—
Mossbauer spectroscopy	Fe valence	Bulk sample	Measures Fe only	—
Organic Chemistry				
GC-MS	Chemical composition, enantiomeric excess, diasteriomer specificity, structural isomer preference, and repeating structural units; lipid biomarkers; isotopes	Mass resolution 1:60,000; 10^{-15}-10^{-18} mole	Development of ionization techniques	Moldowan, Chang, Cotter, Soffen
Chromatography for chirality (capillary zone electrophoresis)	Structure and chirality; enantiomer excess and repeating structural units	Picomoles	Disadvantage is need to derivatize sample	Bada
Laser desorption-laser ionization (TOF, MALDI, ESI)	Intact biomolecules	10^{-20} mole	Measures molecular weight only; need to develop better lasers and improve sample preparation	—
Raman (IR spectroscopy and UV fluorescence)	Presence of organic compounds, pigments, biomineralization; noninvasive	1 μm	Need to develop low-noise detectors	Becker
GC-isotope ratio mass spectrometry (CHONS isotope analysis)	All biogenic elements and isotopic composition	Nanomoles to picomoles	Improve chromatography and ion source	—
Chip chromatography-microarray antibody binding	Single organic molecule	Single molecule	Need further development of sensors, detectors, and arrays	—
Liquid chromatography	Suitable for detecting enantiomer excess, diasteriomer specificity, and repeating structural units	Micromoles	Improve resolution	—
Molecular and Biochemical				
Polymerase chain reaction and sequencing	Detect and sequence DNA and RNA; identify specific taxa	Theoretically can detect a single cell	Develop methods for in vivo PCR on single cells and fossilized cells	Stahl, Ruvkun et al.

continued

TABLE 5.2 Continued

Biosignature and Technique	Measurement for Life Detection	Sensitivity	Limitations and Developments Needed	Workshop Paper by
Nanopores	Size and some structural information about biopolymers	Single molecules	Nanopore technology in development phase; linear molecules only	Meller and Branton, Deamer
Protein-chip, chromatography, stable isotopes	Molecular weight and structure of macromolecules; isotopic composition	Picomoles to femtomoles	Need further development of sensors and detectors	Fogel
Metabolic analysis	Detect biological activity including metabolic pathways and bioenergetic and biocatalytic activities	Not known and would be test specific	Methods in the early development stage	Stahl
Isotopic Analyses (see above for GC-MS and IR spectroscopy)				
C, O, N, and S isotopes by gas source MS	Isotope composition	Picogram	Preservation of signatures (labile compounds)	—
C, O, N, and S (?) isotopes by spectroscopy	Isotope composition	?	Development of new diode lasers and detection systems	—
C and S isotopes by ion probe MS	Isotope composition	Sub-picogram	Improved detection systems and ion sources	—
Transition metal isotopes by MC-ICP-MS and TIMS	Isotope composition	Nanogram	Better understanding of fractionation systematics; improved detection systems and ion sources	Anbar

NOTE: Acronyms used in this table are defined in Appendix A.

of the committee the main issue regarding sterilization is the ability to sample, poststerilization, the remaining level of terrestrial microorganisms to ensure that it is below the value required for a particular mission. Because all terrestrial organisms rely on the same basic biochemistry—specifically and most importantly, the RNA and DNA nucleic acid bases—amplification to detect very small remnant levels of contamination is a suitable approach.

The more difficult challenge is the sterilization itself, which must be done in such a way as to avoid damaging spacecraft components. Sterilization via dry heating in an oven, as performed on the Viking Mars landers, puts harsh demands on spacecraft components and leads to a substantial increase in mission costs and, possibly, the chances of mission failure. Sterilization by particle irradiation may not reach all spacecraft subsystems, particularly when the mission design dictates shielding electronic components from ambient sources of radiation, for example, in the Jupiter system. Irradiation levels may have to be so high and so sustained that protection of optical components, as well as electronics, becomes problematic. Radiation-tolerant bacteria may dictate that irradiation levels exceed even the extraordinary levels expected to be experienced during the prime mission phase of the Europa Orbiter.[1]

Finally, access to all parts of a spacecraft for sampling after sterilization is an unsolved problem. Particularly for compact landers and entry probes, access may mean disassembly and reassembly, increasing mission risk as well as the possibility of recontamination of the spacecraft. Indeed, the very compact Huygens Probe that will land on Titan in 2004 was not sterilized to a high standard on the grounds that the profoundly cold Titan environment would sterilize the lander soon after landing. Yet Titan is itself a target for investigating advanced stages of organic chemistry that on Earth might have led to life.

An illustrative summary of sterilization techniques is given in Table 5.3. The choice of technique will be mission specific and may evolve, for later missions, as more is learned about the planetary target. The principal

TABLE 5.3 Sterilization Techniques in Common Use

Procedure—Target	Technique—Problems
Dry heat—exterior or interiors	105-180 °C for 1 to 300 hours—problems caused by thermomechanical incompatibility between materials can lead to the failure of electronic components, alteration of organics, and volatilization
Wet heat—exterior or interiors	120-134 °C for 3 to 20 minutes—problems caused by steam (e,g., corrosion and water absorption)
Alcohol wipes—exterior surfaces	Isopropyl or ethyl alcohol swabbing—problems arise because interior and encased surfaces (e.g., electronic components) are inaccessible
Ethylene dioxide—exterior or internal exposed surfaces	Toxic gas, 40-70 °C—problems arise because the gas reaches only exposed surfaces, is absorbed by carbon polymers (e.g., rubbers and polyvinyl chloride), and leaves an organic residue
Gamma radiation—exterior or subsurface	Typically 2.5 Mrad—problems encountered include optical changes in glasses, damage exposed to electronics and solar cells, and altered organics
Beta radiation—exterior or near-surface	1-10 MeV—problems arise because of limited penetration
Hydrogen peroxide plasma—exterior or internal exposed surfaces	6 mg per liter of H_2O_2—problems encountered because unexposed surfaces remain untreated
Ultraviolet—exterior surfaces	5,000-20,000 J/m^2—problems arise because unexposed surfaces remain untreated
Methyl bromide, chlorine dioxide, and ozone—exterior or internal exposed surfaces	Toxic gases—problems encountered because unexposed surfaces remain untreated and because the gases may catalyze chemical reactions between metals and other components

SOURCE: Space Studies Board, National Research Council, *Preventing the Forward Contamination of Europa*, 2000, and *The Quarantine and Certification of Martian Samples,* 2002, National Academy Press, Washington, D.C.

conclusion the committee drew from the workshop and from its subsequent deliberations is that the difficult part of sterilization is not the detection of residual terrestrial contamination—it is the sterilization itself.

The committee recommends that studies of future missions to astrobiologically interesting targets include explicit consideration of the types of sterilization for spacecraft systems, subsystems, and components and that sterilization costs be included in a realistic fashion. The committee recommends that special near-term emphasis be given to the issues of sample selection, spacecraft sample handling, and sample characterization. The committee also encourages further work to refine sterilization approaches to minimize impacts on mission costs and success.

DETERMINING IF THERE ARE LIVING ORGANISMS IN A RETURNED SAMPLE

The committee concludes that a number of very sensitive and specific techniques are available for detecting living organisms in a returned sample; however, these techniques depend on the organisms being composed of essentially terrestrial biopolymers. While other techniques exist for detecting a potentially broader suite of nonterrestrial-like (but carbon-based) organisms, their results will not be as definitive. Hence, multiple approaches will be required to establish the presence of life in a definitive fashion, unless such life happens to be essentially terrestrial in nature. There is a pressing need to develop methods for the detection in single cells of evidence of metabolic activity and of specific macromolecules, including an analysis of their chemical structure and isotopic signature.

In considering the search for life on other planets, many workshop participants assumed that "life," as defined, would be broadly like that on Earth. Because the basic functions of catalysis and replication require molecules with a high degree of specificity, life anywhere would rely on macromolecules—indeed complex polymers. Hence it would be based on carbon with its high cosmic abundance and unique propensity for building such molecules. Likewise other cosmochemically abundant elements—hydrogen, oxygen, phosphorus, nitrogen, and sulfur—should be important components of life anywhere. Much less certain is whether extraterrestrial life would use the same polymers for catalysis and replication as life on Earth—namely, proteins based on the biologically common amino acids and the RNA-DNA coupled system. Although it is possible to rationalize why terrestrial biology employs the molecules it does, much simpler life forms or those in a very different chemical environment might employ different molecules. Possibilities include a different set of amino acids, alternatives (or precursors) to RNA involving a peptide backbone, or a sugar-based nucleic acid with different bases (or number of bases). Likewise, the diversity of metabolic processes seen in the bacterial and archaebacterial domains of earthly life may not represent the full spectrum of possible metabolisms that could be encountered on other worlds. Finally, different sets of membrane lipids might be expected as well. The last is an important issue since membranes may be universal in all biochemistries and lipid biosignatures have been a key to tracing biochemical evolution in Earth's rock record.

For these reasons, there is a disconnect between those techniques that have been developed to an exquisite degree of sensitivity to identify terrestrial organisms and those that could provide the greatest probability of detecting exotic life forms from another planet. Most of the chemistry-based techniques discussed in the workshop assumed terrestrial-type biochemistry or something close to it. Although techniques for amplification of nucleic acids have, for example, improved somewhat in the breadth of genetic material amplified, they are still extraordinarily narrow compared with the range of possible encoding schemes one can imagine.

Imaging techniques that reveal structures indicative of processes that would be unexpected in abiotic chemistry, and whose detectable presence is independent of chemispecific amplification techniques, are promising in the sense of being quite general. These techniques are applicable to signs of extinct life as well (see below), but they may be more definitive in identifying extant life since the signatures will be much fresher than for fossils of long-extinct organisms. However, it is difficult to assess how well current experience with terrestrial biosignatures will map onto the range of morphologies or chemistries possible in extraterrestrial environments.

The committee is strongly encouraged by the multidisciplinary efforts to define the possible range of processes indicative of living organisms. Given the extreme difficulty (or impossibility) of inductively describing all possible living processes based on terrestrial biochemistry, no single approach, or even

combination of approaches, will guarantee success on a given sample. Multiple approaches, both chemical (including isotopic and molecular) and microscopic, are key to the successful detection of life in a sample.

With respect to analysis of returned samples to assess possible hazards to terrestrial life, the committee notes that there have been several recent Space Studies Board and other studies on the risks potentially posed by returned samples.[2] Only insofar as the threat to the terrestrial biosphere might come from organisms whose catalytic and reproductive (information-carrying) machinery is virtually identical to our own is the reliance on amplification techniques appropriate. The continuing increase in sensitivity of amplification techniques will certainly provide increased confidence in protocols to assess the threat of forward contamination of other planets. The issue for back contamination, however, is whether organisms might exist that are sufficiently different from terrestrial organisms to escape laboratory detection, yet similar enough to pose a threat to the health of the biosphere. In the debates about life detection and back contamination, this "niche" has not been explored to the extent that it should be—in part because of the difficulties in answering the question.

The committee recommends that a focused study be done in the near future to address the detection of microorganisms with varying degrees of nonterrestrial biochemistry, and the possible threat that such organisms might pose to terrestrial organisms.

There can be no single strategy for sample acquisition since each planetary body presents a unique contemporary geology or chemistry and a unique evolutionary history. Similarly, there can be no single strategy for treatment of returned samples. Instead, the risk factor related to each variety of sample must be assessed, and our experience with lunar return samples can serve as a useful guide in developing policy.

To the extent possible, reasonable efforts (defined through carefully deliberated scientific strategies) should be made to assess the potential for extant life on other planetary surfaces in situ, using robotic missions. The results will markedly increase confidence about the risk factors associated with a given sample that could be returned to Earth for further study and will provide scientific evidence to further justify the expense of a return mission. Since life (or past life) will concentrate in habitats that provide suitable nutrients and chemical or physical conditions, its distribution on any planetary body will be patchy and of varying local abundance. For Mars in particular, the issue of selecting promising sites has been addressed in multiple reports and studies, the most recent being that of COMPLEX.[3] At the same time, in situ analysis of a site will be limited in the capability and flexibility of the experiments that can be employed, compared to those that can be brought to bear on returned samples. The potential phase space of possible life forms and biomarkers is broad, and in situ studies are more likely to miss detection than are studies performed on returned samples. Nonetheless, the combination of site selection and analysis followed by acquisition of the most promising samples for study on Earth is likely to maximize the chances of success in the identification of extant or extinct life.

Appropriate site selection for sample return is critical and will determine the amount of sample required for testing and the need for possible sample concentration. Multiple measurements with different techniques will be required to perform triage on a set of field samples at a given landing site, so as to select the most promising samples for in situ or returned life detection.

Sampling and return methods must be compatible with the requirements imposed by analysis. For example, an activity-based measure (metabolism) will require that samples not be exposed to environmental extremes during recovery and return. A (bio)chemical assessment of structures characteristic of life (e.g., enantiomers) would not place the same constraints on sampling and return. Sample selection, handling, and characterization at a given site must be thought through carefully to ensure an interpretable result in either laboratory or in situ life detection protocols. **The committee recommends that special near-term emphasis be given to the issues of sample selection, spacecraft sample handling, and sample characterization.**

DETERMINING IF LIVING ORGANISMS HAVE BEEN PRESENT AT SOME EARLIER EPOCH AND HAVE LEFT FOSSIL REMNANTS BEHIND IN A RETURNED SAMPLE

The committee concludes that the search strategy for evidence of extinct life must include the identification of suitable landing sites, the selection of the appropriate rock types, and multiple analytical techniques that, in the aggregate, are capable of distinguishing between abiogenic and biogenic signatures. The

assessment of extinct biosignatures will likely require a sample return mission to carry out the sophisticated set of measurements needed to make this determination.

Most of the more general techniques for detecting life, versus the very specific (and potentially more sensitive) approaches of amplifying nucleic acid bases, would be suitable for searching for either extant or extinct life. This is an important point since the majority (but not exclusive) view is that for Mars, the probability is much higher of detecting extinct rather than extant life. Biosignatures may be morphological, chemical, or isotopic. For example, the biological fixation of carbon dioxide into organic carbon preferentially leaves behind the heavier isotope, ^{13}C. Hence, the lower value of $^{13}C/^{12}C$ seen in organic carbon compared to carbonates over geologic time testifies to the lengthy history of life on Earth (3.9 billion years or longer). This seems a very reliable signature of biological processes past or present. However, work remains to be done in fully quantifying the variation of isotopic fractionation in terrestrial microorganisms, and indeed the variation in the isotopic ratio in biologically fixed carbon is large. Also, the preservation of organic carbon in the martian soil is questionable. Identification of isotopic biosignatures in elements more robust against alteration, such as iron, is promising, but more measurements are needed to establish fractionation patterns and decide whether the approach is reliably diagnostic of past biological activity. Morphological signatures, such as mineralogical or textural alteration of minerals by organisms, can reveal the past presence of life when carefully documented and compared to known alteration signatures in the terrestrial rock record. Elimination of false positives in a sample will require multiple types of biosignatures and critical testing of alternatives to the biological hypothesis. The committee particularly commends the work to characterize biosignatures associated with microbe-mineral interfaces on Earth and strongly encourages additional efforts in this regard, especially in extreme environments.

Biosignatures degrade progressively over time. Rates of degradation for a particular kind of biosignature generally depend on the environment; for example, the rates of racemization of many chiral amino acids are accelerated by the presence of water. A key issue then, and one for which much work remains to be done, is to understand the rates and nature of degradation of biosignatures in planetary environments that are likely candidates to be searched. Additional effort on basic mechanisms can be undertaken today and is largely independent of the uncertainties associated with specific, rapidly changing plans (and budgets) for obtaining samples. Application to a particular planetary sample will depend on characterizing, to the extent possible, the environmental conditions to which the biosignatures were exposed over long periods of time.

The committee recommends that attention be given to understanding thoroughly the rates and nature of degradation of biosignatures in planetary environments. Theoretical and experimental studies should be supplemented with comparative analysis of putative samples of extraterrestrial biomarkers (e.g., ALH84001), with a specific eye to better understanding the issue of degradation of signatures of past life. Additionally, the identification and development of new and possibly universal biosignature approaches should be an active area of study.

In the course of the workshop, an issue arose that was not part of the original charter—namely, the availability of the same samples to multiple research groups. The resolution of at least one controversy regarding the interpretation of biosignatures in the SNC meteorite ALH84001 has been hampered by the fact that distinct research groups are analyzing different samples from the meteorite, with evident sample-to-sample variations. Magnetite crystals left behind by bacteria exhibit a combination of properties that are considered indicative of biological manufacture because these properties are not found in abiotically produced crystals (either in nature or in the laboratory). As carefully studied as this phenomenon has been, its one application to an extraterrestrial sample—ALH84001—has led to a broad range of interpretation of the magnetite found therein. Multiple workshop participants held the view that the current mechanism for dissemination of samples and the normal handling practices within research groups have discouraged direct analysis of the same sample by multiple teams. Yet the scientific method encourages, if not demands, that a controversial result obtained on a sample be validated through additional studies, including by alternative research teams with different approaches.

The challenge of having multiple research groups analyze the same sample will be greater for samples returned from extraterrestrial bodies by spacecraft (as opposed to serendipitous meteorite infall), since quarantine could discourage the transfer of samples from one laboratory to another. Nonetheless, the nature of some of the most vigorous workshop debates illustrated the importance of having groups with differing approaches or

predisposed biases examining the very same material. Possible solutions include formation of consortia of researchers from different institutions to perform a particular type of analysis, provision for multi-institutional (repeat) analyses of a select subset of the sample material, and so on.

Because of these concerns, **the committee recommends that any plans for analysis of returned extraterrestrial samples include a provision for repeat analyses of a subset of the same material, preferably by different teams. The committee encourages early development and testing of appropriate protocols using existing samples of high astrobiological interest (e.g., ALH84001).**

DETERMINING IF THERE ARE LIVING ORGANISMS OR FOSSILS IN SAMPLES EXAMINED ROBOTICALLY ON ANOTHER SOLAR SYSTEM BODY

Because of the technical difficulties associated with returning samples to Earth (and potential back-contamination threats), much of the search for life elsewhere may initially be done in situ. It was evident from the workshop presentations that many of the powerful and sensitive techniques for detecting life in the laboratory are not yet available in the form of miniaturizable spacecraft instrumentation and may not be so in the near future. Consequently, in situ life detection approaches are constructed based on a priori hypotheses as to the structural, metabolic, or replicative nature of the organisms searched for. This allows a small, flyable package to be designed—but severely narrows the phase space of possible successful searches and limits the opportunity for adaptively altering the analysis of samples based on initial results.

Because of the continuing rapid improvements in technology, it is not appropriate at this time to recommend a specific set of techniques for in situ life detection, but in situ life detection will require commitment to a small set of potential techniques with significant lead time to ensure that they can be space qualified. Some of the approaches available for the detection of living organisms are available in miniaturized form and are potentially space qualifiable for an in situ life detection mission. The committee encourages continued efforts to develop innovative and miniaturizable techniques for in situ life detection.

It remains unclear as to which environments in our solar system should be searched for signs of life, beyond the general identification of planetary targets (e.g., Mars, Europa, and Titan). In large measure, we do not yet know enough about these bodies to target searches in particular locations. Where might organic molecules be present or have been present within the martian crust? Are there places on the surface of Europa where the putative ocean is accessible to drilling or where the extrusion of oceanic material is continuing today? Where are the interesting organics (liquid or solid) on the surface of Titan? Answering these questions requires additional planetary missions to deepen our understanding of these environments, and there is a natural tension between systematically planning and executing those missions versus pressing on with the search for life itself.

The key to success in the search for life is appropriate site selection. Promising surface or subsurface sites would be ones showing evidence of past or present liquid water, aqueous alteration of minerals, sources of chemical free energy (past or present), and possibly organic molecules.[4] However, terrestrial experience shows that *extant* life will not be confined to sedimentary environments, and biosignatures need not (indeed probably will not) consist of organic matter.

Almost certainly, the most interesting sites from the point of view of the search for life will not be the easiest to get to. **For Mars, this means that landing site selection cannot be based primarily on issues of spacecraft safety. Furthermore, proper site selection will require a series of missions including orbital reconnaissance followed by exploration of selected sites by landed vehicles.** The in situ search for life can serve as a selective precursor for determining which samples should be returned for laboratory analysis, and assessing the potential hazard of back contamination before a sample is brought back to Earth. Site characterization (age, exposure to liquid water, high temperatures, etc.) will be essential as well in order to understand the extent to which degradation of biosignatures, analyzed in situ or on Earth, has occurred.

In the case of Europa, a follow-on to the Galileo mission (most plausibly in the form of an orbiter) is required to identify potential landing sites where access to subsurface liquid water is possible. Finally, for Titan, the Cassini-Huygens mission will provide the necessary data to map the surface distribution of organics and identify whether any particular sites are of interest for future studies of abiotic organic evolution.

Living organisms represent a subset of the organic molecules that exist in the solar system and beyond. Analyses of meteorites, the atmospheres of the giant planets and Titan, and the surfaces of outer solar system bodies reveal a range of abiotic chemical processing of organic molecules. Detection of simple organic molecules (e.g., methane) has been accomplished for the atmospheres of very cool brown dwarf stars, an important precursor to a protocol for the eventual remote spectroscopic assessment of the habitability of extrasolar planets.

Although the vast bulk of the analysis performed to date on extraterrestrial samples demonstrates the non-living origin of these organics, the small enantiomeric excess in the Murchison meteorite remains a contentious puzzle. Whether it suggests the action of abiotic processes to introduce asymmetry into an abiotic stereochemical system, or some sort of postimpact terrestrial process acting on the meteoritic organics, is unresolved. In the case of Mars, the failure of the Viking landers to detect organic molecules was key to the conclusion that the Viking life detection experiments were seeing abiotic processes in a highly oxidizing soil, rather than metabolism. Terrestrial laboratories are capable of detecting and characterizing very small amounts of organic molecules in samples, as well as reliably determining whether the molecules are indigenous to the sample or a terrestrial contaminant. This was of particular value in analyzing the SNC meteorite ALH84001, in which it was found that most of the organic material is a terrestrial contaminant and the remainder resembles primitive meteoritic or interstellar material.

The development of more sensitive techniques to detect organic molecules and characterize the extent to which organic chemical systems may be evolving toward life in a planetary environment is an important priority. Many organic-rich environments in the solar system will be accessible to in situ analysis in the coming couple of decades but are prohibitively difficult targets for sample return (e.g., the surface of Titan). It is therefore desirable to miniaturize the instruments necessary for sensitively detecting and characterizing organic phases so that they can be accommodated on spacecraft dispatched to a variety of solar system targets.

The committee concludes that it is crucial to continue the development of techniques to detect and analyze in situ organic chemical systems of either biotic or abiotic origin, with the goal of increasing the techniques' sensitivity and diagnostic capability.

REFERENCES

1. Space Studies Board, National Research Council, *Preventing the Forward Contamination of Europa*, National Academy Press, Washington, D.C., 2000.

2. See, for example, Space Studies Board, National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002.

3. Space Studies Board, National Research Council, *Assessment of Mars Science and Mission Priorities* [prepublication text], National Academy Press, Washington, D.C., 2001.

4. M.H. Carr in *Water on Mars*, Oxford University Press, New York, 1996, pp. 180-183.

Appendixes

A

Glossary and Acronyms

GLOSSARY

Abiotic	Originating without the participation of a living system.
Aerogel	A nearly transparent, very lightweight material made primarily from silica; the world's lightest solid, weighing as little as three times the weight of air and exhibiting superb insulating properties.
Aerosol	A fine aerial suspension of liquid (mist, fog) or solid (dust, fume, smoke) particles.
Anaerobe	An organism that lives and reproduces in the absence of dissolved oxygen, instead using oxidants such as iron and sulfur compounds in energy metabolism.
Anoxic	Lacking oxygen or other oxidizing agents.
Archaebacteria	A group of prokaryotic microorganisms that are only distantly related to eukaryotes and the other prokaryotes and are members of the domain Archaea.
Autotroph	An organism that can make its own energy without requiring previously formed organic material from the environment.
Biogenic	Of biological origin.
Biopolymer	A large molecule having a repeating structural feature assembled by a living system from building blocks; proteins (built from amino acids) and DNA (built from nucleotides) are two examples.
Carbonate	A mineral having the carbonate ion, such as calcite (calcium carbonate), siderite (iron carbonate), and magnesite (magnesium carbonate).

Catalyst	A substance that enhances the rate of a chemical reaction by lowering its activation energy, without itself being changed.
Chemoautotrophic	Producing organic matter by using the energy obtained from oxidation of certain chemicals; using carbon dioxide as the only source of carbon.
Chemolithotrophic	Capable of generating metabolically useful energy by the oxidation of inorganic compounds.
Chert	A hard sedimentary rock made up of very-fine-grained or amorphous silica.
Chirality	Handedness; a property of an object. An object is chiral if it cannot be superimposed on its mirror image.
Diagenesis	All of the changes that occur to a fossil (or more generally any sediment) after initial burial; includes changes that result from chemical and physical as well as biological processes.
E_h	A measure of the oxidative potential of the environment.
Enantiomers	A pair of objects (molecules, crystals) that are non-superimposable mirror images of each other.
Enantiopurity	In a mixture of enantiomers, the ratio of one enantiomer to the other.
Eukaryote	An organism having a membrane-bound nucleus and usually other organelles.
FastDNA ML	A program to analyze the relationships between nucleic acid sequences.
Fluorogenic	Capable of producing a new moiety that generates fluorescence.
Flow cytometry	A process of automated cell analysis that utilizes detection of cells flowing in a fluid stream.
Fossiliferous	Referring to any rock (usually sedimentary) that contains fossils.
Fractionation	Separation of a complex mixture into fractions, each of which is enriched in one of the components of the mixture.
Gas chromatography	Chromatographic technique in which the stationary phase is a solid or an immobile liquid and the mobile phase is gaseous. The gaseous samples are separated based on their differential adsorption to the stationary phase.
Genomics	The science that studies the entire genetic content of an organism.
Heterotrophic	Using only organic matter for energy and growth.
Hydrolase	Any member of the class of enzymes that catalyze the cleavage of a chemical bond with the addition of water.

Levorotatory	A term used to describe an optically active substance that rotates the polarization of plane-polarized light in a left-handed (counterclockwise) direction.
Lipid	An organic molecule that is not soluble in water and often forms membranes.
Lipopolysaccharide	A composite of lipids and sugars, found in biological membranes from terrean organisms.
Lithobiont	An organism inhabiting hard rock substrates.
Magnetic biomineralization	The formation of magnetic bodies by biological action.
Metabolism	The processes or chemical changes in a cell by which food is built up (anabolism) into living protoplasm and by which protoplasm is broken down (catabolism) into simpler compounds with the exchange of energy.
Methanogenesis	The biological production of methane.
Microautoradiography	The technique of obtaining photographic images of microscopic samples by using neutron, x, or gamma radiation emitted from the sample.
Monomer	A building block of a polymer, including a biological polymer. Amino acids are monomers of polypeptides (proteins) and nucleotides are monomers of nucleic acids.
Muramic acid	A component of bacterial cell walls.
Nanopore	A pore in a membrane barrier, defined by having a diameter of several nanometers or less.
Oligotroph	A microorganism specifically adapted to grow under conditions of low nutrient supply.
Organic	Derived from living systems; more recently, given the fact that all known living systems contain carbon in reduced form (i.e., not carbonate), "organic" has come to mean "containing reduced carbon."
Peptides	Any of various natural or synthetic compounds containing two or more amino acids linked by the carboxyl group of one amino acid and the amino group of another.
Phylogeny	Ordering of species into higher taxa and the construction of evolutionary trees based on genetic relationships.
Pigment	Coloring matter in cells and tissues.
Planetesimals	The planetary bodies that formed the building blocks of all solar system planets and satellites.
Prion	A small proteinaceous infectious agent that resists inactivation by procedures that modify nucleic acids.

Ribosome	A minute, round particle composed of RNA and protein found in the cytoplasm of living cells and active in the synthesis of proteins.
Self-assembly	A process by which biomolecules such as proteins, nucleic acids, and lipids aggregate into more complex structures.
Tholin	The reddish tar-like organic residue created in simulations of the action of ultraviolet radiation on gases typically found in planetary environments in the outer solar system.
Vesicle	A microscopic volume defined by a boundary structure; examples include self-assembled vesicles bounded by a membranous lipid bilayer, and small cavities formed in volcanic rock by entrapment of a gas bubble during solidification.

ACRONYMS

ADP	Adenosine diphosphate.
AMP	Adenosine monophosphate.
ATP	Adenosine triphosphate.
CHONS	Containing carbon, hydrogen, oxygen, nitrogen, and sulfur.
CT	Computerized tomography.
DAPI	4′,6-Diamidino-2-phenylindole; a DNA dye that fluoresces (“blue”) when exposed to ultraviolet light and is used to stain the nuclear or background DNA of the cell in FISH assays.
DNA	Deoxyribonucleic acid; the genetic biopolymer of most terrean organisms.
EDX	Electron diffraction.
EDXA	Energy-dispersive x-ray analyzer.
EELS	Electron energy-loss spectrometry.
EM	Electron microprobe.
ESEM	Environmental scanning electron microscopy.
ESI	Electrospray injection; a way of generating ions for mass spectrometry that involves making droplets of a solution containing the analyte and evaporating it.
FA	Fluorescent antibody.
FISH	Fluorescent in situ hybridization; the detection of highly specific DNA probes that have been hybridized to either interphase or metaphase chromosomes using fluorescence microscopy.

GC-MS	Gas chromatography-mass spectrometry; an analytical tool that separates molecules by chromatography and then analyzes them for their mass.
IDP	Interplanetary dust particle.
IR	Infrared.
LSCM	Laser scanning confocal microscope.
MALDI	Matrix assisted laser desorption and ionization; a way of elevating large molecules into the gas phase to permit their analysis in a mass spectrometer that involves embedding these in a matrix, and then putting energy into the matrix to sputter it and the molecule of interest into the "gas phase."
MPN	Most probable number.
mRNA	Messenger RNA.
PCR	Polymerase chain reaction.
rRNA	Ribosomal RNA.
RNA	Ribonucleic acid.
S	Svedberg; a unit of sedimentation coefficient, equal to 10^{-13} second.
SAED	Selected area electron diffraction.
SEM	Scanning electron microscopy; images objects as small as 1 nanometer.
SIMS	Secondary ion mass spectrometry.
SNC meteorites	Three small classes of basaltic meteorites (shergottites, nakhlites, and chassignites) thought to have been ejected from Mars's surface during an impact.
TEM	Transmission electron microscopy.
TOF	Time of flight; a type of mass spectrometry that assesses the mass of an ion by the time that it takes to move in an electric field.
TPF	Terrestrial Planet Finder.
UV	Ultraviolet.
XAFS	X-ray absorption fine structure.
XRD	X-ray diffraction.
XRF	X-ray fluorescence.

B

Workshop Agenda

APRIL 25, 2000

I. INTRODUCTION

8:20 a.m.	Welcome and Overview of Local Astrobiology Activities Wesley T. Huntress, Jr., Carnegie Institution of Washington
8:45	Gerald A. Soffen, Goddard Space Flight Center
9:10	Norman R. Pace, University of Colorado
9:35	David W. Deamer, University of California, Santa Cruz
10:00	Break

II. SAMPLE RETURN

10:15	Donald Brownlee, University of Washington
10:35	Kenneth H. Nealson, Jet Propulsion Laboratory
10:55	Christopher F. Chyba, SETI Institute
11:15	Christopher McKay, NASA Ames Research Center
11:35	James F. Kasting, Pennsylvania State University
11:55	Lunch

III. DETECTING EXTANT LIFE

12:50 p.m. Chris Jacobsen, State University of New York, Stony Brook

1:20 William W. Barker, University of Wisconsin

1:50 Amit Meller, Rowland Institute at Harvard

2:20 Marilyn L. Fogel, Carnegie Institution of Washington

2:40 Break

2:55 Joseph L. Kirschvink, California Institute of Technology

3:25 Robert J. Cotter, John Hopkins University School of Medicine

3:55 Steven A. Benner, University of Florida

4:25 Panel Discussion on Techniques

Panelists: Christopher McKay, Chair
Chris Jacobsen
William W. Barker
Amit Meller
Robert J. Cotter
Steven A. Benner
Stephan Morse

5:00 Adjourn

APRIL 26, 2000

8:30 a.m. David A. Stahl, University of Washington

9:00 Gary Ruvkun, Massachusetts General Hospital

9:15 John Rummel, NASA Office of Space Science

IV. DETECTING EXTINCT LIFE

9:50 Luann Becker, University of California, Santa Barbara

10:20 J. Michael Moldowan, Stanford University

10:50 Break

11:05 Sherwood Chang, SETI Institute

11:35	Ariel D. Anbar, University of Rochester
12:05 p.m.	David McKay, NASA Johnson Space Center
12:35	Lunch
1:35	Sherry L. Cady, Portland State University
2:05	Panel Discussion Panelists: Marilyn L. Fogel, Chair David McKay Hayes Griffith
3:05	Break
3:20	Wesley T. Huntress, Jr., Carnegie Institution of Washington
3:50	Jeffrey L. Bada, Scripps Institution of Oceanography
4:20	Panel Discussion Panelists: John Kerridge, Chair Jeffrey L. Bada Kenneth H. Nealson Marilyn L. Fogel Jonathan I. Lunine

V. WRAP UP

5:00	Conclusion John Baross, University of Washington
5:20	Adjourn

C

Workshop Papers

NOTE: The papers in this appendix are published essentially as received from their authors. They therefore should be regarded as a record of the workshop proceedings and not as a refereed work. Where possible, typographical errors, egregious errors of fact, and non-pertinent remarks have been redacted by the Committee on the Origins and Evolution of Life.

Listed below by session, the papers are reproduced on the CD-ROM of the full report but are not included in the printed report owing to space limitations.

SESSION 1: INTRODUCTION TO THE DETECTION OF LIFE

SESSION 2: SAMPLE RETURN

SESSION 3: DETECTING EXTANT LIFE

SESSION 4: DETECTING EXTINCT LIFE

Session 1: Introduction to the Detection of Life

HISTORY OF LIFE DETECTION APPROACHES

Gerald A. Soffen
Goddard Space Flight Center
National Aeronautics and Space Administration

Abstract

The history of life detection (search for extraterrestrial life) began with the invention of the telescope. Astronomer Perceval Lowell stimulated public interest in the canals of Mars from his personal observations. With the advent of the National Aeronautics and Space Administation (NASA) in 1958, the search for extraterrestrial life became a U.S. national priority. Telescopic and spectroscopic observations were made of planets and interstellar space for detection of evidence for life in the 1960s. The development of numerous in situ instruments was supported by NASA in the period 1960-1975. These involved detection of form, function, and chemistry. The Viking experiments to Mars in 1976 carried numerous instruments and performed observations and experiments on the surface and atmosphere to directly detect living organisms or their organic products or debris. Terrestrial meteorites that have been recovered have been examined for possible signs of life. The Search for Extraterrestrial Intelligence (SETI) program has been listening for coherent radiation, searching for signals from other technological civilizations.

Introduction

NASA was initiated in 1958, in response to the Soviets having launched their Sputnik. On May 21, 1961, President Kennedy announced a decision to "put a man on the Moon before the end of the decade," despite the urgings of a number of U.S. scientists who were arguing for instrumented planetary missions. Homer Newell headed the first Space Science Office (OSS) and realized the needs of the new agency to invoke some practical as well as pure biology into the program. "NASA, (is) concerned with life sciences in a variety of ways . . . medical support for manned spaceflight, . . . life support systems, . . . aviation medicine . . . [and] exobiology (the search for and the study of extraterrestrial life) . . . Only space biology and exobiology could be regarded as pure science . . . " is quoted from a chapter entitled "Life Sciences: No Place in the Sun" in Newell's book on the history of space science. The decade of the 1960s was used by the exobiologists mostly to develop techniques of life detection.

Life Detection

The exobiologists agreed that Mars is the most likely planet in the solar system, other than the Earth, to support living organisms, and that if there was any martian life it would most likely be microbial in nature. At that time, there was serious attention to what was called "the wave of darkening" on Mars, which some planetologists interpreted as possible biological microorganisms that were quiescent during the dry part of the season and became active when water became available. Another spectroscopic observation was the "Sinton Bands," absorption at 3.58 μm and 3.69 μm, which suggested possible organic material; the bands were subsequently discovered to be caused by deuterium in the Earth's atmosphere.

Wolf Vishniac is credited to be the first to invent a device for monitoring microbial growth on another planet. Under a NASA grant in 1961, Vishniac built a laboratory model of a machine that would self-inoculate a small soil sample into liquid growth media. This was illuminated, and the subsequent increase in the number of organisms was measured by the changing opacity of the growing inoculation. This technique of measuring cloudiness by forward light scattering (nephelometry) to measure growth rate had been previously shown. Changes in pH as a function of time were also measured. The device called the "Wolf Trap" was developed and field-tested on Earth, but was not included onboard the Viking missions that landed the first life detection experiments on Mars in 1976.

Another life detection instrument funded by NASA utilized a radioactive tracer, ^{14}C, to measure the respiratory products of a growing culture of microorganisms. Gilbert Levin, having used this technique for the rapid

detection of coliform organisms in sewage, modified the growth media and the inoculation technique and built the first ^{14}C detector instrument for measuring respiration and growth of microbes on a foreign planet. He named this instrument "Gulliver," and the concept was used as one of the three life detection experiments onboard the 1976 Viking missions. This was called the "labeled release" (LR) part of the experiment. The results are still subject to interpretation and strongly debated.

A number of other concepts were developed during the 1960s. These included the Abbreviated Vidicon Microscope, gas chromatography and mass spectroscopy (GC-MS), J-band (detection of a reaction between an organic dye and organic macromolecules such as proteins or nucleic acids), ultraviolet spectroscopy (absorption at 1800 angstroms due to peptide linkages), optical activity, Multivator (photomultiplier to detect biochemistry of biological activity using substrates), adenosine 5'-triphosphate (ATP) detection using luciferase to measure light, detection of redox potential, ^{18}O and ^{15}N as tracers in metabolic reactions, gas exchange (to measure metabolism), and $^{14}CO_2$ uptake (to measure organic formation or photosynthesis). There was no shortage of ideas, but the actual development of these into practical experiments to be carried out on Mars required a more directed project than is normally done under research and development fiscal constraints.

In 1968, NASA decided that Mars was the most likely planet to have developed indigenous life. The agency commissioned the Viking missions to perform instrumented exploratory missions on Mars to take pictures of the surface, determine the nature of its indigenous organic compounds, perform life-detection experiments, analyze the atmosphere, and perform measurements of the meteorology and seismometry of Mars.

Venus was considered too hot (temperatures of over 600˚C) for the existence of living organisms. The Moon, with its intense ionizing irradiation, was not considered a likely site. Also, there were plans for lunar surface samples to be returned to Earth by the astronauts in 1969.

In 1969 the Viking missions to Mars (two identical spacecraft) were planned. Each of the dual spacecraft consisted of an orbiter, to serve as both the bus and the communication relay, and a soft lander. The lander contained cameras, life detection experiments, chemical analysis of the atmosphere and soil, and other instruments to measure the physical nature of the surface and atmosphere. In anticipation of finding organic material on the martian surface, great emphasis was placed on the organic analysis of collected surface samples as it might bear on the question of martian biogenesis.

Besides the camera, which might have detected macroscopic life, there were three experiments performed on the Mars surface and subsurface samples to detect viable microbial activity. Since Mars is nearer the asteroid belt (where organic-bearing carbonaceous chrondrites come from), it was logical that the Mars surface must contain organic material coming from the asteroid belt. The question then was to distinguish the indigenous organic materials on Mars and place them in two categories:

1. Biological or chemical through de novo synthesis; or
2. Deposition by meteoric infall.

The three life detection experiments on the Viking missions were to detect metabolism, growth, or organic synthesis. They used ^{14}C tracers and gas exchange techniques. LR inoculated soil samples with a dilute solution of nutrients (formic, glycolic, and lactic acids, glycine and alanine in both of their optical isomers). A second, called PR (for pyrolytic release), inoculated Mars soil samples with gaseous ^{14}CO and $^{14}CO_2$, while exposing this to simulated martian sunlight. If these gases were incorporated into the putative life, then by measuring the pyrolisate following exposure and subsequent combustion of the soil sample for ^{14}C, this would be a test for metabolic processes (perhaps photosynthesis!). The third test, GEX (for gas exchange), was to examine the gases evolved from a rich mixture of nutrients, which was inoculated with Mars soil samples.

Each of these experiments was tested repeatedly on both of the Mars landing sites in the northern hemisphere, 7,000 km apart. The organic analysis (related to the biological search) of the Mars soil sample was performed with sensitive pyrolytic GC-MS. The device had a mass range from 12 to 250 atomic mass units and sensitivity in the range of parts per billion. It sampled the martian atmosphere and soils similar to those used for the biological tests. Despite the success of the operation of this instrument, *no organic material was found on Mars at either of the landing sites*.

In the case of the three biology experiments, all gave results, but the interpretation of the results was inconclusive. The PR experiment gave one anomalous unrepeatable result and several negative ones. The LR experiment yielded some evolution of CO_2 but not enough to be explained by reproducing organisms. The GEX evolved O_2 from the soil sample when exposed to water vapor, but no metabolic gases when the nutrient was added. This result led to an interpretation that the Mars surface includes a superoxide material over most of its surface deposited by the global dust storms. This oxidizing material (highly reactive) presumably is formed by the action of unfiltered solar ultraviolet radiation splitting the H_2O and freeing the oxygen to form metal peroxide. This would account for the absence of organic material (from meteorite deposition) due to its destruction by the martian peroxide.

The conclusion reach by the Viking Biology Team was that "there is not conclusive evidence for life on Mars." They left open the door to other experiments in other places and the possibility of fossils, should there be no contemporary life on Mars. In the succeeding decade the interest in life on Mars waned, and those interested in exobiology turned to focusing on a better understanding of how life got started on Earth.

One high-risk, high-payoff effort in exobiology is the search for extraterrestrial intelligence. This employs the use of large antennas to listen for coherent signals of extraterrestrial origin. The argument for this is that other living systems may have developed a technological capability of communicating, using the electromagnetic spectrum, that we should be able to detect by listening to the wavelengths being used. Given the dimensions of space, a search of the whole cosmos would be extremely ambitious. An unquestioned directed coherent signal detected would be considered by many as one of the greatest discoveries of mankind! Through SETI, there have been a number of efforts over the past several decades to detect coherent signals that are extraterrestrial. To date, none have been detected. Since they require unusual types of technology, most of those efforts have been supported through nongovernment funds.

Acknowledgment

NASA has initiated and developed the program of extraterrestrial life detection over the past 40 years. The work has been done by scientists and engineers in the NASA laboratories, university laboratories, and scientists working in the private sector. Some efforts were performed by scientists in the Soviet Union and other countries, but that work is poorly documented.

Bibliography

W.A. Bonner, N.E. Blair, and F.M. Darbis, "Experiments on the Abiotic Amplification of Optical Activity," *Origins of Life* 11:119-134, 1981.

J. Chela-Flores and F. Raulin (eds.), *Exobiology: Matter, Energy, and Information in the Origin and Evolution of Life in the Universe,* Proceedings of the Fifth Trieste Conference on Chemical Evolution, Kluwer Academic Publishers, Dordrecht, Boston and London, 1998.

C. Chyba, P.J. Thomas, L. Brookshaw, and C. Sagan, "Cometary Delivery of Organic Molecules to the Early Earth, *Science* 249:366-373, 1990.

H.S.F. Cooper, *The Search for Life on Mars: Evolution of an Idea,* Holt, Rinehart and Winston, New York, 1976.

D. Deamer and G.R. Fleischaker (eds.), *Origins of Life: The Central Concepts,* Jones and Bartlett Publishers, Boston, 1994.

S.J. Dick, *Life on Other Worlds: The 20th Century Extraterrestrial Life Debate,* Cambridge University Press, Cambridge, Massachusetts, 1998.

E.C. Ezell and L.N. Edzell, *On Mars, Exploration of the Red Planet 1958-1978,* Scientific and Technical Information Branch, NASA SP 4212, Washington, D.C., 1984.

D. Goldsmith and T. Owen, *The Search for Life in the Universe,* Benjamin/Cummings Publishing Co., Menlo Park, California, 1980.

J.B.S. Haldane, "The Origin of Life" (1929) reprinted in J.D. Bernal, *The Origin of Life,* Weidenfeld and Nicolson, London, 1967, pp. 242-249.

N.H. Horowitz, *To Utopia and Back: The Search for Life in the Solar System,* W.H. Freeman and Co., New York, 1986.

H.P. Klein, "The Viking Biological Investigation: General Aspects," *Journal of Geophysical Research* 82:4677-4680, 1977.

H.E. Newell, *Beyond the Atmosphere: Early Years of Space Science,* Scientific and Technical Information Branch, National Aeronautics and Space Administration, NASA SP-4211, Washington, D.C., 1980.

A.I. Oparin, *The Origin of Life,* Dover Publications, Inc., New York, 1938.

W.J. Schopf, *The Cradle of Life,* Princeton University Press, Princeton, New Jersey, 1999.

G.A. Soffen et al., "Scientific Results of the Viking Mission," *Science* 194:1274-1353, 1976.

G.A. Soffen et al., "Scientific Results of the Viking Project," *Journal of Geophysical Research* 82:3959-4681, 1977.

Space Studies Board, National Research Council, *The Search for Life's Origins,* National Academy Press, Washington, D.C., 1990.

THE NATURE OF BIOCHEMISTRY IN THE UNIVERSE

Norman R. Pace
Department of Molecular, Cellular, and Developmental Biology
University of Colorado

Abstract

The search for life beyond Earth requires understanding the basic chemical requirements for life and the fundamental molecular structures upon which life is likely to be based. Life on Earth is a self-replicating, evolving system based on the element carbon, and life elsewhere is highly likely to be based on carbon as well. Life anywhere will be based on macromolecules (polymers) because of the high degree of specificity required in carrying out reactions in self-sustaining living organisms and the demand for storage of large amounts of information required for living organisms. Because of their ubiquity and simplicity, amino acids, purines, and pyrimidines are likely to be the universal monomeric foundation of polymers upon which life is based. Differences in evolutionary systems will lie at the higher-order levels, the structures of the large molecules assembled from the simple units, and the mechanisms through which they are assembled and in which they participate. Techniques for detecting life beyond Earth could take advantage of the expected universality of the foundations of biology, but must account for likely variations associated with diverse evolutionary pathways.

Introduction

Humans have long speculated about the possibility of life in settings other than Earth. Only in the past few centuries, however, have we been able to conceive of the specific nature of such settings; other planets around our own Sun, and solar systems like our own elsewhere in the physical universe. Speculation on the nature of life elsewhere generally has paid little heed to constraints imposed by the nature of biochemistry, however. A century of fanciful science fiction writings has resulted not only in social enthusiasm for the quest for extraterrestrial life, but also in fanciful notions of the chemical and physical forms that life can take, what the nature of life can be.

At the current stage of the exploration of life in the solar system we are, for the first time, confronting realistically the simple question: How to detect life regardless of its nature and origin? As we undertake detection of extraterrestrial life on nearby bodies in the solar system such as Mars and Europa, or terrestrial life on outbound or returning missions, it is instructive to try to put constraints on what the nature of life can be. The search for life elsewhere than Earth will be conducted at the chemical level, so we need to try to understand what are the basic chemical requirements for life and what are the forms that life can take.

What Is Life?

An early question that we must pose, indeed one which in the last analysis requires definition, is: What is life? Most would agree that self-replication is the fundamental goal of the life process. Most would also agree that the definition of life should include the capacity for evolution. Indeed the mechanism of evolution, natural selection, is a consequence of the competing drives for self-replication that are manifest in all organisms. The definition based on those processes, then, would be that life is any self-replicating, evolving system.

The processes of self-replication and evolution are not reliably detectable, however, so we need to incorporate into the definition of life information on the nature of the chemical reactions that provide the basis for self-replication and, consequently, evolution. Based on the expected properties of molecules likely to be needed to replicate an evolving entity, life that we encounter anywhere in the universe, and can recognize, is likely to be composed of organic chemicals that follow the same general principles as our own terrestrial kind of organic-based life. The operational definition of life then becomes the following: Life is a self-replicating, evolving system based on organic chemistry. This is what we need to search for.

Why Organic Chemistry?

The basic drive of life is to make more of itself. In order to replicate, life must capture energy and transform that energy into the chemistry of new life.[1] The many processes required to faithfully propagate a free-living organism necessarily require high degrees of specificity in the many interactions of the molecules that carry out the reactions. Such specificity requires information, in the form of complex molecular structure—large molecules. The molecules that serve terrestrial organisms typically are very large macromolecules, such as DNA, the repository of genetic information, and proteins. It is predictable that life, wherever we encounter it, will be composed of macromolecules.

Only two of the natural atoms, carbon and silicon, are known to serve as the backbones of molecules sufficiently large to carry biological information. Carbon, however, because of its electronic structure is unique beyond silicon in many ways. One important reason is that carbon, unlike silicon, can engage in the formation of chemical bonds with many other kinds of atoms, to thereby gain the chemical versatility required to conduct the many types of chemical reactions required for biological metabolism and propagation. Beyond carbon as a backbone atom, life—at least as we know it—requires hydrogen, oxygen, nitrogen, phosphorus, sulfur, and a host of metals, such as iron, magnesium, zinc, and others. The various organic functional groups and metals provide the enormous diversity of chemical reactions necessarily catalyzed by a living organism. Silicon, in contrast, is limited in the different atoms with which it interacts, and the large silicon molecules are monotonous compared to the infinitely combinatorial universe of organic macromolecules.

In addition, the electronic properties of carbon, unlike silicon, allow carbon to share readily with other atoms so-called double bonds or even triple bonds, chemical bonds that allow the capturing and delocalization of electronic energy. Carbon-containing compounds, therefore, can be highly polarized and thereby capture energy, and transfer this chemical energy in order to do work or to produce new chemicals in a catalytic manner. The potential polarizability of organic compounds contributes also to the specificity of intermolecular interactions, since ionic and van der Waals complementarities can shift to mesh with or to repulse one another.

The likelihood that life throughout the universe is carbon based is further encouraged by the fact that carbon is one of the most abundant of heavier elements. Astronomical studies find complex organic compounds strewn throughout interstellar space. More specifically, the common occurrence of carbonaceous meteorites speaks to an organic-rich beginning of our own solar system. Since life depends on the properties of elements heavier than hydrogen and helium, it is expected to occur only in association with second- or later-generation stars. This is because virtually all elements other than hydrogen and helium, including carbon, are manufactured within stars and must be expelled at the end of a star's lifetime.

The Universal Nature of Biochemistry

Life builds simple organic molecules such an amino acids and sugars from simple inorganic molecules: carbon dioxide, water, ammonia, phosphate, sulfur, and metals. The simple organic molecules then are used as building blocks for large molecules. Amino acids, for instance, are used to construct the long chains of proteins; simple sugars combine with purine and pyrimidine bases and phosphate to construct the nucleic acids. It seems logical that evolution of any organic-based life form would similarly result in the construction of complex molecules as repeating structures of simple subunits. Indeed, it seems likely that the basic building blocks of life anywhere will be similar to our own, in the generality if not the detail. Thus, the 20 common amino acids are the simplest carbon structures imaginable that can deliver the functional groups used in life, with properties such as repeating structure (the peptide unit), reactivity with water, and intrinsic chirality. Moreover, amino acids are formed readily from simple organic compounds and occur in extraterrestrial bodies such as meteorites, so they are likely to form in any setting that results in the development of chemical complexity necessary for life.

Similarly, the five-carbon sugars used in nucleic acids are likely to be repeated themes, perhaps in part because those are the smallest sugars that can cyclize and thereby confer spatial orientation on other molecules, for instance, the purines and pyrimidines that comprise the genetic information of terrestrial organisms. Further, because of unique abilities of purines and pyrimidines to interact with one another with particular specificity, those subunits, too, or something very like them, are likely to be common to life. Differences in evolutionary systems

likely will lie at the higher-order levels, the structures of the large molecules assembled from the simple units, and the mechanisms through which they are assembled and in which they participate.

Themes that are necessarily common to life extend beyond the building blocks. Energy transformation is a critical issue. The processes of life require capture, from physical or chemical processes, of adequate energy to conduct the chemical transformations requisite for life. There are only two such energy-obtaining processes that can support "primary productivity," the production of biological materials from inorganic carbon dioxide.

One general process, termed "chemoautotrophy," involves the oxidation and concomitant reduction of geochemical compounds. For instance, methanogenic organisms gain energy for growth by use of hydrogen as a source of high-energy electrons that are transferred to carbon dioxide, forming the waste product methane. Other microbes might use hydrogen sulfide as an energy source, respiring with oxygen to produce sulfuric acid. The earliest life on Earth probably relied on chemoautotrophic metabolism.

The second general process for obtaining energy, photosynthesis, uses light energy to generate energetic electrons that can be used to accomplish biochemical tasks. Photosynthesis arose early in the history of terrestrial life and probably drives most primary productivity on Earth today. The actual extent of terrestrial primary productivity remains unknown, however, since there currently is little information on chemoautotrophic metabolism that may be distributed through Earth's crust, wherever the physical conditions permit.[2]

The requirements of biological energy-gathering strategies constrain the sites where life is to be sought. For example, a setting appropriate for chemoautotrophic life might be indicated or contraindicated by the occurrence or lack of an appropriate mix of oxidized and reduced chemicals. A setting for photosynthesis-based life requires sufficient light of appropriate quality. The light must be sufficiently energetic to support biosynthesis, but not so energetic as to be chemically destructive. These considerations constrain photosynthesis-based life to the spectral zone of about 300 to 1500 nm in wavelength. Below that habitable wavelength zone the light energy is sufficient to destroy organic molecules. Above that zone the light energy is probably insufficient to drive biological reactions.

Although terrestrial life and life that might arise independently of Earth are expected to use many similar, if not identical, building blocks, they also are expected to have some biochemical qualities that are unique. This expectation is because even the different evolutionary lines of terrestrial evolution also have engendered novelties unique to those lines. Thus, Archaea invented the biochemistry of methanogenesis, and the property of chlorophyll-based photosynthesis was invented among the phylogenetic domain Bacteria. Considering the variety of Earth's life, novelty, as well as commonality, must be expected elsewhere.

The expected similarity of chemistry in life's processes, regardless of the setting and origin of the life, assists in life detection in the extraterrestrial setting because it predicts that terrestrial chemicals are useful targets for analysis in any setting. On the other hand, the expected similarities of terrestrial and alien life forms complicate to some extent the interpretation of positive results. Thus, analyses of simple terrestrial-like biochemical compounds possibly could not distinguish clearly between an alien life form and a terrestrial contaminant or some organic compounds that might arise abiotically. Discrimination between different and the same origins for organisms would require analysis of macromolecules and genes. Particularly, the nature of the genetic information and the details of the information would be telling.

A Genetic Definition of Terrestrial Life

All terrestrial life is genetically related in an evolutionary past extending to 4 billion years ago or longer. All life on Earth is derived ultimately from a common ancestry. We see this relatedness in the many common structural and mechanistic features that constitute the molecular core of all cells. This relatedness is quantitatively explicit in the now-emerging maps of the course of evolution, phylogenetic trees based on gene sequences. Even in the absence of other biochemical information, genetic sequences could provide criteria to identify the evolutionary source of DNA-containing organisms and to distinguish terrestrial organisms from one another or from extraterrestrial.

The gene sequence-based overview of terrestrial biological diversity is embodied in universal phylogenetic trees such as that shown in Figure 1.[3] The construction of such a relatedness diagram is conceptually simple. Pairs

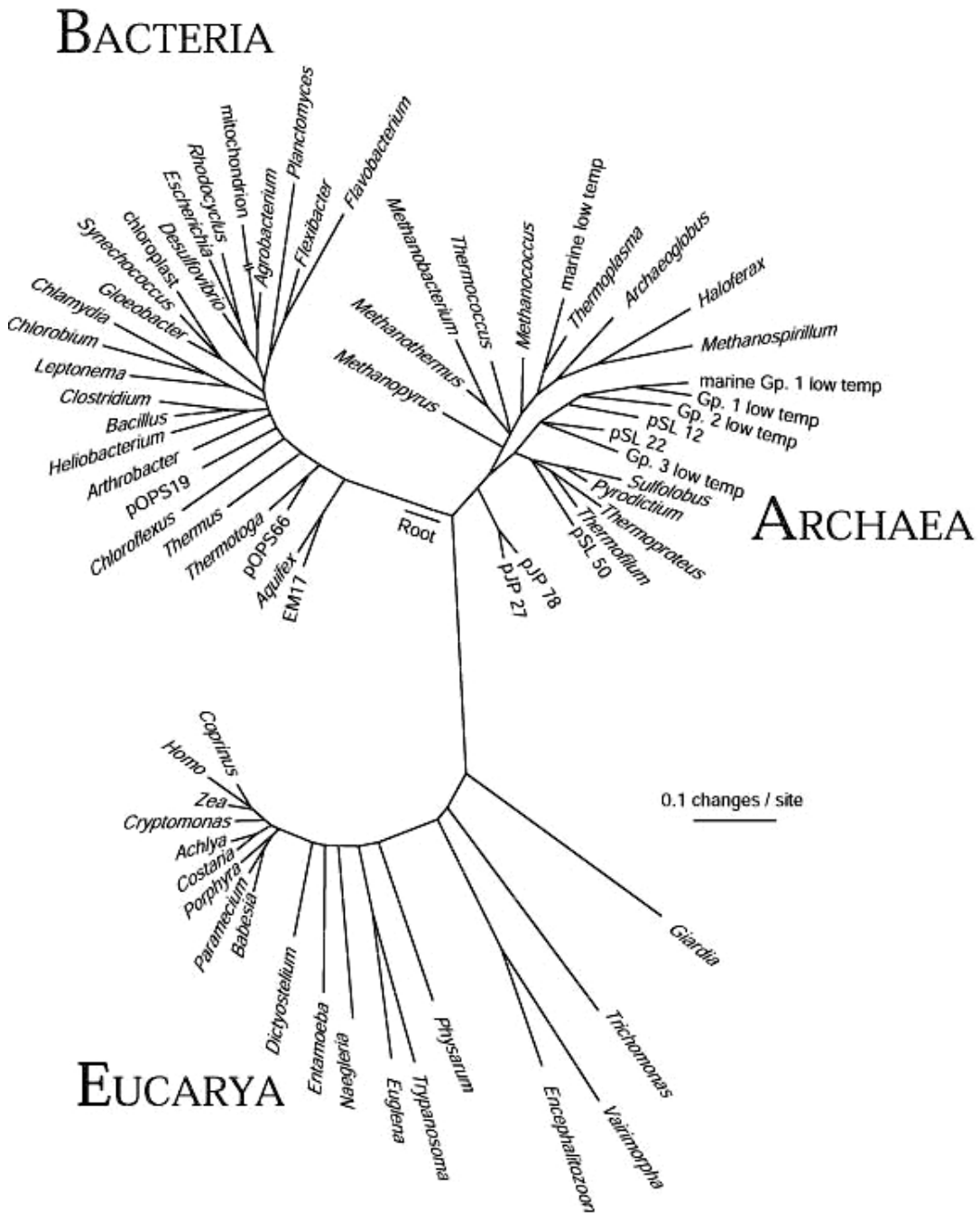


FIGURE 1. Universal phylogenetic tree based on small-subunit ribosomal RNA sequences. Sixty-four rRNA sequences representative of all known phylogenetic domains were aligned and a tree was constructed using the program FASTDNAML, correcting for multiple and back mutations. That tree was modified to the composite one shown by trimming lineages and adjusting branch points to incorporate results of other analyses. Evolutionary distance (sequence difference) between the species shown is read along line segments. The scale bar corresponds to 0.1 change per nucleotide. SOURCE: N.R. Pace, "A Molecular View of Microbial Diversity and the Biosphere," *Science* 276:734-740, 1997.

of corresponding sequences from different organisms are aligned, and the number of differences between the pairs of sequences is taken to be some measure of the "evolutionary distance" that separates the organisms that contributed the sequences. Pair-wise differences between many organisms can then be used to infer relatedness trees, maps of sequence change on the evolutionary paths leading to the modern-day sequences.

The phylogenetic tree shown in the figure is based on small-subunit ribosomal RNA (rRNA) sequences, but the same topology results from comparing sequences of any other genes involved in the central, nucleic acid-based information-processing systems of cells. On the other hand, phylogenetic trees based on metabolic genes, those involved in manipulation of small molecules and in interaction with the environment, commonly are not congruent with the rRNA version. Significant incongruities in phylogenetic trees made with different molecules are generally thought to reflect lateral transfers of genes or even the intermixing of genomes in the course of evolution.[4]

The intrinsic commonalities in sequences of fundamental genes that occur in all organisms provide for the detection and incisive identification of organisms of terrestrial origin. This is because organisms with the same evolutionary origin are expected to contain the sequences that are universally present in the relatedness group. Conversely, organisms with independent origin are unlikely to have evolved identical genetic sequences, even if the chemical structures of the subunits that comprise the genetic information were identical. Thus, in gene sequences we can recognize the terrestrial kind of life and distinguish it from life derived from a different evolutionary origin even in the face of substantial biochemical similarity. This will become an important issue if life is discovered on another body in the solar system.

It is now clear from meteorite studies that bodies can be transported from one planet to another—for instance, from Mars to Earth—without excessive heating. In principle, life of the terrestrial kind, such as spores and cysts of microbes, could have survived such transport and seeded the solar system over the eons, wherever conditions occur that are permissible to life.

Biochemistry and Molecular Biology in Life Detection

Techniques in biochemistry and molecular biology are applicable to the detection and evaluation of life, regardless of its evolutionary origin. Thus, knowledge of the chemical requirements for metabolism can point to chemical settings where life may be found. Highly sensitive methods for the detection of simple biochemical compounds potentially are useful in detecting signatures of life and metabolism, and thereby for directing further analyses.

The interpretation of results based on simple organic compounds can be compromised by abiotic sources, however. For example, carbonaceous meteorites contain amino acids and other chemicals that might generally be considered as indicative of life. Tests for suites of simple biochemical compounds or assays for chirality could remove potential ambiguity. Tests for specific macromolecules such as proteins or nucleic acids can be unambiguous in the detection of life. Available methods for detecting macromolecules are, however, generally far less sensitive than methods for detection and identification of small molecules and, in any case, are entirely dependent upon a general knowledge of the kind of life to be detected.

Techniques of molecular biology are based primarily on the properties of nucleic acid sequences. Thus, analytical tools require a clear notion of the nature of the target. Molecular probes based on terrestrial gene sequences are unlikely to be applicable to organisms of extraterrestrial origin. On the other hand, techniques such as the polymerase chain reaction can provide exquisitely sensitive tests for terrestrial organisms, and so serve for analysis of contaminants on spacecraft bound for potentially habitable sites. Nucleic acid-based tools are potentially useful not only for detection, but also for procedures that require identification, such as to track sources of contamination.

CHANCE AND NECESSITY IN BIOMOLECULAR CHEMISTRY: IS LIFE AS WE KNOW IT UNIVERSAL?

Steven A. Benner
Departments of Chemistry and Anatomy and Cell Biology
University of Florida

Abstract

Experimental and theoretical work in organic and biological chemistry suggests the following four general principles for detection of life in nonterrestrial environments:

1. A universal genetic molecule acting in water must have a repeating charge, a structural feature of an organic molecule that is easily detected by simple probes.
2. A single biopolymer life form, presumably characteristic of all new life, will recruit cofactors with structures suited for binding to it. These can be used as confirmatory signatures of nonterrestrial life.
3. Intermediates in metabolic pathways have distinctive thermodynamic relationships, and these can be recognized in a nonterrestrial sample.
4. Organic molecules that serve as biosignatures undergo predictable diagenesis. On Mars, we may already have found some of these.

Introduction

Life is defined as a self-sustaining chemical system capable of undergoing Darwinian evolution. Despite this, organic chemists and their expertise have too often been absent from the NASA effort to define the search for life. This paper reviews four topics where an understanding of the reactivity of organic molecules can contribute to our search for life on other planets.

Universal Biosignatures Based on the Chemistry of Genetic Molecules

To sustain Darwinian evolution, a biopolymeric system must have two properties. First, it must be capable of directing its own reproduction. Second, it must be able to suffer mutation without disrupting the physical and chemical properties that are essential for reproduction.

Polymers that display the second property have come to be known as COSMIC-LOPER ("Capable of Suffering Mutation Independent of Concern over Loss of Properties Essential for Replication").[5,6] COSMIC-LOPER behavior is scarce in organic chemistry. Typical biopolymers (proteins, for example) generally change, often dramatically, their physical properties (solubility, for example) even with small numbers of changes in their sequence.[7,8] The "textbook" case is sickle cell hemoglobin, where a single amino acid substitution has created a variant that undergoes partial precipitation.

Experiments with structurally modified nucleic acids have suggested that the polyelectrolyte (polyanion or polycation) structure of DNA and RNA, derived from the repeating phosphate group in the backbone, gives them their COSMIC-LOPER behavior.[9]

Phosphate groups force the interaction surface between strands as far distant from the backbone as possible, to the Watson-Crick "edge" of the nucleobases. In nonionic analogues of DNA, sugar-sugar interstrand interactions, sugar-backbone interstrand interactions, interactions between the sugar and backbone groups of one strand and the Hoogsteen edge of the nucleobases on the other, Hoogsteen-Hoogsteen interstrand interactions, and Watson Crick-Hoogsteen interstrand interactions, all become important. This means that nonionic oligonucleotide analogues have rich intermolecular conformational properties (just like peptides). Conversely, the repeating polyanion in DNA constrains interstrand recognition to a small number of possible interactions, permitting the rule-based nature of strand-strand interaction represented by the rules "A pairs with T; G pairs with C."

Phosphates keep the DNA molecule from folding on itself, allowing it to act as a template. The statistical mechanical theory of polymers suggests that the polyanionic backbone will cause natural oligonucleotides to adopt an extended structure.[10] Nonionic oligonucleotide analogues have rich intramolecular conformational properties, folding like peptides.

Electronic distribution in a molecule is described as an infinite series (monopole + dipole + quadrupole + ...), with the first nonvanishing term dominating. The repeating monopole (charge) in DNA makes dipolar interactions (hydrogen bonding) secondary to its global physical properties. The physical behavior of a DNA molecule is therefore largely the same regardless of its sequence. An encoding molecule needs physical properties that are largely independent of its sequence. One does not want to mutate a gene to get a better protein, only to discover that the mutant DNA gene precipitates.

From these experiments has come a working hypothesis that may guide our search for universal chemical structures in single-biopolymer systems and in encoding biopolymers in all forms of life: As a universal chemical characteristic, living systems must contain at least one biopolymer having a repeating charge, either polyanionic or polycationic (in water), because these structures are mutable without creating dysfunctional physical properties (such as precipitation). This biopolymer will perform both catalytic and repository roles (in a one-biopolymer system) and the information repository role (in a two-biopolymer system).

This hypothesis is particularly useful because it suggests a way to detect chemical remnants of life in non-terrean samples: One searches for biopolymers or their fragments with regularly spaced positive or negative charges. This turns out to be a rather simple structural feature to search for experimentally. A chip having an absorbent with a regularly spaced negative or positive charge will bind the polycation or polyanion (respectively) more tightly than competing monocations or monoanions, and provides a convenient analytical tool for detecting such materials.

Models for the Origin of Life

Modern life on Earth is based on two biopolymers, one specialized to do genetics (DNA), and the other specialized to do catalysis (proteins). Virtually all models for how life emerged are based on the notion that a single-biopolymer performed both roles in early life forms. A decade ago, we suggested that the transition from a single biopolymer life form to a two-biopolymer system might have been slow on Earth, and discussed evidence for this hypothesis that could be found in contemporary metabolism.[11] This suggests that single-biopolymer life forms would be more abundant in the universe.

The need for the polymer in a single-biopolymer Darwinian system both to be capable of searching mutation space independent of concern over the loss of properties essential for replication (COSMIC-LOPER) and to confer fitness through catalytic activity, generates competing (and occasionally contradictory) demands on the physical behavior and reactivity of the biopolymer, specifically:

- A biopolymer specialized to be a catalyst optimally has many building blocks, so that it can display a rich versatility of chemical reactivity; a biopolymer specialized to store information should have few building blocks, as a way of ensuring faithful replication. The inverse relation between fidelity and the number of building blocks is suggested both by theory[12] and experiment.[13]
- A biopolymer specialized to be a catalyst must fold easily so that it can form an active site; a biopolymer specialized to store information does not fold easily, so that it can serve as a template.
- A biopolymer specialized for catalysis must be able to change its physical properties rapidly with few changes in its sequence, enabling it to explore "function space" during divergent evolution; a biopolymer specialized to encode information must have physical properties largely unchanged even after substantial change in its sequence, so that the polymer remains acceptable to the enzymes required for replication (the COSMIC-LOPER property).

The contradiction between the structures or properties required for catalysis and those required for information storage creates problems. At the very least, any biopolymer forming a single-biopolymer Darwinian system

must generate a compromise between these goals. A two-biopolymer system allows the two biopolymers to specialize for genetic and catalytic roles, respectively. Thus, if an RNA world existed and if it had more than the four standard building blocks, one would have expected the number of building blocks to have been reduced after the breakthrough to translation to allow specialization of nucleic acids for encoding function. Further, once invented, proteins would have been expected to acquire functionality rapidly to match that not already available in RNA cofactors. The "palimpsest" of modern metabolism can be read exactly as such.[14]

Reconstruction of the metabolism of the putative RNA world on early Earth suggests that cofactors having structural features analogous to the structure of the catalytic biopolymer might be one way to solve these contradictions. Should non-terrean samples become available and a putative genetic biopolymer identified based on its repeating charge, a rational chemical approach to confirm the suspicion of non-terrean life would be to identify small organic molecules that are plausible building blocks of the biopolymer carrying appended reactive groups not found in the biopolymer itself. Using the RNA world model, such molecules would include reduced nicotinamide-adenine dinucleotide (NADH), flavin-adenine dinucleotide (FAD), *S*-adenosylmethionine, coenzyme A, and adenosine 5'-triphosphate (ATP), inter alia, presumed to be vestiges of a time when terrean life was struggling to resolve the contradicting demands between genetics and catalysis in RNA as its primordial single biopolymer life form.

Alternatives to Terrean Molecular Biochemistry

The disadvantages of a single-biopolymer life form center on the difficulty of finding a single molecular system that can satisfy competing and contradicting demands imposed by its genetic and catalytic roles, respectively. Single biopolymer life forms have advantages, however. For example, they require fewer resources; one can, for example, imagine an RNA-based organism that requires fewer sulfurs than a two-biopolymer organism. Single-biopolymer organisms can also be very small since they do not need the translation machinery, which occupies over 70 percent of the volume of a classical microorganism.

Single biopolymer life systems can also be sustained by very small metabolic repertoires. We recently proposed a 50-step metabolism that could support an autotrophic RNA organism.[15] Should samples be available from non-terrean life and a putative genetic biopolymer identified based on its repeating charge, those with an understanding of organic chemical reactivity would be able to suggest pathways to generate its building blocks, in much the same way as an understanding of organic reactivity was used to hypothesize metabolic pathways in terrean organisms during the great classical period of chemistry that dominated the biochemical sciences from 1930 until about 1960, and included such figures as Robinson, Krebs, Woodward, and Bloch. Intermediates in these pathways could then be sought using the mass spectrometric analytical tools of the new millennium.

Two features are expected to distinguish biochemical pathways composed of steps catalyzed by macromolecules from nonbiological pathways. First, steps catalyzed by macromolecular catalysts surmount problems with regio- and stereochemistry with far greater ease than steps catalyzed by smaller, nonbiological catalysts. This is transparently obvious in the "design" displayed by metabolic paths. They do not "select" intermediates with much of a concern of competing reactivity.

The high specificity of macromolecular catalysts also permits the thermodynamics of metabolic pathways to be different from nonbiological chemical pathways.[16] Except for those catalyzing regulated steps or the last step in a reaction sequence, metabolic steps can be approximately isoenergetic. This allows natural biochemical pathways to be more efficient than multistep synthetic sequences not involving enzymes. Because most of the steps in a natural biochemical pathway have equilibrium constants near unity, the starting materials and reagents need not be extremely reactive. Further, an enzyme-based reaction sequence can be extended indefinitely with essentially no extra energetic cost, allowing major rearrangement of the atoms in a starting material in one pot to yield a product with an entirely different structure. Because the final step in a biosynthetic pathway is exergonic, the yields are close to quantitative. The high specificity of natural enzymes allows such pathways to proceed without the chemical chaos that would result should the enzymes catalyze even small amounts of undesired side reactions.

Should non-terrean samples become available, a putative genetic biopolymer be identified, the associated cofactors found, and a metabolic pathway identified, that pathway might be identified as being "biological" by its design without major concern for regio- and stereoselectivity issues. The thermodynamics of the pathway could then be identified to see if they fit the biological model.

Organic Molecules on the Surface of Mars

Ultimately, the search for organic vestiges of life must involve experiments on non-terrean material. In designing these experiments, we must recognize that organic matter of nonbiogenic origin is abundant and may generate a large signal beneath which a smaller signal of biogenic organic material must be sought. When trying to identify this signal, an understanding of organic chemical reactivity will be needed to predict the diagenesis of nonbiogenic organic material.

On Mars, for example, meteorites almost certainly deliver a large amount of nonbiogenic organic material to Mars. Gas chromatography-mass spectrometry (GC-MS) on the Viking 1976 Mars missions did not detect these.[17] This suggested that the martian regolith might hold a potent oxidant that converts all organic molecules to carbon dioxide rapidly relative to the rate at which they arrive. A different conclusion emerged in light of what is known about the oxidation of organic compounds generally and the nature of organics likely to come to Mars via meteorite.[18] In particular, nonvolatile salts of benzenecarboxylic acids, and perhaps oxalic and acetic acid, should be metastable intermediates of meteoritic organics under oxidizing conditions. Salts of these organic acids would have been largely invisible to GC-MS.

Approximately 2 kg of meteorite-derived mellitic acid may have been generated per square meter of martian surface over 3 billion years. How much remains depends on decomposition rates under martian conditions. Since available data do not require that the surface of Mars be very strongly oxidizing, some organic molecules might be found near the surface of Mars, perhaps in amounts sufficient to be a resource. Future missions should seek these.

However, large amounts of organic molecules will almost certainly complicate the search for organics from (an entirely hypothetical) martian life. In principle, the experimental procedure will begin by creating an inventory of all organic molecules in a martian sample. Those that can be attributed to diagenesis of meteoritic organics are then subtracted from the sample. The residuals are candidates for being Mars derived. As models for the diagenesis will be sensitive to the nature of inorganic species on the planet that might catalyze various transformations, the list of possible diagenesis products will almost certainly change as more is learned about the chemical structure of the martian regolith. Additional nonbiological processes for making reduced carbon (such as the ultraviolet-derived fixation of carbon monoxide via ultraviolet-based photochemistry) must also be considered, together with the fact that biological molecules on the surface of Mars have presumably also undergone diagenesis.

Acknowledgment

Work described in this paper was supported in part by the National Aeronautics and Space Administration through its Exobiology and Astrobiology programs.

SELF-ASSEMBLY PROCESSES: STEPS TOWARD LIFE'S ORIGINS

David W. Deamer
Department of Chemistry and Biochemistry
University of California, Santa Cruz

Abstract

A fundamental property of life is the capacity for polymer synthesis in a confined space, using free energy and nutrients available in the environment. In contemporary cellular life, two polymers are central to this process: Nucleic acids store and express genetic information, and proteins have structural and catalytic functions. In order to have the capacity for evolution, the two polymers are necessarily linked through a genetic code and translation process that couples mutational changes to catalytic function. The origin of cellular life presumably occurred by self-assembly of organic compounds on the prebiotic Earth into encapsulated molecular systems capable of catalyzed polymer synthesis. Although it is unlikely that nucleic acids and proteins as such were components of the first living systems, analogous polymers were produced by an as yet unknown synthetic pathway, which were capable of interacting in such a way that evolution was possible. Laboratory models of such systems offer a promising approach to test hypothetical scenarios for the origin of cellular life.

Introduction

Nucleic acid replication, transcription, and protein synthesis are characteristic of all contemporary forms of life, but such highly evolved processes could not have played a role in the steps leading to the origin of molecular systems having the properties of the living state. Instead, we must consider simpler physical processes that are collectively referred to as self-assembly. Certain organic molecules have properties that allow them to spontaneously organize into larger structures, a common example being the self-assembly of amphiphilic soap molecules into soap bubbles. All living cells are defined by membranes, and the same forces that act between soap molecules also stabilize membrane boundaries between the cytoplasm and the external environment.

Two other self-assembly processes play central roles in all life today. The first is the folding of amino acid polymers into highly ordered structures of functional proteins. This folding process occurs when amino acids are linked into proteins on ribosomes, and the folded state is stabilized by physical forces acting between the amino acids that compose the polymer. If protein-like molecules were somehow produced on the early Earth, they would also have the capacity to fold into a variety of structures, some of which could perform catalytic functions in primitive forms of life.

The second basic self-assembly process involves base pairing in nucleic acids, which is stabilized by hydrogen bonding between complementary bases. The resulting intra- and intermolecular forces produce structures such as hairpins and helices in DNA and RNA. Base pairing also plays a role in catalyzed DNA replication when nucleotides in solution bind specifically to complementary bases in templates. All life today depends on such self-assembly processes, and the earliest forms of life must have had primitive versions incorporated in their molecular systems.

Our current understanding of self-assembly processes in contemporary cellular life leads to a variety of hypothetical scenarios about how life can begin on a planetary surface.[19] Given the presence of liquid water, there is little doubt that mixtures of organic compounds present on prebiotic Earth would become organized into more complex systems by self-assembly. Such microscopic molecular systems can be thought of as countless natural experiments that would occur globally for tens of millions of years prior to the origin of life. The next step toward life would take place when a few such systems happened to contain the particular set of molecules that allowed capture of energy and nutrients from the environment to be used for polymer synthesis. As noted earlier, polymer synthesis defines growth in all living systems today, and a molecular system capable of such reactions is well on its way toward the living state. Significantly, if one of the growing systems contained molecules that could be used as templates to direct further growth, a second polymeric molecule could be synthesized that was a replica of the

first molecule, thereby passing the information content of one molecule to a second molecule. This would represent the origin of replication, an essential feature of the definition of life.

The last step in the origin of life would occur when a growing, replicating system began to use the sequence of monomers in a molecule similar to a nucleic acid, to direct the sequence of monomers in a second kind of molecule such as a protein. This represents the origin of the genetic code and translation, and the beginning of life as we know it today. It also marked the beginning of biological evolution, because encapsulated molecular systems containing two different interacting molecules such as nucleic acids and proteins have the potential to undergo mutational change followed by selection.

Laboratory Simulations of Self-Assembled Protocells

How can we test the set of conjectures outlined above? One approach is to carry out laboratory simulations of molecular systems that might plausibly be present at some stage leading to the origin of life. If a simulation happened to produce a self-assembled molecular system with certain properties of the living state, the result would guide our thinking about pathways by which life could have begun on early Earth. In this brief review, one such system will be described in which amphiphilic molecules produce encapsulated microenvironments containing protein catalysts and nucleic acid templates.

Amphiphiles are defined as molecules having both hydrophilic and hydrophobic functional groups. Examples include fatty acids, detergents, and virtually all lipids such as phospholipids and cholesterol that are components of biological membranes. All amphiphiles are surface active, forming monomolecular layers at air-water interfaces, and some amphiphiles have the additional property of assembling into bilayers, which provide the permeability barrier that is essential to all biological membranes. For instance, when phospholipids are isolated from membranes and dispersed in aqueous phases, they produce spherical lipid bilayer vesicles called liposomes, which are in the size range of small bacteria.[20] Standard liposome preparations are able to capture macromolecules such as enzymes and nucleic acids, but their bilayers are relatively impermeable to polar and ionic solutes. Because growth and reproduction require the efficient transport of nutrients across the cell membrane, contemporary cells employ complex protein assemblies to catalyze the transport process.

Before such proteins had evolved, what mechanism was available to transport the nutrients required for cell growth? This has been a conceptual barrier to research progress on the origin of cellular life, but new approaches have at least partially resolved the problem. Modern lipids are highly evolved products of several billion years of evolution and typically contain two hydrocarbon chains 16 to 18 carbons in length. However, much simpler amphiphilic molecules, including fatty acids, can also form reasonably stable vesicles composed of bilayer membranes.[21,22] Furthermore, permeability is strongly dependent on chain length, so that shortening the chains of a given membrane lipid dramatically increases permeation rates of ionic solutes.[23] Such liposomes can encapsulate enzymes, nucleic acids, and other macromolecules, yet they are sufficiently permeable to allow influx of smaller solutes that are potential substrates. This property has permitted systems of encapsulated catalysts and nucleic acids to be used as models of primitive cell-like structures.[24,25]

Before going on, it will be useful to define the properties of such model systems, referred to here as protocells. An ideal experimental model of a protocell should have the following properties:

- There must be a simple mechanism for encapsulating macromolecules that could plausibly occur on the prebiotic Earth.
- An encapsulated polymer such as a nucleic acid must be capable of replication by a template-directed polymerization process. The replication must be imperfect so that errors can occur in the genetic information, thereby allowing selection of variations that lead to evolution within the system.
- A catalytic activity must be present that is somehow linked to the replication process, so that variations in replication affect the rate or efficiency of the catalyzed reaction. The catalyst must also be part of the growth and replication process.
- The replicating catalytic system must be maintained within a membrane-defined volume so that selection of variations can lead to “speciation” of the encapsulated genetic material.

• The boundary membrane itself must be able to grow. This could be accomplished either by accumulation of amphiphiles from the environment or by conversion of precursor molecules into amphiphiles. Furthermore, the growth must somehow be coupled to the internal replication process so that it neither lags behind nor gets too far ahead of polymer synthesis.

• There must be a mechanism that allows the system to separate into two or more smaller structures at some point in the growth process, and the smaller structures in turn should incorporate the capabilities of the larger system.

Anyone with a biological background would recognize that this list of properties could also be used to define a living cell. However, the system remains a model, even though in its final version it would approach the definition of life. The reason is that virtually no metabolism occurs. Instead, all of the substrates and energy required for the growth process are provided by the experimental conditions. Even with this limitation, a laboratory version of a protocell would be a useful self-assembled molecular system that incorporates most of the processes defining life.

Progress Toward a Model Protocell

In early experiments, an RNA polymerase called polynucleotide phosphorylase (PNPase) was encapsulated either in liposomes composed of dimyristolyphosphatidylcholine (DMPC)[26] or in vesicles prepared from oleic acid.[27] This enzyme does not depend on a template to synthesize RNA. Instead, it can use nucleotide diphosphates such as adenosine 5'-diphosphate (ADP) as both an energy source and a monomer to be incorporated into RNA. In a typical experiment with DMPC, the enzyme was captured in liposomes by a simulated tide pool cycle in which a mixture of the enzyme and lipids was first dried, then rehydrated in the reaction medium.[28] Under these conditions up to half of the original enzyme present can be encapsulated in the lipid vesicles that are produced upon rehydration. DMPC contains relatively short acyl chains, 14 carbons in length, and bilayers composed of DMPC have transient defects that allow ionized solutes such as ADP to cross the membrane barrier and supply the encapsulated polymerase with substrate. When ADP was provided to the encapsulated enzyme, vesicles containing the polymerase synthesized substantial amounts of RNA that could be detected as labeled bands in gels. The RNA could also be visualized inside the liposomes when stained with the fluorescent dye ethidium bromide and then observed by fluorescence microscopy. RNA was synthesized even if a protease was present in the medium, which demonstrated that the lipid bilayer protected the polymerase in the vesicle interior. The experiments with oleic acid vesicles show that even the simplest known membrane-forming lipid—a pure fatty acid—is able to function as a bounded environment for the enzyme. Again, the ionic substrate ADP is able to penetrate the bilayer membranes fast enough to supply the enzyme with substrate for RNA synthesis.

The encapsulated RNA polymerase provides a partial answer to the question of substrate permeation in a primitive cell. That is, a model cell composed of lipids maintain a catalyst and its RNA product in a protected environment, yet still provides access to a external nutrient and chemical energy in the form of ADP. Similar problems of substrate permeation must have been solved by the earliest forms of cellular life.

The next obvious step toward a laboratory model of a protocell is to encapsulate a system capable not only of simple polymerization, but also transcription. The viral enzyme T7 RNA polymerase can use DNA as a template to produce RNA with a transcribed sequence. Using the same conditions described above for PNPase, T7 RNA polymerase was encapsulated together with a specific DNA template in liposomes. Upon addition of four triphosphonucleotides, the encapsulated polymerase was able to synthesize RNA with a sequence transcribed from the template DNA.

In the future, it should be possible to build on this progress by capturing both a reverse transcriptase and the T7 RNA polymerase in liposomes. This represents a significant hurdle, since it requires two functional enzymes, two primers, and a template to be present in the same liposome. It also requires eight different substrates (four ribonucleotide triphosphates and four deoxyribonucleotide triphosphates) to cross the membrane barrier at a rate sufficient to supply the encapsulated enzymes with substrates. Although this is a challenging problem, the payoff of a functioning encapsulated polymerase will be significant, because ribozymes could then be included as

templates for the reaction. Ribozymes have the potential to act both as catalysts and as carriers of genetic information, and have been proposed as a primitive genetic material.[29] In one laboratory model of a replicating RNA system, a reverse transcriptase first copies a DNA strand from a specific ribozyme, and a second polymerase makes multiple copies of the RNA from the DNA, thereby amplifying the original RNA strand thousands of times as the cycle is repeated. Significantly, the ribozyme itself can also evolve under these conditions when faced with a suitable selective pressure. For example, Beaudry and Joyce found that it was possible to produce a specific catalytic site on a ribozyme by continuously selecting for that site with biochemical hurdles, a kind of molecular breeding carried out in the test tube.[30] Wilson and Szostak went on to show that a specific catalytic site could be selected from a mixture of trillions of random RNA sequences, similar to the kind of selection that would have occurred in a population of early molecules competing for a resource.[31] In such systems, the test tube acts as a macroscopic encapsulated environment that maintains the components in contact with one another while the investigator adds monomers and an energy source (nucleoside triphosphates) through the "channel" at the top of the test tube.

Further investigations of encapsulated replicating catalytic systems will help us to better understand what happened over 3.5 billion years ago as self-assembled molecular systems first began to grow, reproduce, and evolve toward the earliest forms of microbial life. It must be noted here that we have no information yet about what kinds of polymers and catalysts in the prebiotic environment could have played the roles of the nucleic acids, enzymes, and lipids that are components of the laboratory models described above. This is where our knowledge ends, and further progress in understanding the origin of life must wait until we know more about what monomers, polymers, and polymerization reactions are plausible under prebiotic conditions.

Relevance to Life Detection

Given that terrestrial life has a system of replicating polymers at its core, it is reasonable to assume that life elsewhere might also incorporate linear polymers as a structural and functional framework. It follows that life detection, for instance, in the europan ocean, could involve a search for linear ionic polymers that have been released into the environment by extant life, just as plasmids and nucleic acid fragments are released by many terrestrial microorganisms. The signal would be unambiguous, because there are no inorganic examples of linear ionic polymers resembling nucleic acids or proteins.

Until recently, instruments capable of detecting and identifying single linear ionic polymers in solution have not been available. However, recent advances in nanopore technology offer this promise, and the paper by Meller and Branton in Session 3 provides a detailed description of the remarkable sensitivity of a nanoscopic pore (nanopore) for characterizing linear polymers in solution. Earlier work established that individual single-stranded DNA and RNA molecules can be detected by a nanopore that is linked to appropriate amplification and signal processing capabilities.[32–34] The prototype nanopore used in these studies is α-hemolysin, a 33-kD protein isolated from *Staphylococcus aureus,* which self-assembles in lipid bilayers to form a channel with a relatively large pore.[35] The limiting aperture of the nanopore—1.5-nm diameter—is formed by a ring of alternating lysine and glutamate amino acid residues at the top of the stem, and this is followed by a 2.2-nm stem that penetrates the lipid bilayer (Figure 1A, adapted from Song et al.[36]).

To produce a nanopore, a single α-hemolysin channel is embedded in a planar lipid bilayer.[37] Unlike most membrane channels, α-hemolysin remains open at neutral pH ranges in 1.0 M KCl, and passes a steady ionic current in the range of 120 picoamperes (pA) with an applied voltage of 120 mV. When a linear molecule is driven through the pore by an electric field, it produces a characteristic blockade of the ionic current. The amplitude, duration, and modulation of the blockade all provide information about the molecule.

Figure 1B shows a single strand of poly(dC) (a synthetic DNA hompolymer) being translocated through the nanopore. The DNA molecule nearly fills the pore and inhibits the free flow of ions being driven by the imposed voltage.

Typical blockades of poly(dC) and poly(C) are shown in Figure 1C. It is clear that small changes in the structure of the DNA would likely have detectable effects on the amount of ionic current during a blockade and thereby produce a modulation of the blockade amplitude or duration. Furthermore, because of the tight fit,

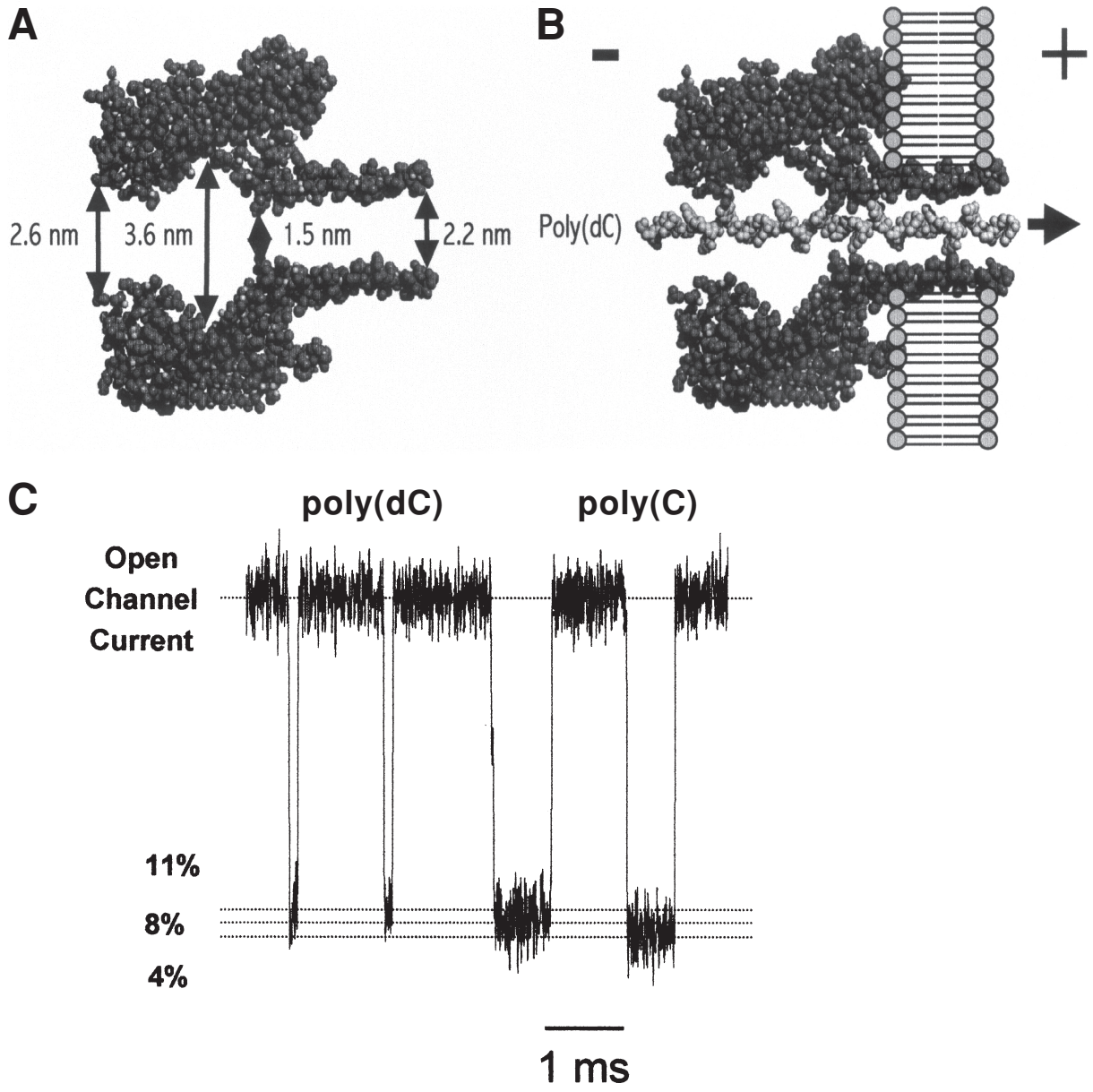


FIGURE 1. DNA and RNA blockades of the hemolysin pore. A pore in a lipid bilayer membrane separates two compartments containing a salt solution such as 1.0 M KCl. A voltage imposed across the bilayer causes an ionic current to flow through the pore of the channel. When anionic DNA is added to the *cis* side (negative) it can be captured by the large electrical field at the pore and driven through to the *trans* side. Figure 1A (adapted from L. Song, M.R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, and J.E. Gouaux, "Structure of Staphylococcal α-Hemolysin, a Heptameric Transmembrane Pore," *Science* 274:1859-1866, 1996) illustrates the hemolysin pore in cross section with main dimensions given in nanometers. Figure 1B shows a single strand of poly(dC) DNA traversing the pore. Ionic current blockades produced by single molecules of poly(dC) and poly(C) 100-mers are shown in Figure 1C. The blockade reduces the open channel current by approximately 90 percent (I/Io = 0.1), and for poly(dC) the duration ranges from 100 to 200 microseconds, or ~ 1 microsecond per nucleotide to traverse the length of the pore. Note that the poly(C) blockades have significantly longer durations, suggesting that RNA experiences greater molecular "friction" as it traverses that nanopore.

variations in primary and secondary structure would also be likely to affect the duration of the blockade, as demonstrated experimentally by Akeson et al. and Meller et al.[38,39] These modulations of amplitude and duration provide a signature by which approximately a variety of DNA and RNA molecules have been detected and identified with a high degree of accuracy. If similar signals were produced by a nanopore instrument analyzing melted ice from the europan ocean, it would be difficult to explain the results as anything other than a positive biomarker for extant life.

Summary

Terrestrial life is fundamentally polymeric, and life elsewhere would be likely to incorporate organic polymers for information storage, catalytic, and structural functions. It follows that detection of linear ionic polymers by a suitably sensitive instrument would provide an unambiguous signature of extant life. Nanopore technology offers a new approach to single-molecule detection. A nanopore is able to detect single molecules of linear ionic polymers such as nucleic acids, and to identify them according to unique signatures related to electrical signals that are generated when the molecule is driven through the pore. Nanopores can also be designed to detect particles ranging in size from nanoscopic to microscopic, and a nanopore instrument would therefore be useful for extant life detection missions to Europa and Mars.

DETECTING LIFE ON EXTRASOLAR PLANETS

James F. Kasting
Department of Geosciences
Pennsylvania State University

Abstract

NASA's proposed Terrestrial Planet Finder (TPF) mission (and the European Space Agency's [ESA's] Darwin mission) may eventually provide the capability of obtaining low-resolution thermal-infrared spectra of extrasolar planet atmospheres. One of the primary goals of these missions is to determine whether such spectra provide indirect evidence for life. A TPF-like interferometer looking at Earth from a distance would be able to detect CO_2, H_2O, and O_3. O_3 (ozone) is a potential indicator for life, since it is formed photochemically from O_2, and most of Earth's O_2 comes from photosynthesis. Exceptions to this rule (i.e., planets with high abiotically produced O_2 levels) are possible and must be considered. CH_4 (methane) is a potential bioindicator in atmospheres resembling that of early Earth, prior to the rise of O_2. Methane is an ambiguous indicator of biological activity, however, because it could also have significant abiotic sources from impacts and volcanism. Hence, other indicators of life on early-Earth-type planets need to be identified.

Introduction

A possible strategy for detecting life spectroscopically is to look for examples of extreme disequilibrium in planetary atmospheres. Sagan et al. credit Lederberg with suggesting this idea originally.[40,41] Lovelock made the concept more concrete by pointing out that in Earth's atmosphere, O_2 is many orders of magnitude out of thermodynamic equilibrium with reduced gases like CH_4 and N_2O.[42] All three of these gases are produced either exclusively or primarily by the biota. Lovelock argued that biological fluxes are needed to maintain this degree of disequilibrium. One has to be careful with this argument as planetary atmospheres are *always* out of thermodynamic equilibrium because they are being constantly irradiated by high-energy ultraviolet photons from their parent stars. Mars, for example, has 0.1 percent O_2 in its atmosphere, which is not in equilibrium with CO_2 and N_2, and Venus has clouds of sulfuric acid, which are not in equilibrium with SO_2 and H_2O. The idea is still useful, however. It just needs to be backed by detailed photochemical modeling to verify that certain atmospheric compositions are truly indicative of biological forcing.

Possible Indicators for Life

If one were to look at our own solar system from a great distance using an interferometer such as those proposed for TPF or Darwin, one would see a great difference between the atmospheres of Venus, Earth, and Mars. At low spectral resolution, Venus and Mars show only the strong 15-μm band of CO_2. By contrast, Earth exhibits bands of both CO_2 and H_2O, along with a pronounced absorption band at 9.6 μm caused by O_3. As argued by several authors,[43–45] O_3 is formed photochemically from O_2, and most of our O_2 comes from photosynthesis. Thus, under most circumstances, detection of O_3 by itself is fairly strong evidence for life. Two possible exceptions to this conclusion have been identified.[46] The first is a runaway greenhouse planet like Venus, where rapid loss of hydrogen from a water-rich atmosphere could result in abiotic O_2 buildup. The second is a frozen planet like Mars that also lacks volcanism. The absence of liquid water at the surface would inhibit oxygen loss by weathering, whereas the lack of volcanism would eliminate oxygen loss by reaction with reduced volcanic gases. Oxygen left behind by H_2O photodissociation followed by hydrogen escape could therefore accumulate indefinitely in such a planet's atmosphere. As mentioned previously, Mars itself has 0.1 percent O_2 in its atmosphere as a result of this process. Mars might have even more O_2 if the planet were slightly larger, so that it did not lose oxygen to space, but still not so large that it would have active volcanism. Despite assertions to the contrary,[47] there is no theoretical upper limit to the amount of O_2 that could build up in this manner.

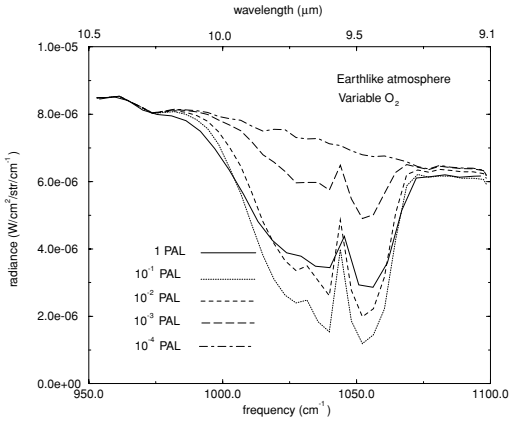


FIGURE 1. Synthetic spectra of an Earth-like planet containing various amounts of atmospheric O_2. SOURCE: T.L. Schindler and J.F. Kasting, "Synthetic Spectra of Simulated Terrestrial Atmospheres Containing Possible Biomarker Gases," *Icarus* 145:262-271, 2000.

One attractive point about using O_3 as a bioindicator is that it is a very sensitive barometer for O_2. A substantial ozone layer can develop at relatively low O_2 levels because of the nonlinear nature of ozone photochemistry.[48] Figure 1 shows simulated spectra in the 9- to 10-μm region for an Earth-like planet with various amounts of O_2. The 9.6-μm ozone band is quite strong for all O_2 levels exceeding 10^{-2} PAL (times the present atmospheric level). These calculations are preliminary because the stratospheric temperature profile has not been calculated self-consistently. Nevertheless, it appears that ozone should be a good indicator of O_2 down to very low O_2 levels.

Figuring out what biogenic gases to look for in an atmosphere resembling that of present Earth is relatively simple. A more difficult task is to determine what gases to look for in an early-Earth type atmosphere. Most authors,[49–52] but not all,[53,54] believe that atmospheric O_2 levels increased dramatically some time between 2.0 Ga and 2.4 Ga. This presumably represents the time at which net O_2 production from photosynthesis followed by organic carbon burial exceeded the rate of supply of reduced volcanic gases.[55] If so, the atmosphere prior to that time should have been almost completely anoxic. Some O_2 should have been present at high altitudes as a consequence of CO_2 photolysis, but the ground-level concentration should have been vanishingly small.[56] The column depth of O_3 would have been much less than is required to produce a strong 9.6-μm band.

In the absence of O_2, however, certain reduced biogenic gases could have been much more abundant. N_2O is not one of these. It photolyzes rapidly at the same wavelengths at which O_2 absorbs and, hence, may have been less abundant in the distant past than it is today.[57] CH_4 is a much better candidate. Its photolysis cutoff is at ~145 nm, so it is relatively stable against photolysis even in a low-O_2 atmosphere. (Most of the solar photons capable of dissociating CH_4 are in one particular line, Ly α, at 121.6 nm.) Today, CH_4 is destroyed mostly by reaction with the hydroxyl radical, OH. Since the atmosphere contains abundant OH, the lifetime of CH_4 is only about 12 years, so the abundance of CH_4 is relatively small, ~1.6 ppmv (parts per million by volume). In an anoxic atmosphere, OH would have been consumed by reaction with molecular hydrogen:

$$H_2 + OH \rightarrow H_2O + H.$$

So the lifetime of CH_4 would have been much longer. Photochemical calculations by Brown predict that the CH_4 lifetime would have been of the order of 104 years.[58,59] Thus, a methane flux equivalent to the present biological source, 550 Tg of CH_4 per year,[60] could have produced an atmospheric CH_4 mixing ratio in excess of 10^{-3} (Figure 2).

Methane is a good gas to look for in an extrasolar planet atmosphere because it has a strong absorption band

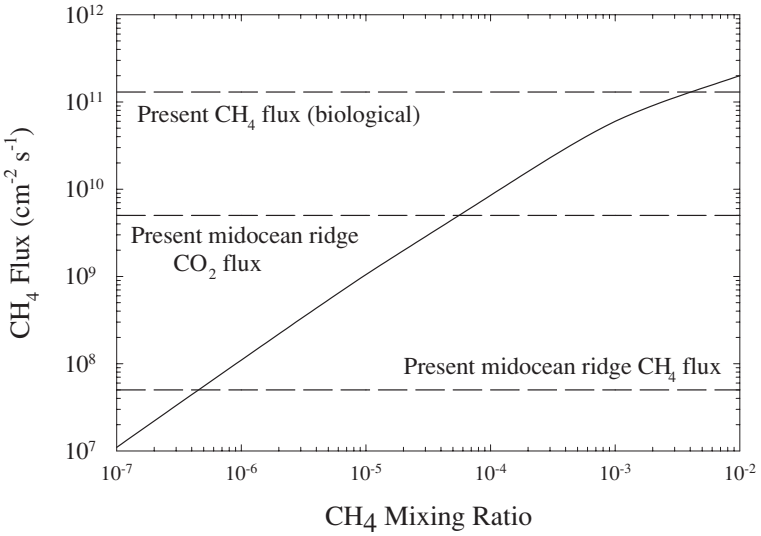


FIGURE 2. CH_4 destruction rate in an anoxic atmosphere as a function of CH_4 concentration. SOURCE: After J.F. Kasting and L.L. Brown, "Setting the Stage: The Early Atmosphere as a Source of Biogenic Compounds," in *The Molecular Origins of Life: Assembling the Pieces of the Puzzle,* A. Brack (ed.), Cambridge University Press, New York, 1998, pp. 35-56.

at 7.7 μm that is within the proposed spectral range for TPF.[61] It should be observable if present at a concentration ≥100 ppmv.[62] Thus, it could be seen on an early-Earth-type planet if methane was being produced there at the same rate that it is on Earth today. Therein lies ambiguity, however. First, we do not know what the methane production rate would have been on early Earth. Methanogenic bacteria are thought to be evolutionarily ancient,[63] but the anaerobic ecosystem on early Earth would have been very different from the modern ecosystem. All methanogens can generate methane from the reaction

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O.$$

Since both CO_2 and H_2 should have been abundant on the early Earth,[64] methanogens could presumably have flourished in a variety of surface environments. Still, we cannot say precisely what biogenic methane flux would be expected.

A further complication is that methane also has appreciable abiotic sources. (We do not include methane generated from fossil fuels, since these sources are ultimately biogenic.) Today, about 1 percent of the carbon emanating from midocean ridge vents is in the form of CH_4.[65] The other 99 percent of the carbon is outgassed as CO_2. On an early Earth with a more reduced mantle, however, most of the carbon outgassed at the ridges might have been released as CH_4. The abiotic source would then be within a factor of 25 of the present biological source (Figure 2). But the intensity of midocean ridge volcanism might also have been much higher in the past as a consequence of higher geothermal heat flow. At 4 Ga, geothermal heat flow should have been four to five times higher than today.[66,67] The rate of volcanism ought to scale somewhere between linearly and quadratically with heat flow.[68] Thus, at the higher end, the abiotic CH_4 flux could have equaled the present biological CH_4 flux. Although these crude estimates for abiotic CH_4 generation may be somewhat high, it is clear that we will have to be careful in using methane as a bioindicator. Further research on anoxic atmospheres and anaerobic ecosystems is needed to try to identify other gases that might be used as bioindicators in early-Earth-type atmospheres.

Acknowledgment

This work was supported by a grant from NASA's Exobiology Program.

REFERENCES FOR PAPERS IN SESSION 1

1. F.M. Harold, *The Vital Force: A Study of Bioenergetics,* W.H. Freeman and Co., New York, 1986.
2. T. Gold, *The Deep Hot Biosphere,* Springer-Verlag/Copernicus, New York, 1998.
3. C.R. Woese, "Interpreting the Universal Phylogenetic Tree," *Proc. Natl. Acad. Sci. USA* 97:8392-8396, 2000.
4. N.R. Pace, "A Molecular View of Microbial Diversity and the Biosphere," *Science* 276:734-740, 1997.
5. S.A. Benner and C.Y. Switzer, "Chance and Necessity in Biomolecular Chemistry: Is Life as We Know It Universal?" in *Simplicity and Complexity in Proteins and Nucleic Acids,* H. Frauenfelder, J. Deisenhofer, and P.G. Wolynes (eds.), Dahlem Workshop Report, Dahlem University Press, Berlin, 1999, pp. 335-359.
6. S.A. Benner, "The Molecular Origins of Life: Assembling Pieces of the Puzzle," *Science* 283:2026, 1999.
7. K. Johnsson, R.K. Allemann, and S.A. Benner, "Designed Enzymes: New Peptides That Fold in Aqueous Solution and Catalyze Reactions," in *Molecular Mechanisms in Bioorganic Processes,* C. Bleasdale and B.T. Golding (eds.), Royal Society of Chemistry, Cambridge, U.K., 1990, pp. 166-187.
8. K. Johnsson, R.K. Allemann, H. Widmer, and S.A. Benner, "Synthesis, Structure and Activity of Artificial, Rationally Designed Catalytic Polypeptides," *Nature* 365:530-532, 1993.
9. C. Richert, A.L. Roughton, and S.A. Benner, "Nonionic Analogs of RNA with Dimethylene Sulfone Bridges," *Journal Am. Chem. Soc.* 118:4518-4531, 1996.
10. D.A. Brant and P.A. Flory, "The Configuration of Random Polypeptide Chains. I. Experimental Results," *Journal Am. Chem. Soc.* 87:2788-2791, 1965.
11. S.A. Benner, A.D. Ellington, and A. Tauer, "Modern Metabolism as a Palimpsest of the RNA World," *Proc. Natl. Acad. Sci. USA* 86:7054-7058, 1989.
12. E. Szathmary, "What Is the Optimum Size for the Genetic Alphabet," *Proc. Natl. Acad. Sci. USA* 89:2614-2618, 1992.
13. M.J. Lutz, H.A. Held, M. Hottiger, U. Hübscher, and S.A. Benner, "Differential Discrimination of DNA Polymerases for Variants of the Non-standard Nucleobase Pair Between Xanthosine and 2,4-Diaminopyrimidine, Two Components of an Expanded Genetic Alphabet," *Nucleic Acids Research* 24:1308-1313, 1996.
14. S.A. Benner, A.D. Ellington, and A. Tauer, "Modern Metabolism as a Palimpsest of the RNA World," *Proc. Natl. Acad. Sci. USA* 86:7054-7058, 1989.
15. S.A. Benner, "How Small Can a Microorganism Be?" in Space Studies Board, National Research Council, *Size Limits of Very Small Microorganisms: Proceedings of a Workshop,* National Academy Press, Washington, D.C., 1999, pp. 126-135.
16. A. Moradian and S.A. Benner, "A Biomimetic Biotechnological Process for Converting of Starch to Fructose: Thermodynamic and Evolutionary Considerations in Applied Enzymology," *Journal Am. Chem. Soc.* 114:6980-6987, 1992.
17. K. Biemann, J. Oro, P. Toulmin III, L.E. Orgel, A.O. Nier, D.M. Anderson, P.G. Simmonds, D. Flory, A.V. Diaz, D.R. Rushneck, J.E. Biller, and A.L. Lafleur, "The Search for Organic Substances and Inorganic Volatile Compounds in the Surface of Mars," *Journal of Geophysical Research* 82:4641-4658, 1977.
18. S.A. Benner, K.G. Devine, L.N. Matveeva, and D.H. Powell, "The Missing Organic Molecules on Mars," *Proc. Natl. Acad. Sci. USA* 97:2425-2430, 2000.
19. H.J. Morowitz, *Beginnings of Cellular Life,* Yale University Press, New Haven, Connecticut, 1992.
20. A.D. Bangham, "The Liposome as a Membrane Model," in *Permeability and Function of Biological Membranes,* L. Bolis, A. Katchalsky, R.D. Keynes, W.R. Lowenstein, and B.A. Pethica (eds.), North-Holland, Amsterdam, 1970, pp. 195-206.
21. W.R. Hargreaves and D.W. Deamer, "Liposomes from Ionic, Single-Chain Amphiphiles," *Biochemistry* 17:3759-3768, 1978.
22. P. Walde, R. Wick, M. Fresta, A. Mangone, and P.L. Luisi, "Autopoietic Self-reproduction of Fatty Acid Vesicles," *Journal Am. Chem. Soc.* 116:11649-11654, 1994.
23. S. Paula, A.G. Volkov, A.N. Van Hoek, T.H. Haines, and D.W. Deamer, "Permeation of Protons, Potassium Ions and Small Polar Molecules Through Phospholipid Bilayers as a Function of Membrane Thickness," *Biophys. J.* 70:339-348, 1996.
24. D.W. Deamer, "The First Living Systems: A Bioenergetic Perspective," *Microbiol. Mol. Biol. Rev.* 61:239-262, 1997.
25. P.L. Luisi, "About Various Definitions of Life," *Orig. Life Evol. Biosph.* 28:613-622, 1998.
26. A. Chakrabarti, G.F. Joyce, R.R Breaker, and D.W. Deamer, "RNA Synthesis by a Liposome-encapsulated Polymerase," *Journal Mol. Evol.* 39:555-559, 1994.
27. P. Walde, A. Goto, P.A. Monnard, M. Wessicken, and P.L. Luisi, "Oparin's Reactions Revisited: Enzymatic Synthesis of Poly(adenylic acid) in Micelles and Self-reproducing Vesicles, *Journal Am. Chem. Soc.* 116:7541-7547, 1994.
28. R. Shew and D. Deamer, "A Novel Method for Encapsulating Macromolecules in Liposomes," *Biochim. Biophys. Acta* 816:1-8, 1983.
29. G.F. Joyce, A.W. Schwartz, S.L. Miller, and L.E. Orgel, "The Case for an Ancestral Genetic System Involving Simple Analogues of the Nucleotides," *Proc. Natl. Acad. Sci. USA* 84:4398-4402, 1987.
30. A.A. Beaudry and G.F. Joyce, "Directed Evolution of an RNA Enzyme," *Science* 342:255-257, 1992.
31. C. Wilson and J.W. Szostak, "In Vitro Evolution of a Self-alkylating Ribozyme," *Nature* 374:777-782, 1994.
32. J.J. Kasianowicz, E. Brandin, D. Branton, and D.W. Deamer, "Characterization of Individual Polynucleotide Molecules Using a Membrane Channel," *Proc. Natl. Acad. Sci. USA* 93:13770-13773, 1996.
33. M. Akeson, D. Branton, J.J. Kasianowicz, E. Brandin, and D.W. Deamer, "Microsecond Time-scale Discrimination Among Polycytidylic Acid, Polyadenylic Acid, and Polyuridylic Acid as Homopolymers or as Segments Within Single RNA Molecules," *Biophys. J.* 77:3227-3233, 1999.

34. A. Meller, L. Nivon, E. Brandin, J. Golovchenko, and D. Branton, "Rapid Nanopore Discrimination Between Single Polynucleotide Molecules," *Proc. Natl. Acad. Sci. USA* 97:1079-1084, 2000.

35. H. Bayley, "Triggers and Switches in a Self-assembling Pore-forming Protein," *Journal Cell. Biochem.* 56:177-182, 1994.

36. L. Song, M.R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, and J.E. Gouaux, "Structure of Staphylococcal α-Hemolysin, a Heptameric Transmembrane Pore," *Science* 274:1859-1866, 1996.

37. J.J. Kasianowicz, E. Brandin, D. Branton, and D.W. Deamer, "Characterization of Individual Polynucleotide Molecules Using a Membrane Channel," *Proc. Natl. Acad. Sci. USA* 93:13770-13773, 1996.

38. M. Akeson, D. Branton, J.J. Kasianowicz, E. Brandin, and D.W. Deamer, "Microsecond Time-scale Discrimination Among Polycytidylic Acid, Polyadenylic Acid, and Polyuridylic Acid as Homopolymers or as Segments Within Single RNA Molecules," *Biophys. J.* 77:3227-3233, 1999.

39. A. Meller, L. Nivon, E. Brandin, J. Golovchenko, and D. Branton, "Rapid Nanopore Discrimination Between Single Polynucleotide Molecules," *Proc. Natl. Acad. Sci. USA* 97:1079-1084, 2000.

40. C. Sagan, W.R. Thompson, R. Carlson, D. Gurnett, and C. Hord, "A Search for Life on Earth from the Galileo Spacecraft," *Nature* 365:715-721, 1993.

41. J. Lederberg, "Signs of Life: Criterion-System of Exobiology," *Nature* 207:9-13, 1965.

42. J.E. Lovelock, "A Physical Basis for Life Detection Experiments," *Nature* 207:568-570, 1965.

43. J.R.P. Angel, A.Y.S. Cheng, and N.J. Woolf, "A Space Telescope for Infrared Spectroscopy of Earth-like Planets, *Nature* 322:341-343, 1986.

44. A. Leger, M. Pirre, and F.J. Marceau, "Search for Primitive Life on a Distant Planet: Relevance of O_2 and O_3 Detections," *Astron. Astrophys.* 277:309-313, 1993.

45. J.R.P. Angel and N.J. Woolf, "Searching for Life on Other Planets," *Scientific American* 274:60-66, 1996.

46. J.F. Kasting, "Habitable Zones Around Low Mass Stars and the Search for Extraterrestrial Life," *Origins of Life* 27:291-307, 1997.

47. J. Rosenqvist and E. Chassefiere, "Inorganic Chemistry of O_2 in a Dense Primitive Atmosphere," *Planet. Space Sci.* 43:3-10, 1995.

48. M.I. Ratner and J.C.G. Walker, "Atmospheric Ozone and the History of Life," *Journal Atmos. Sci.* 29:803-808, 1972.

49. P.E. Cloud, "A Working Model of the Primitive Earth," *Am. J. Sci.* 272:537-548, 1972.

50. J.C.G. Walker, C. Klein, M. Schidlowski, J.W. Schopf, D.J. Stevenson, and M.R. Walter, "Environmental Evolution of the Archaean-Early Proterozoic Earth," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983.

51. J.F. Kasting, "Earth's Early Atmosphere," *Science* 259:920-926, 1993.

52. H.D. Holland, "Early Proterozoic Atmospheric Change," in *Early Life on Earth,* S. Bengtson (ed.), Columbia University Press, New York, 1994, pp. 237-244.

53. H. Ohmoto, "Evidence in Pre-2.2 Ga Paleosols for the Early Evolution of Atmospheric Oxygen and Terrestrial Biota," *Geology* 24:1135 1138, 1996.

54. H. Ohmoto, "When Did the Earth's Atmosphere Become Oxic?" *Geochemical News* 93(Fall):13, 1977.

55. J.C.G. Walker, C. Klein, M. Schidlowski, J.W. Schopf, D.J. Stevenson, and M.R. Walter, "Environmental Evolution of the Archaean-Early Proterozoic Earth," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983.

56. J.F. Kasting, "Earth's Early Atmosphere," *Science* 259:920-926, 1993.

57. J.F. Kasting and T.M. Donahue, "The Evolution of Atmospheric Ozone," *Journal of Geophysical Research* 85:3255-3263, 1980.

58. L.L. Brown, *Photochemistry and Climate on Early Earth and Mars,* Ph.D. dissertation, Pennsylvania State University, State College, Pa., 1999.

59. J.F. Kasting and L.L. Brown, "Setting the Stage: The Early Atmosphere as a Source of Biogenic Compounds," in *The Molecular Origins of Life: Assembling the Pieces of the Puzzle,* A. Brack (ed.), Cambridge University Press, New York, 1988, pp. 35-56.

60. M. Prather, R. Derwent, D. Ehhalt, P. Fraser, E. Sanhueza, and X. Zhou, "Other Trace Gases and Atmospheric Chemistry," in *Climate Change 1994: Radiative Forcing of Climate Change and an Evaluation of the IPCC IS92 Emission Scenarios,* J.T. Houghton et al. (eds.), Cambridge University Press, Cambridge, U.K., 1995, pp. 72-126.

61. C.A. Beichman, N.J. Woolf, and C.A. Lindensmith, *The Terrestrial Planet Finder (TPF): A NASA Origins Program to Search for Habitable Planets (JPL Publication 99-3),* NASA Jet Propulsion Laboratory, Pasadena, California, 1999, Chapter 4.

62. T.L. Schindler and J.F. Kasting, "Synthetic Spectra of Simulated Terrestrial Atmospheres Containing Possible Biomarker Gases," *Icarus* 145:262-271, 2000.

63. C.R. Woese and G.E. Fox, "Phylogenetic Structure of the Prokaryotic Domain: The Primary Kingdoms," *Proc. Natl. Acad. Sci. USA* 74:5088, 1977.

64. J.F. Kasting, "Earth's Early Atmosphere," *Science* 259:920-926, 1993.

65. J.A. Welhan, "Origins of Methane in Hydrothermal Systems," *Chem. Geol.* 71:183-198, 1988.

66. D.L. Turcotte, "On the Thermal Evolution of the Earth," *Earth Planet. Sci. Lett.* 48:53-58, 1980.

67. G.F. Davies, G.F. "Heat and Mass Transport in the Early Earth," in *Origin of the Earth,* H.E. Newsom and J.H. Jones (eds.), Oxford University Press, New York, 1990, pp. 175-194.

68. N. Sleep, private communication.

Session 2: Sample Return

SAMPLE RETURN FROM PRIMITIVE BODIES

Donald Brownlee
Department of Astronomy
University of Washington

Abstract

This paper discusses the collection of samples from primitive solar system bodies. A comet or asteroid sample return mission, though expensive, can be only half the price of a major "flagship," planetary mission. In addition to mission-targeted bodies, some 20,000 meteorites have landed on Earth at no cost. Over half of known meteorites found on Earth have been recovered from Antarctica. Meteorites are a wonderful resource, though they have some limitations. In addition to the normal meteorites, there are also the invisible meteorites—the cosmic dust particles—which are smaller than normal meteorites but are interesting because they provide an alternate and more representative sampling of bodies in the solar system.

Introduction

Most of the information we have about primitive solar system bodies comes from spectral reflectivity, which is a useful source of information but is not tied to astrobiology or mineralogy in a straightforward way. Asteroids exhibit different spectral reflectance types, and these have been studied in great detail. One of the most abundant is the so-called C-type, in which the dark material is typically attributed to carbon-rich materials, but in fact any material such as metal or sulfide can make a C-type spectrum. There are also types called P and D, which increase in reflectivity into the red region of the spectrum. In some cases there are matches with spectral reflectance of certain meteorite types. The most successful match is that of Vesta (and the Vestoids that are associated with Vesta) with the so-called howardite-eucrite-diogenite achondrite meteorites.

The meteorites come from the asteroid belt, with a few exceptions that appear to have come from Mars or the Moon. Most meteorites are delivered from the 1:3 resonance (referring to the ratio of the orbital period of the asteroids there to the orbit period of Jupiter) between 2.3 and 2.2 AU. There is a range of meteorite types that come from this small band of the solar system. The asteroid belt delineates the transition between the terrestrial planet and gas giant domains of the solar system, and there indeed appears to be a stratigraphy in the asteroid belt. The main belt is dominated by the C-type, the inner part is dominated by the E- and R-types, and the outer part is dominated by the P- and D-types.

The asteroids in the mid-belt commonly show OH in their reflectance spectrum—denoting the presence of hydrated minerals. Those farther out are thought to be very primitive—the common explanation for P- and D-types is that they have a high organic content, which is responsible for their dark, red higher reflectances. But these do not show OH. It is clear that the closer asteroids were heated above the melting point of water ice.

The black-body temperature today in the middle of the asteroid belt is about 180 K. In the primitive nebula, neither insolation nor the background gas could produce high enough temperatures to melt ice. There must have been an energy source that heated the asteroids and had a radial dependency. One of the most popular explanations is heating from short-lived radioisotopes such as ^{26}Al and ^{60}Fe. This imparts a radial dependence because the half-lives of these isotopes are compared to the accretion time of asteroidal-sized bodies, and plausibly those on the inner edge of the belt would form before those on the outer edge. Whatever the explanation, there was a heat source that had a radial dependence. In the 1:3 resonance, which is the source of many of our meteorites, bodies may have formed with a fair amount of ice in them and the ice then melted because of this mysterious heat source. The resulting liquid water then modified these rocks. Many of the primitive meteorites we have were thus relatively warm wet rocks for millions of years, and their parent asteroids may have had a much higher content of water in them than we see today.

In addition to meteorites, there are small particles, called cosmic dust particles, that come from both asteroids and comets. Some of these have quite unusual properties, including very high carbon content—up to 50 percent by

weight—much higher than meteorites. They can be collected from Earth's stratosphere as material rains down from space. It would be particularly attractive if we could collect dust particles directly from the tail of a known, documented comet. That is the role of the Stardust mission currently in space.

Sample Return Missions: Stardust

Figure 1 shows the Stardust spacecraft, which is a Discovery mission, with the collector and then the canister (on top), which is coming back to Earth in 2006. Stardust is collecting dust right now—very small particles that are believed to be streaming through the solar system from interstellar space. In 2004 Stardust will fly through the coma of comet Wild-2, collecting dust as close as 150 km from the comet's nucleus as well as making in situ measurements with a mass spectrometer. After the Wild-2 encounter, the sample canister will eject from the spacecraft and parachute down to a landing in Utah in 2006.

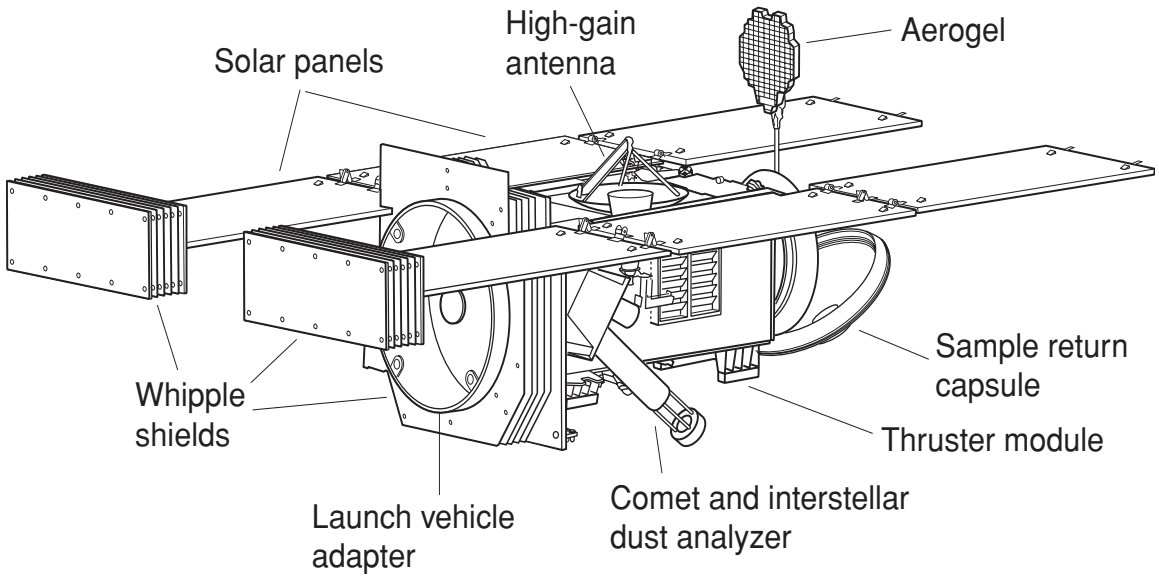


FIGURE 1. Schematic of the Stardust spacecraft with sample return capsule and other key parts marked.

Meteorites in Astrobiology

Meteorites do have an amazing variety of types, despite the fact that they come from a relatively limited range of semi-major axes in the solar system. Some of them have been heated to silicate melting temperatures, and some to the melting point of ice. While those objects clearly have been altered, others have been very well preserved.

One of the remarkable things about some meteorites is that there has been thermal alteration of material leading to significant changes in mineralogical structure—equilibration of one mineral to another. But the most chemically primitive meteorites, in terms of the highest abundance of volatile materials, remarkably seem to be the ones most affected by aqueous alteration—contact with liquid water. Therefore, from an astrobiology standpoint, it is interesting that meteorites have come from such a wealth of different environments that existed in one small strip of the solar system. They're all extremely old rocks—they go back to the origin of the solar system—and they were the most organic-rich materials that we know. How this fits into the astrobiology picture is as yet unknown. Certainly as we learn more about the relationships among meteorites, interplanetary dust, and their parent bodies (comets and asteroids), we will get a clearer picture of the evolution of organic material during the early history of the solar system.

MARS SAMPLE RETURN: LIFE DETECTION AT ALL LEVELS

Kenneth H. Nealson
Center for Life Detection
Jet Propulsion Laboratory

Abstract

The Mars sample-return (MSR) missions are presently in a state of reorganization as a result of the recent reports by the committees that examined the failed missions (Mars Climate Orbiter [MCO] and Mars Polar Lander [MPL]). There is little doubt that MSR is still an important and viable component of the Mars Exploration Program, and remains a central goal of the program, but the exact nature of the mission (e.g., the time of launch, the architecture of the mission, the international partners) cannot be specified. However, the basic components of sample return will remain the same: spacecraft assembly, launch, transit to Mars, landing on Mars, sample retrieval, launch of the sample to Mars orbit, sample retrieval in Mars orbit, return to Earth, Earth entry, sample retrieval and containment, and sample analysis. Life detection will play a prominent role throughout the mission, from the point of view of planetary protection, science protection, and life detection of terrestrial life forms, either extant or extinct.

Introduction

The MSR missions have been dominated by a desire for rapid sample return for several years, with a program for sample return aimed at the year 2008. This mission design included two separate launches (2003 and 2005) of lander-rover combinations that would retrieve samples (soil samples, drilled rock cores, and unconsolidated martian surface materials), place the samples into a sample container called an OS (orbiting sample), and launch the OS into low Mars orbit using the Mars Ascent Vehicle (MAV). After the second sample was placed in orbit, an orbiter was to be sent to fetch both samples and place them into the Earth Entry Vehicle (EEV), which would deliver them to the surface of the Earth via a hard landing at a site to be determined. Some variation on this was expected to occur with a sample returned to Earth at the target date of 2008.

With the unfortunate loss of two Mars missions (MCO and MPL), both the architecture and the timing of the MSR program are being reexamined. At this point in time it is difficult to present any details of the missions. What can be said with some authority is that MSR is still a major component of the Mars program, and the return of a pristine sample from the surface of Mars remains a major goal.

While neither the exact nature of the mission nor the timing of the MSR effort can be specified, it is possible to identify many parts of the mission and of the total program that will occur, and to discuss the role that life detection will play, in fact must play, in the various stages.

Mission Components

The components of the mission that will almost certainly occur in any future mission are shown in Table 1, although the details surrounding them may vary considerably.

For the purpose of the discussion, we divide the issue of life detection into two parts: earthly life, and extraterrestrial life. For the former, standard methods and logic can be used, while for the latter, other methods (so-called non-Earthcentric methods) must be employed.

The need for these analyses has been clearly noted by previous task groups of the NRC in reports presented in 1992 and 1997.[1,2] In these reports it was noted that while earthly life is not likely to prosper on Mars, in the interest of protecting the science of the mission, it is necessary to fastidiously clean any spacecraft that will land on the surface of Mars. This so-called science protection is of great importance to avoid false positives during in situ life detection experiments, as well as those that might occur in returned samples as the result of "roundtrippers"—earthly contaminants that survive the transit in both directions.

TABLE 1 Mission Components

Mission Phase	Activities Relevant to Life Detection
Prelaunch	Spacecraft fabrication and assembly
	Cleaning and sterilization
	Bioburden and cleanliness assessment[a]
Launch	n/a
	Bioburden and cleanliness assessment[a]
Transit to Mars	n/a
Landing on Mars	n/a
Mars activities	Sample retrieval
	Sample transfer to orbiting sample (OS) container
	Launch of Mars Ascent Vehicle
	Release of OS to Mars orbit
	(in situ science and analyses on Mars[a])
Retrieval of OS and transfer to Earth Entry Vehicle	n/a
Delivery to Earth by Earth Entry Vehicle	n/a
Retrieval of sample	Validation of sample integrity[a]
	Prelanding site analyses[a]
Sample containment	n/a
Sample analysis	Presentations in this volume[a]

[a]Parts of the mission discussed in the remainder of this presentation.

Assessment of Bioburden

The technology for detecting very low levels of bacteria, especially if they do not grow in culture media, is severely limited. One method that shows promise is that of fluorescence analyses of spacecraft surfaces using deep ultraviolet-excited broadband fluorescence. The advantage of the deep ultraviolet for this purpose is that no other stains need to be added, and yet at 248 or 224 nm, there is little fluorescence from minerals or metals, so that the signal-to-noise (S/N) ratio of the method is quite high. Once fluorescent spots are seen, they can be interrogated by more definitive methods (e.g., ultraviolet-Raman spectroscopy or other approaches) that can identify various groups of molecules indicative of earthly life at the appropriate size scale. Such an approach allows one to reduce the "search space" by a general scanning method, saving the time-consuming Raman methods for those few spots where fluorescence is seen. Such methods should allow one to accurately (and perhaps robotically) assess the bioburden of spacecraft surfaces, one of the key challenges for life detection in MSR.

Another issue with regard to bioburden assessment relates to the types and numbers of various organisms and groups of organisms that are present. It seems likely that one of the best approaches will be that of molecular genetics, but with the low numbers of organisms present on the surfaces after cleaning, even these methods are severely challenged to deliver definitive results. This would seem to be an area that is ripe for improvement and adaptation to bioburden assessment, as recommended by the 1992 National Research Council report.[3]

Molecular Tagging of Earthly Organisms

Another approach being tested is that of developing molecular tags for labeling bacteria that may be attached to the spacecraft and end up in the sample chamber or interfere with in situ life detection experiments. Such methods, if successful, would allow treatment of the parts of the spacecraft that will come into contact with the Mars sample, so that any organisms (dead or alive, intact or fragmented) would be labeled and thus identifiable as of terrestrial origin. For organisms in hard-to-reach places and/or shielded from the ultraviolet light of the sun during the mission, this method would be particularly valuable. Several approaches to this tagging method are now under consideration.[4,5]

In Situ Science

With the potential restructuring and delay of the sample-return mission, it is conceivable that more emphasis will be put on in situ measurements on the martian surface. For all such studies, should they be done as independent missions or done as part of an MSR effort, the potential interference by contaminants from Earth will be a factor. For this reason, both an accurate knowledge of the bioburden and a tagging method that could identify earthly contaminants, would enhance the integrity of the mission immensely.

Sample Handing and Analysis

When samples are returned to Earth for analysis, presumably new approaches will be employed, using non-Earth-centric methods for life detection. Such methods are not yet available but are under development in several laboratories.[6,7] A combination of non-Earth-centric, standard terrestrial life methods and screening for tagged materials might constitute a suitable approach for samples when returned.

Conclusions

There are a number of ways in which life detection will be of importance in the Mars program. The prospect of success will be greatly aided by improvements in methods for non-Earth-centric life detection, development of tagging methods, and improvements in molecular methods for terrestrial life detection. With these accomplishments, it should be possible to use a combination of these approaches to ensure both planetary protection and science protection adequate for the upcoming missions.

Acknowledgments

The help of many agencies in support of our work in life detection is gratefully acknowledged: the National Science Foundation in support of planetary atmospheric signatures; Department of Energy Natural and Accelerated Bioremediation Research program in support of methods for studying life associated with mineral interfaces; NASA Exobiology Program; and NASA Astrobiology Program for support in the development of biosignatures.

SEARCHING FOR LIFE ON EUROPA FROM A SPACECRAFT LANDER

Christopher F. Chyba
SETI Institute and
Department of Geological and Environmental Sciences
Stanford University

Abstract

An in situ search for life on Europa using a spacecraft lander should examine ice that appears to have been liquid recently and that seems most likely to have been derived from the ocean. An important lesson from the Viking missions to Mars is that searches for extraterrestrial life on Europa should establish the geological and chemical context needed to inform the results. The amount of sample available for life detection analysis could be maximized by the melting and filtration (and possibly the evaporation) of ice. In the case of sample return missions, both pristine and concentrated samples should be collected. Sample acquisition from below the radiation processing depth on Europa is essential. Sample acquisition and handling on Europa present difficult technical challenges.

Introduction: Definitions of Life

There is no broadly accepted general definition of life in the scientific community. A wide variety of definitions have been suggested, including metabolic, thermodynamic, biochemical, and genetic definitions. Each of these definitions fails in so far as it seems to include entities or phenomena that we do not wish to consider to be alive or excludes entities that are.[8] To these definitions can be added others; Monod suggested that a necessary part of a definition of living entities must include the notion of purpose or "teleonomy,"[9] whereas Shapiro and Feinberg propose that life should be defined as "the activity of a biosphere."[10] These definitions have their own important problems and in any case are unlikely to prove useful in a remote search for life. One must also be careful not to conflate (as I am somewhat guilty of here) the distinct ideas of "a living entity" and "life."[11,12]

One working definition for life that has attracted attention in the origins-of-life community is what we call the "chemical Darwinian" definition,[13] that "life is a self-sustained chemical system capable of undergoing Darwinian evolution."[14] The word "chemical" has the effect of excluding computer "life" by fiat. But however useful the Darwinian definition may be for interpreting laboratory experiments, or guiding thinking about how "the origin of life" on early Earth is to be conceived ("the origin of life is the same as the origin of evolution" is a common corollary), it too is unlikely to be of utility in a remote search for life. How long do we wait to determine if a candidate entity is capable of undergoing Darwinian evolution?

In assessing the possibilities of life in various locations in the solar system, we instead fall back on the less ambitious, but practically useful, notion of "life as we know it," meaning life based on a liquid water solvent, a suite of "biogenic" elements (most famously carbon present as organic molecules), and a useful source of free energy. From the point of view of in situ experiments to be conducted at these sites, what we need are definitions (or implicit definitions) that prove useful in a remote exploration context. I suggest that there are useful insights to be gained from the Viking search for life on Mars about how to conduct future searches for life and the role that varying definitions of life might play.

The Viking Search for Life on Mars

The Viking biology package contained three experiments,[15,16] each of which can be described as a search for evidence of metabolism in martian soil samples. That is, the Viking biology package implicitly adopted a metabolic definition of life. One of the experiments, the labeled-release experiment, gave especially provocative results. Indeed, with respect to the labeled-release results, the head of the Viking biology team wrote in 1978, "If

information from other experiments . . . had not been available, this set of data would almost certainly been interpreted as presumptive evidence for biology."[17]

Why then is it largely held in the scientific community that Viking failed to find life on Mars? There are several reasons. Theoretical modeling of the martian atmosphere and regolith suggests the production of oxidants (e.g., hydrogen peroxide) by the action of ultraviolet light, and these seem more or less able to account for the results of the three biology package experiments.[18] In this view, while the biology package hoped to find unambiguous evidence of martian biology, it instead was initially misled by unanticipated martian nonbiological chemistry.

Perhaps most important, however, was the failure of the Viking gas chromatograph-mass spectrometer (GC-MS) to find any organic molecules (released in stages at temperatures up to 500°C) in the martian soil at the part-per-billion level for molecules containing three or more carbon atoms (and at the part-per-million level for molecules containing one or two carbons).[19] Although not intended as a life-detection experiment, the GC-MS provided a search for life that implicitly assumed a biochemical definition: no (detected) organics, no life. In effect, a metabolic search for life yielding apparently positive results was undercut by the negative results of a de facto search based on biochemistry.

The interpretation of the labeled-release results as due to the action of martian oxidants is still debated and may be premature.[20,21] It is a remarkable fact that, a quarter century after Viking, it is still the case that the chemical oxidant hypothesis for the biology package results remains untested on the martian surface. Nevertheless, the extraordinary claim of life on Mars must defer to a plausible chemical explanation in the absence of more compelling evidence.

Lessons from Viking

With the benefit of 25 years' hindsight, I suggest that there are a number of lessons to be learned from the Viking experience on Mars for future remote spacecraft searches for life:

1. If the payload permits the luxury of more than one life detection experiment, a remote search for life is best conducted with experiments that in effect assume contrasting definitions of life.
2. Nevertheless, it is unlikely that, for example, the first Europa lander will permit more than 10 or 20 kg of science payload. In this case, it seems unlikely for the first lander that more than one life detection experiment will be present. In this case, the Viking experience suggests that the biochemical definition trumps other definitions. In the absence of a compelling case based on organic chemistry, it is unlikely that a biological interpretation of other experimental results will be accepted. At a minimum, then, we want to understand the organic chemistry present in our sample.
3. It is crucial to establish the geological and chemical context within which any biological experiments will be conducted. Had the presence of abundant oxidants in the martian soil already been demonstrated, very different experiments would have been flown in the Viking biology package.
4. The importance of negative results is axiomatic. Searches for life are best designed, where possible, to provide valuable information even if the searches fail to find any life.
5. We must nevertheless temper these conclusions with the realization that exploration need not, and often cannot, be hypothesis testing. Much of what we do in planetary missions is simple exploration.

Where Should We Land on Europa?

A europan lander is included as an upcoming mission in the current draft NASA Roadmap for solar system exploration. It is not premature to begin thinking about where a first lander mission should set down. At this time, however, it is difficult to give more than general guidelines to the answer to this question. Information from the Europa Orbiter mission will be crucial in helping us decide where to land. We should consider the option of a lander mission that would have the flexibility to launch to Europa prior to the full results from the Orbiter and be able to take advantage of the Orbiter data returned.

The first lander should touch down at a location that we think represents a site where liquid water from Europa's ocean has recently reached the surface. However, it is difficult on the basis of current knowledge to determine with confidence where these sites may be (or even if any exist). Current models for Europa's surface geology are still evolving rapidly, and it may yet be several years before they settle down and, perhaps, converge. (Even then, of course, there is no guarantee that they will converge to the correct model.) When first described,[22] chaos regions of Europa seemed possibly to provide candidate locations where the ocean may have reached the surface through catastrophic melt-through events. Now, however, models of viscous creep in Europa's ice argue against this explanation.[23] If lenticulae are in fact the expressions of solid-state diapirism in Europa's ice,[24] they too may prove poor locations for the first lander. Whether large cracks represent sites where ocean water reaches Europa's surface on a diurnal basis remains controversial, but if so they might be of special interest for a search for life.[25] It is unclear how to interpret "ponds" on Europa's surface that seem to indicate the eruption of liquid water from some source below the surface.[26,27] However, if we had to choose a site for the first europan lander based on Galileo data alone, and assuming the ability to target a region only kilometers across, we might well decide to land in such a place.

Information from the Orbiter would play a major role in choosing among the various geomorphological models and selecting the most appropriate landing site. It now appears likely, in light of theoretical models, geological observations, and perhaps most importantly, Galileo magnetometer results, that an ocean exists beneath kilometers or tens of kilometers of Europa's surface ice.[28,29] The first lander need not wait for the Orbiter to determine the presence of an ocean—the presence of an ocean, if not its detailed characteristics, now seems likely—but we would certainly want to make use of results from the Orbiter for landing site selection. Consistent with the recommendations of the National Research Council's Committee on Planetary and Lunar Exploration in *A Science Strategy for the Exploration of Europa,*[30] we should regard europan exploration as analogous to that of Mars, demanding a systematic program of exploration. Yet we do not require all the results of each Mars mission to be in hand before designing, building, and launching a subsequent mission. Rather, we recognize that Mars is a target of such importance that it will require multiple missions over many decades for its exploration, and we therefore interweave these missions in a way that incorporates new knowledge, as it becomes available, into an ongoing program.

Life Detection on Europa

A search for life on Europa should examine ice that appears to have been liquid recently and, in the best case, that seems most likely to have been derived from the ocean. Prior to or simultaneous with any experiments to search for life itself, however, a suite of measurements intended to establish chemical context should be performed. These would include determining the abundances of the major cations and anions present, the salinity, the pH, an analysis of the volatiles (e.g., CO_2, O_2, CH_4) present in the water, and a search for organic molecules. In fact, the latter probably represents the highest-priority life detection experiment to be conducted. If sufficient payload were available, additional experiments might include high-sensitivity searches for certain specific indicative organic molecules (such as amino acid enantiomers), a determination of key stable isotope ratios (e.g., $^{12}C/^{13}C$), or even fluorescent microscopy. But the chemical context should be established simultaneously or first.

What life detection sensitivity is required at Europa? Models cannot answer this question. Gaidos et al. have emphasized the difficulty of identifying sources of chemical disequilibrium on an ice-covered world and the concomitant difficulty of imagining large biomasses.[31] Photosynthesis is extremely difficult on Europa, though not entirely excluded.[32] Estimates of the biomass that could be supported by possible europan hydrothermal vents are very uncertain,[33] and the communication of biomass at the base of a 100-km-deep ocean with Europa's surface is uncertain. Ecosystems supported by the production of oxidants and organics in Europa's surface ice seem likely to yield low cell densities (see "Appendix" below).[34]

Because of these uncertainties, any search for life on Europa should scan a large amount of material in a manner that chooses particular sites for subsequent high-sensitivity investigation and/or take advantage of the opportunity to concentrate sample by melting and filtering (or perhaps evaporating) Europa's ice. I examine only the latter option in more detail here; both possibilities bear further investigation.

Sample Concentration and Handling

The amount of sample available for life detection on Europa should be maximized by the melting and filtration (or possibly evaporation) of ice. The capability to perform this sort of sample concentration is likely to be needed for both in situ exploration and sample return missions. In the case of sample return missions, both pristine and concentrated samples should be returned.

Moreover, sample acquisition from some depth into Europa's surface is essential. At a minimum, sampling should take place below the radiation-processing depth, and preferably below the impact gardening depth.[35] Certainly this means that sample acquisition should take place more than a centimeter below the surface (assuming densities of 1 g cm^{-3}) and preferably at depths greater than 10 cm.

Additionally, attention must be paid to the challenges of sample acquisition and handling for chemical or biological analyses. Whether the sample is acquired from the ice directly, from melting ice, or from melting ice and concentrating its contents, sample acquisition on Europa presents difficult technical challenges not previously encountered, with implications for technology development.

How much ice can we hope to process during a surface lander mission with a duration of about one month (the length of time likely to be permitted by the intense radiation environment at Europa's surface)? The energy required to melt 1 kg of ice on Europa, starting at an average surface temperature of 100 K, is given by

$$\Delta E = H_{fusion} + \int_{100}^{273} C(T)dT.$$

Here $H_{fusion} = 3.3 \times 10^5$ J kg^{-1} is the heat of fusion of ice and $C(T)$ is the temperature-dependent specific heat, which in J kg^{-1} K^{-1} for an absolute temperature T is $C(T) = 7.04\ T + 185$, giving $\Delta E = 6 \times 10^5$ J kg^{-1}. For a dedicated spacecraft power source of 20 W (chosen for this example to be comparable to the total electric power expected to be available for the science payload of the planned Europa Orbiter mission),[36] this calculation might suggest that ~100 liters is a likely upper limit for how much water could be melted and filtered during a month-long mission. However, a single dedicated radioisotope thermoelectric generator (RTG) "brick" could likely provide an order of magnitude more thermal power than this,[37] so these numbers are strongly dependent on decisions yet to be made regarding the power that a lander could in fact dedicate to the task.

Melting and filtration has drawbacks, however. In particular, filtration alone will not capture soluble organics. Some of these could be captured through an adsorption column, with the necessary additional mass requirement. Alternatively, rather than filtering the water, one could imagine evaporating it—though vaporized organics would have to be captured in this case. Evaporation would require substantially more energy than melting; the relevant heat of vaporization for water is $H_{vapor} = 2.5 \times 10^6$ J kg^{-1}, or nearly eight times the heat of fusion.[38] All told, evaporation would require about $\Delta E = 3 \times 10^6$ J kg^{-1}, or five times as much energy (or five times less sample processed) as in the calculations above.

However, these calculations neglect the power requirements of the sampling system that would core into Europa's ice, withdraw samples, and introduce them into a melting chamber. This sampling system would likely pose substantial challenges and could limit the total amount of sample acquired over a mission lifetime to values lower than those suggested above. Melting directly at the surface (without withdrawing samples into a chamber) poses much greater power requirements due to heat conduction out into the ice. Indeed, the initiation of melting-sublimation at Europa's ice would require about a kilowatt of thermal power.[39]

Appendix: Radiation-powered Life on Europa

I recently estimated the biomass that could be supported by mixing the ice irradiation products HCHO and H_2O_2 into Europa's ocean.[40] For growth limited by either energy or the carbon from HCHO, I found steady-state biomasses of $\sim 2 \times 10^{10}$ g and $\sim 4 \times 10^7$ g, respectively. E.J. Gaidos, K.N. Nealson, and J.L. Kirschvink of the California Institute of Technology have kindly informed me of a calculational error in the higher estimate. I correct that error here and briefly discuss its implications.

The calculation should be modified as follows: I estimate the efficiency, φ, for microbial biomass (dry weight) production by dividing the dry mass that can be produced per mole of adenosine 5'-triphospate (ATP), Y_{ATP}, by the energy required for ATP production, E_{ATP}.[41] For a variety of microorganisms growing anaerobically or aerobically, $Y_{ATP} \sim 10$ g mol^{-1}.[42] Typically, $E_{ATP} \sim 10$ kcal mol^{-1},[43] giving $\varphi \sim 1$ g kcal^{-1}. Were all the available energy used by microorganisms, this value for φ would lead, following Chyba (2000), to a steady-state biomass ~5×10^8 g. If microorganisms utilized only 10 percent of the available energy,[44] this value would be reduced by a factor of 10.

A biomass of ~10^8 g corresponds to ~5×10^{21} aquatic cells.[45] Were these cells distributed evenly throughout Europa's putative ocean—an unlikely scenario—average cell densities would be only about 1 cell per liter. Even if this water reached the surface and froze, such low cell densities would render life detection extremely difficult. For example, for an instrument (perhaps fluorescent high-performance liquid chromatography [HPLC]) with a sensitivity of ~10^5 cells, tens of thousands of liters of ice would have to be melted and filtered to yield sufficient sample for a detection. For comparison, a melter probe of 12-cm cross section that descended into Europa's ice (such as those being developed at the Jet Propulsion Laboratory[46]) and captured the resulting meltwater could provide about 10^3 liters of water for every 100 m of penetration depth.

These requirements could be greatly lessened if organisms were strongly concentrated in nutrient-rich regions near the ice-water interface,[47] as might be expected by analogy to the variable distribution of terrestrial microbes.[48,49] For example, if the microorganisms maintained themselves within the upper 10 m of the ocean, ice derived from this layer could have concentrations of ~10 cells cm^{-3}, requiring only ~10 liters of meltwater to be filtered. Biomass production at hydrothermal vents may also be possible at the bottom of Europa's ocean,[50] a possibility not considered further here. These uncertainties emphasize the importance of establishing chemical and geological context as an early and ongoing step in any search for life on Europa. This search would be aided by a choice of landing site where water from the ocean seemed most likely to have reached the surface, and by an ability to concentrate and examine as much sample as practical.

Note added in proof: More recent estimates have been made by Chyba and Hand.[51]

Acknowledgments

This work was supported in part by the NASA Exobiology Program and a Presidential Early Career Award for scientists and engineers.

SAMPLE RETURN FROM TITAN FOR EXOBIOLOGY

Christopher McKay
Ames Research Center
National Aeronautics and Space Administration

Abstract

Titan, the largest moon of Saturn is a natural environment for studies of the abiotic synthesis of organic molecules. Remote sensing and laboratory simulations have provided a basis for understanding Titan's organic chemistry. Soon in situ analysis will be conducted by the Huygens Probe. Further in situ and possibly sample return missions may be needed to provide a detailed understanding of how the atmospheric methane and nitrogen are converted to organic, possibly prebiotic, molecules.

Background

The surface pressure of Titan is 50 percent greater than that of Earth. The temperature at the surface is close to 94 K, decreasing to about 71 K at the tropopause, which is located at an altitude of 40 km.[52,53] Above the tropopause, the temperature rises rapidly to 160-180 K. The atmosphere is composed primarily of N_2 with less than 8 percent CH_4.[54,55] These molecules are the parent species for an active photochemistry resulting in the production of many hydrocarbons and nitriles in the stratosphere. Further chemistry and phase changes in the thermal environment of the stratosphere lead to the production of an extensive system of organic clouds and aerosols.

Titan has been associated with organic chemistry since early observations of Titan revealed that it had an atmosphere with methane present. The presence of methane and the reddish appearance of the satellite lead to the suggestion that solid organic aerosols were being produced in Titan's atmosphere by photochemistry. These observations prompted laboratory simulations in which gas mixtures with the composition of Titan's atmosphere irradiated with ultraviolet light, electrical discharge, or energetic electrons have produced a solid organic material—termed tholin.

The organic haze is not uniformly distributed on Titan and changes with season.[56,57] The particles in the main haze deck are probably fractal in structure with an equivalent volume radius of 0.2 micron. The haze material is organic and, if similar to laboratory tholin, has a C/N ratio in the range of 2 to 4 and a C/H ratio of about unity.[58] The haze significantly affects the thermal balance of Titan, causing an antigreenhouse effect that cools the surface by 9 K.[59] Titan's faintly banded appearance suggests strong zonal winds in the lower stratosphere. Condensate clouds of ethane or methane, if present, are thin, patchy, or transient. In infrared wavelengths the surface can be detected through the haze.[60]

Laboratory simulations have been carried out in an effort to reproduce the solid organic material thought to compose the Titan haze. The optical properties of the laboratory material—tholin—match the broad features of the geometric albedo spectrum of Titan.[61] If tholin provides a good analogue for the Titan haze then we can conclude that the haze is composed of refractory organics that, once condensed, do not evaporate and are ultimately deposited on Titan's surface.

The following are key questions about Titan's haze and clouds:[62]

- What produces the detached haze at high altitude?
- What is the cause of the seasonal variation? It is not production itself and appears to be related to dynamics.
- Is the haze material at high altitude, particularly the detached haze layer, of different composition than lower in the atmosphere? Are there seasonal changes in composition (e.g., does the C/N ratio vary with altitude and latitude)?
- How is nitrogen incorporated into the haze?

- What is the efficiency of haze particles as condensation nuclei for the various stratospheric hydrocarbons and nitriles and tropospheric methane?
- Is the lower atmosphere cleared of haze by rainout?
- What are the compositions, altitudes, and particle sizes associated with stratospheric condensate clouds? At what latitudes are they located, and at what times of the year?
- What are the frequency of occurrence, areal extent, and altitudes of tropospheric methane clouds?
- What is the relationship between the existence of methane clouds and the degree of methane supersaturation?

And, directly relevant to the upcoming Cassini mission and attempts to image the surface of Titan:

- What are the optical properties of the haze and any condensation clouds in the 1- to 3-micron range?

Role of Sample Return

A sample return has the potential to return significant information that can address the questions listed above. In particular, a sample return could directly address the elemental composition and the organic molecules that constitute the haze. Detailed isotopic studies that would be relevant to the formation mechanisms of the haze could be readily done with a returned sample but would be difficult to determine in situ.

However, laboratory simulations can provide insights into the formation mechanisms. Moreover, in situ analysis of Titan's atmosphere is simplified because the atmosphere provides a medium for a descent probe or an aerobot. Many of the questions listed above could be answered with such in situ studies.

In conclusion, it would seem that a sample return from Titan should not be considered at this time. Further in situ experiments are warranted as well as continued laboratory simulations. If these point to enduring mysteries about Titan's organic haze, then such mysteries would warrant a sample return mission.

PLANETARY QUARANTINE

John D. Rummel
Office of Space Science
National Aeronautics and Space Administration

Abstract

NASA's commitment to exploring space while avoiding the biological contamination of other solar system bodies and protecting the Earth against potential harm from materials returned from space dates from the early beginnings of the space program, and in some ways from before that time. NASA has developed policies and regulations to achieve this commitment, and depends on advice from the U.S. National Research Council (NRC) and others in fulfilling it. NASA imposes constraints on individual missions depending on the target-body of exploration, and the nature of the planned contact. In applying NRC reports and other advice on planetary quarantine to real-world missions, NASA faces a number of practical problems and questions. These focus on the survival and detectability of Earth organisms, both on Earth and under conditions on or under other solar system bodies, and the potential for life to exist elsewhere and to be detectable by us, either on those bodies or in samples returned to Earth. Implementing a satisfactory and successful planetary quarantine effort demands a great deal of both sophistication and accuracy in assessing the presence and viability of Earth life, while straining the boundaries of life detection as practiced when searching for unknown life forms in poorly understood materials returned from other planets.

Introduction

The concept of planetary quarantine (now generally referred to as planetary protection) has had a long history—if only by suggestion—dating at least from nineteenth-century discussions of panspermia. The implications of planetary quarantine (or a lack thereof) were perhaps most famously explored in H.G. Wells's *War of the Worlds*—where invading martians were killed-off by Earth germs—although its introduction into the practice of spaceflight, in a practical sense, was to wait until the post-Sputnik era. Specifically, quarantine standards were adopted by the International Council of Scientific Unions (ICSU) in 1958,[63,64] and later the U.S. National Academy of Sciences made the practice of planetary quarantine a specific recommendation in its 1958-1960 studies.[65] From the first, the distinctions between the concept and its achievement were obvious to spacecraft engineers and scientists,[66] but by the early 1970s, NASA policy and practice had reached a sufficient state of pragmatism that the Viking missions to Mars were able to be implemented successfully, while protecting both Mars and the spacecraft's biology package from contamination by Earth organisms.

But the issue that concerned the Viking missions was one of "forward contamination"—the potential exportation of Earth organisms to another world. There were serious questions raised about the potential contamination of Mars and the potential to compromise the search for life there in a direct fashion, but overall, the issues associated with Viking were of concern chiefly to the science community. More recently, however, a series of robotic sample-return missions have been under study or are under way in NASA's program. Without the confounding presence of human astronauts, these missions have been or are being assessed with respect to their potential to return biological contamination to Earth—referred to as "back contamination"—and unlike the Viking missions, these missions are potentially of compelling interest to each and every resident on planet Earth. Indeed, the questions associated with both sorts of missions have recently become more focused—both because of our recent appreciation of the wide survival limits of terrestrial life and because of our growing awareness of just how pervasive and diverse microbial life can be. Such an expanded perspective is making the implementation of NASA's policy a more challenging proposition—both in theory and in practice.

Planetary Protection Policy

With planetary quarantine provisions having been recommended by scientific groups with membership from all spacefaring nations, there has been a top-level agreement that planetary protection provisions should be employed for all interplanetary missions. The specific nature of each nation's policy and its implementation have not been coordinated until quite recently. Increasingly, however, there has been a move toward elaborating an international standard, based on the current standard of the ICSU Committee on Space Research (COSPAR), on which NASA policy also is based.

NASA Policy

NASA's current planetary protection policy was established in 1967 and revised beginning in 1984. It is embodied in NASA Policy Directive NPD 8020.7,[67] which sets out the policy and its scope, including the protection of planetary bodies for future exploration, and of Earth from extraterrestrial sources of contamination. Two subsidiary documents elaborate the policy's implementation. NPG 8020.12 is intended to delineate a uniform set of planetary protection requirements for all NASA robotic extraterrestrial missions,[68] while NPG 5340.1 provides the basic procedures for performing microbial assays for assessing contamination levels of spacecraft.[69]

The current NASA policy with respect to forward contamination is focused on preserving future science opportunities, especially for biological and organic constituent exploration, and with respect to back contamination the policy addresses the protection of the Earth. The exact policy statement is the following:[70]

> The conduct of scientific investigations of possible extraterrestrial life forms, precursors, and remnants must not be jeopardized. In addition, the Earth must be protected from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from another planet or other extraterrestrial sources. Therefore, for certain space-mission/target-planet combinations, controls on organic and biological contamination carried by spacecraft shall be imposed in accordance with directives implementing this policy.

This policy covers all spaceflight missions that may intentionally or unintentionally carry Earth organisms and organic constituents to the planets or other solar system bodies and any mission intended to return to Earth from another solar system body. The policy also requires that if NASA is to participate in non-NASA missions, those missions must follow COSPAR policy.

In NPD 8020.7, stewardship of the policy is given to the Associate Administrator for Space Science, while the management of the policy is delegated to the Planetary Protection Officer (PPO), who works on the Associate Administrator's behalf. Under this practice, the PPO is responsible for prescribing standards, procedures, and guidelines to achieve policy objectives. The PPO also certifies for each mission that all measures have been taken to meet policy objectives, including recommendations, as appropriate, of relevant regulatory agencies and any statutory requirements, and that international obligations assessed by the Office of the General Counsel and the Office of External Relations have been met and international implications have been considered. In order to make these certifications and to achieve the other goals of the policy, the PPO conducts reviews, inspections, and evaluations of plans, facilities, equipment, personnel, procedures, and practices of NASA organizational elements and NASA contractors, and by "taking actions as necessary to achieve conformance with applicable NASA policies, procedures, and guidelines."[71] NASA also seeks advice and recommendations on planetary protection "from both internal and external advisory groups, but most notably from the Space Studies Board (SSB) of the National Academy of Sciences [National Research Council]."[72]

Working both directly and through the Associate Administrator for Space Science, the PPO imposes constraints on program and project managers to meet the biological and organic contamination control requirements of the NASA policy in the conduct of research, development, test, preflight, and operational activities associated with solar system exploration missions. These constraints may require some missions to reduce spacecraft biological contamination, amend spacecraft operating procedures, provide an inventory of spacecraft organic constituents and organic samples from spacecraft construction, and comply with restrictions on the handling and methods by which extraterrestrial samples are returned to Earth. Throughout, of course, documentation of

spacecraft flyby operations, impact potential, and the location of landings or impacts of spacecraft on other bodies is required.

Another role for the PPO that is growing increasingly more important is in the area of education and outreach. Engineers working on robotic spacecraft are as a group not very familiar with the challenges of dealing with microbial life or organic contamination. A first group for education activities has thus been spacecraft designers and constructors. The rest of the science community, both space scientists and others dealing with the interdisciplinary questions related to living systems, are targeted for education in terms of both the challenges of dealing with planetary protection questions and the opportunities in doing so. Finally, the public (and its elected representatives) needs to be informed about the issues associated with both forward and back contamination. The back-contamination issue, in particular, involves a variety of cost-benefit questions that require a close alliance between the PPO and mission personnel in the area of public information.

International Policy Standards

Under Article IX of the Outer Space Treaty that entered into force on October 10, 1967,[73] there are international obligations placed on spacefaring nations. The treaty states:

> . . . parties to the Treaty shall pursue studies of outer space including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose . . . .

Some aspects of the early development of international standards for planetary protection are covered in C.R. Phillips' *The Planetary Quarantine Program: Origins and Achievements*.[74] From its inception, much of the international discussion of planetary protection issues has been held under the aegis of COSPAR, which is one of the nongovernmental organizations that reports regularly to the United Nations Committee on the Peaceful Uses of Outer Space (COPUOS). COSPAR policy and U.S. policy are similar, in that they are both based on a model originally proposed by DeVincenzi and Stabekis.[75] While the U.S. policy has been elaborated in a series of documents, COSPAR policy has been promulgated only as resolutions, or as a series of endorsements by the COSPAR Council and Bureau of papers presented at COSPAR's regular meetings. To make its planetary protection policy more readily available and to engender focused and regular consideration of an international standard, COSPAR formed the Planetary Protection Panel in 1999. At the COSPAR 2000 meeting in Warsaw, Poland, a consolidated COSPAR planetary protection policy was presented and distributed as a draft through the Planetary Protection Panel.[76]

As future activities in planetary exploration increasingly involve multinational partnerships, agreed international standards in planetary protection will be of greater practical, as well as philosophical, importance.

Critical Decision Areas for Future Missions

There are significant decisions upcoming in solar system exploration that are related to the issues of forward and backward contamination. That these decisions need to be made in the near future is indicative of the more frequent missions that are now envisioned, as well as the growing realization about the potential for life to exist elsewhere, even in the extreme environments of extraterrestrial worlds in the solar system.

Forward Contamination of Europa

One example of a decision on forward contamination deals with future missions to Europa—perhaps Jupiter's most compelling satellite—and the planned Europa Orbiter mission in particular. With a likely liquid water ocean under a crust of water-ice which may be only a few kilometers thick, Europa may harbor environments that could be used by Earth organisms if they were to be introduced into them. Consequently, it is anticipated that an

upcoming advisory report by the SSB will suggest a fundamentally conservative approach to the introduction of contamination to a europan ocean.[77]

With respect to Europa, it will be important not to introduce a live, Earth organism into an environment where it might grow and spread. Since our knowledge of europan environments (and Earth organisms) is currently quite incomplete, contamination will best be avoided if any spacecraft that may crash or land on the surface of Europa is free of living cells. Such a decontamination may be accomplished prelaunch, or the characteristics of the environment in jovian orbit may be such that (through, say, radiation) live organisms will die after some finite period. Hence, critical issues for a nondecontaminated Europa orbiter include the stability of its final orbit, the amount of time an orbit can be maintained, and the probability of maintaining that orbit if the spacecraft fails. These issues trade off against the level to which the orbiter is cleaned prior to launch and the redundancy built into the spacecraft systems. Both additional levels of redundancy or bioload reduction during processing represent a cost to the flight project, and either may have other implications in terms of mission operations or the use of certain parts or facilities in the construction, assembly, and encapsulation of the spacecraft prior to launch.

It should also be noted that the detection and monitoring of contamination by Earth organisms will have to be done with extreme thoroughness and care. New technologies to enable the quick examination, identification, and control of terrestrial contamination will enable the assured processing and planetary protection status of future solar system exploration spacecraft.

Mars Sample Return—Back Contamination?

An interesting juxtaposition in implementing a planetary protection policy pertains to one of the basic motivations of space exploration. Humans are interested in the possibility of life elsewhere, but the potential for life on a planet complicates exploration missions. The chance of discovering other life forms particularly complicates missions that return samples of other bodies to Earth—where even organisms from other terrestrial continents have caused major ecological disturbances.[78]

Nonetheless, sample-return missions may be essential to understanding other bodies such as Mars, so NASA is looking for ways to accomplish these missions robotically without back contamination. In 1997, the SSB reported advice to NASA on provisions for Mars sample return missions,[79] complementing its 1992 report on the prevention of forward contamination on Mars,[80] and completing the initial update of planetary protection recommendations made by the SSB after the Viking missions of the mid-1970s.[81] More recently, the SSB has provided recommendations on provisions to be taken to protect Earth when conducting sample return missions to small bodies of the solar system, including places as disparate as Europa, asteroids, and comets.[82]

The case for Mars is instructive as a model for the general problem of returning samples from another world where extraterrestrial life may exist, and it doesn't matter whether the life on that other body had a separate origin or is somehow related to life already on Earth. The SSB's 1997 Mars report, *Mars Sample Return: Issues and Recommendations,* provides a careful look at the subject of sample return, and conservative guidelines for the handling of samples from Mars.[83] The basic recommendations of the report were the following:

- Samples returned from Mars should be contained and treated as though potentially hazardous until proven otherwise;
- If sample containment cannot be verified en route to Earth, the sample and spacecraft should be either sterilized in space or not returned to Earth;
- Integrity of sample containment should be maintained through reentry and transfer to a receiving facility;
- Controlled distribution of unsterilized materials should occur only if analyses determine that the sample does not contain a biological hazard; and
- Planetary protection measures adopted for the first sample return should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body.

These recommendations provide the backdrop for planning return missions from Mars, but it should be emphasized that they are far from complete. Even if the NRC recommendations are fully accepted by NASA (and

to date, they have been), each of the terms in these recommendations must be defined and the processes specified in terms pertinent to spacecraft engineers and operators—which is not a simple undertaking. Among the considerations that affect the whole mission are the following:

1. Reduction and/or characterization of spacecraft bioload to accomplish forward contamination goals and minimize the potential for Earth organisms to make the round trip and be misidentified as Mars organisms when the samples are returned;
2. Selection of Earth landing sites to minimize possible dangers and to ensure that the potential for life in a returned sample does not introduce nontechnical issues that are incompatible with mission goals and effective sample analysis and assessment;
3. Reliable isolation of the exterior of the sample return container from the martian surface during mission operations and subsequent containment of martian samples so that they are not released inadvertently on Earth (a high level of operational reliability, especially in the critical phase of Earth entry, and the ability to certify containment prior to committing to Earth entry, will be necessary); and
4. An acceptable means of establishing the absence of a biohazard in the sample, prior to its release for further scientific analysis.

This last requirement will involve a suite of tests that must be done within containment on Earth or with a sterilized sample (see Box 1). Note that the certification of a sterilization method for an unknown life form is, in and of itself, a conceptual challenge.

Box 1. Issues in Returned Sample Analysis and Testing

Considerations:

- What criteria must be satisfied to show that the samples do not present a biohazard?
- What will constitute a representative sample for testing?
- What is the minimum allocation of sample material required for analyses exclusive to the protocol, and what physical or chemical analyses are required to complement biochemical or biological screening of sample material?
- Which analyses must be done within containment, and which can be accomplished using sterilized material outside of containment?
- What facility capabilities are required to complete the protocol?
- What is the minimum amount of time required to complete the protocol?
- How are these estimates likely to be affected by technologies brought to practice by two years before sample is returned?

Practical Planetary Quarantine

Unlike John Snow's famous 1854 study of the London cholera outbreak, where the expected source of the contagion was traced to the Broad Street pump by mapping cases, NASA intends to be proactive in preventing cross-contamination before cases occur. Any practical program of planetary protection that will actually accomplish its objectives must take into account the very ignorance of extraterrestrial conditions that the solar system exploration program is trying to amend and therefore must be somewhat conservative in its approach. Life detection as a general issue becomes focused on the detection of terrestrial contamination, a very pragmatic question to the contamination-control worker, followed by the more esoteric question of detecting extraterrestrial life, if it exists. In both cases, the implementation of planetary protection requirements involves the potential for interference both with mission operations and with the nature of the science that a mission can accomplish. Contamination control measures (either spacecraft cleaning or operational restrictions) may compromise some

science objectives, while the containment of returned samples and the completion of a biohazard protocol may introduce delays into the further distribution of samples to the science community.

Nonetheless, the costs of planetary protection are worth paying. If it occurs, planetary cross-contamination is a genie that almost certainly could not be put back into its bottle—and given the potential assessment of the benefits of space exploration versus the costs incurred by the introduction of any new harmful biological entity onto Earth, the cost of not conducting a sound planetary protection program is much greater than any of the real costs of implementing contamination controls. Increasingly as well, ethical considerations are becoming much more obvious and compelling in our assessment of a future for humans,[84] and their robots, in the universe as a whole.

Acknowledgments

Thanks to Jonathan Lunine and John Baross for inviting this paper, and to Joan Esnayra and David H. Smith for waiting patiently for the manuscript.

REFERENCES FOR PAPERS IN SESSION 2

1. Space Studies Board, National Research Council, *Biological Contamination of Mars: Issues and Recommendations,* National Academy Press, Washington, D.C., 1992.

2. Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations,* National Academy Press, Washington, D.C., 1997.

3. Space Studies Board, National Research Council, *Biological Contamination of Mars: Issues and Recommendations,* National Academy Press, Washington, D.C., 1992.

4. W.C.W. Chan and S. Nie, "Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection," *Science* 281:2016-2018, 1998.

5. M. Bruchez, M. Moronne, P. Gin, S. Weiss, and A. P. Ativisatos, "Semiconductor Nanocrystals as Fluorescent Biological Labels," *Science* 281:2013-2016, 1998.

6. K. Nealson and P. Conrad, "Life: Past, Present, and Future," *Phil. Trans. R. Soc. Lond.* B 354:1923-1939, 1999.

7. P. Conrad and K. Nealson, "A Non-Earthcentric Approach to Life Detection," *Astrobiology* 1:15-24, 2001.

8. C.F. Chyba and G.D. McDonald, "The Origin of Life in the Solar System: Current Issues," *Annu. Rev. Earth Planet. Sci.* 23:215-249, 1995.

9. J. Monod, *Chance and Necessity,* Alfred A. Knopf, Glasgow, 1970.

10. R. Shapiro and G. Feinberg, "Possible Forms of Life in Environments Very Different from the Earth," in *Extraterrestrials: Where Are They?* B. Zuckerman and M.H. Hart (eds.), 2nd ed., Cambridge University Press, Cambridge, U.K., 1995, pp. 165-172.

11. G.R. Fleischaker, "Origins of Life: An Operational Definition," *Orig. Life Evol. Biosph.* 20:127-137, 1990.

12. C.F. Chyba and G.D. McDonald, "The Origin of Life in the Solar System: Current Issues," *Annu. Rev. Earth Planet. Sci.* 23:215-249, 1995.

13. C.F. Chyba and G.D. McDonald, "The Origin of Life in the Solar System: Current Issues," *Annu. Rev. Earth Planet. Sci.* 23:215-249, 1995.

14. G.F. Joyce, "The RNA World: Life Before DNA and Protein," in *Extraterrestrials: Where Are They?* B. Zuckerman and M.H. Hart (eds.), 2nd ed., Cambridge University Press, Cambridge, U.K., 1995.

15. H.P. Klein, "The Viking Biological Experiments on Mars," *Icarus* 34:666-674, 1978.

16. N.H. Horowitz, *To Utopia and Back: The Search for Life in the Solar System,* W.H. Freeman and Co., New York, 1986.

17. H.P. Klein, "The Viking Biological Experiments on Mars," *Icarus* 34:666-674, 1978.

18. N.H. Horowitz, *To Utopia and Back: The Search for Life in the Solar System,* W.H. Freeman and Co., New York, 1986.

19. K. Biemann, J. Oro, P. Toulmin III, L.E. Orgel, A.O. Nier, D.M. Anderson, P.G. Simmonds, D. Flory, A.V. Diaz, D.R. Rushneck, J.E. Biller, and A.L. Lafleur, "The Search for Organic Substances and Inorganic Volatile Compounds in the Surface of Mars," *Journal of Geophysical Research* 82:4641-4658, 1977.

20. See, for example, G.V. Levin and R.L. Levin, "Liquid Water and Life on Mars," *Proceedings of the SPIE—The International Society for Optical Engineering* 3441:30-41, 1998.

21. See, for example, H.P. Klein, "Did Viking Discover Life on Mars?" *Orig. Life Evol. Biosph.* 29:625-631, 1999.

22. M.H. Carr, M.J.S. Belton, C.R. Chapman, M.E. Davies, P. Geissler, R. Greenberg, A.S. Mcewen, B.R. Tufts, R. Greeley, R. Sullivan, J.W. Head, R.T. Pappalardo, K.P. Klaasen, T.V. Johnson, J. Kaufman, D. Senske, J. Moore, G. Neukum, G. Schubert, J.A. Burns, P. Thomas, and J. Veverka, "Evidence for a Subsurface Ocean on Europa," *Nature* 391:363-365, 1998.

23. D.J. Stevenson, "Limits on the Variation of Thickness of Europa's Ice Shell," *Lunar Planet. Sci.* abstr. 1506, CD-ROM, Lunar and Planetary Institute, Houston,Texas, 1999.

24. R.T. Pappalardo, J.W. Head, R. Greeley, R.J. Sullivan, C. Pilcher, G. Schubert, W.B. Moore, M.H. Carr, J.M. Moore, M.J.S. Belton, and D.L. Goldsby, "Geological Evidence for Solid-state Convection in Europa's Ice Shell," *Nature* 391:365-368, 1998.

25. R. Greenberg, P. Geissler, B.R. Tufts, and G.V. Hoppa, "Habitability of Europa's Crust: The Role of Tidal-Tectonic Processes," *Journal of Geophysical Research* 105:17551-17562, 2000.

26. R.T. Pappalardo et al., "Does Europa Have a Subsurface Ocean? Evaluation of the Geological Evidence," *Journal of Geophysical Research* 104:24015-24055, 1999.

27. C. Thomas and L. Wilson, "Photoclinometric Analysis of Resurfaced Regions on Europa," *Lunar Planet. Sci.* abstr. 1328, CD-ROM, Lunar and Planetary Institute, Houston, Texas, 1999.

28. R.T. Pappalardo, J.W. Head, R. Greeley, R.J. Sullivan, C. Pilcher, G. Schubert, W.B. Moore, M.H. Carr, J.M. Moore, M.J.S. Belton, and D.L. Goldsby, "Geological Evidence for Solid-state Convection in Europa's Ice Shell," *Nature* 391:365-368, 1998.

29. K. Khurana, M.G. Kivelson, D.J. Stevenson, G. Schubert, C.T. Russell, R.J. Walker, and C. Polanskey, "Induced Magnetic Fields as Evidence for Subsurface Oceans in Europa and Callisto," *Nature* 395:777-780, 1998.

30. Space Studies Board, National Research Council, *A Science Strategy for the Exploration of Europa,* National Academy Press, Washington, D.C., 1999.

31. E.J. Gaidos, K.H. Nealson, and J.L. Kirschvink, "Life in Ice-covered Oceans," *Science* 284:1631-1633, 1999.

32. R.T. Reynolds, S.W. Squyres, D.S. Colburn, and C.P. McKay, "On the Habitability of Europa," *Icarus* 56:246-254, 1983.

33. T.M. McCollom, "Methanogenesis as a Potential Source of Chemical Energy for Primary Biomass Production by Autotrophic Organisms in Hydrothermal Systems on Europa," *Journal of Geophysical Research,* 104:30729-30742, 1999.

34. C.F. Chyba, "Energy for Microbial Life on Europa," *Nature* 403:381-382, 2000.

35. See, for example, J.F. Cooper et al., "Energetic Ion and Electron Irradiation of the Icy Galilean Satellites," *Icarus* 149:133-159, 2001.

36. National Aeronautics and Space Administration (NASA), *Europa Orbiter Mission and Project Description,* <http://outerplanets.larc.nasa.gov/outerplanets/>, 1999.

37. W. Zimmerman and P. Shakkotai, "Hot Objects on Europa's Surface," white paper presented to the National Research Council's Task Group on the Forward Contamination of Europa, July 1-2, 1999.

38. R.T. Reynolds, S.W. Squyres, D.S. Colburn, and C.P. McKay, "On the Habitability of Europa," *Icarus* 56:246-254, 1983.

39. W. Zimmerman and P. Shakkotai, "Hot Objects on Europa's Surface," white paper presented to the National Research Council's Task Group on the Forward Contamination of Europa, July 1-2, 1999.

40. C.F. Chyba, "Energy for Microbial Life on Europa," *Nature* 403:381-382, 2000.

41. C.F. Chyba, "Energy for Microbial Life on Europa," *Nature* 403:381-382, 2000.

42. A.H. Stouthamer, "The Search for Correlation Between Theoretical and Experimental Growth Yields," in *Microbial Biochemistry,* J.R. Quayle (ed.), *Int. Rev. Biochem.* 21:1-47, 1979.

43. R.K. Thauer, K. Jungermann, and K. Decker, "Energy Conservation in Chemotrophic Anaerobic Bacteria," *Bacteriol. Rev.* 41:100-180, 1977.

44. B.M. Jakosky and E.L. Shock, "The Biological Potential of Mars, the Early Earth, and Europa," *Journal of Geophysical Research* 103:19359-19364, 1998.

45. W.B. Whitman, D.C. Coleman, and W.J. Wiebe, "Prokaryotes: The Unseen Majority," *Proc. Natl. Acad. Sci. USA* 95:6578-6583, 1998.

46. Zimmerman and P. Shakkotai, "Hot Objects on Europa's Surface," white paper presented to the National Research Council's Task Group on the Forward Contamination of Europa, July 1-2, 1999.

47. R. Greenberg, P. Geissler, B.R. Tufts, and G.V. Hoppa, "Habitability of Europa's Crust: The Role of Tidal-Tectonic Processes," *Journal of Geophysical Research* 105:17551-17562, 2000.

48. See, for example, M.T. Madigan, J.M. Martinenko, and J. Parker, *Brock Biology of Microorganisms,* Prentice Hall, Upper Saddle River, New Jersey, 1997.

49. See, for example, W.B. Whitman, D.C. Coleman, and W.J. Wiebe, "Prokaryotes: The Unseen Majority," *Proc. Natl. Acad. Sci. USA* 95:6578-6583, 1998.

50. T.M. McCollom, "Methanogenesis as a Potential Source of Chemical Energy for Primary Biomass Production by Autotrophic Organisms in Hydrothermal Systems on Europa," *Journal of Geophysical Research,* 104:30729-30742, 1999.

51. C.F. Chyba and K.P. Hand, "Life Without Photosynthesis," *Science* 292:2026-2027, 2001.

52. E. Lellouch, A. Coustenis, D. Gautier, F. Raulin, N. Dubouloz, and C. Frere, "Titan's Atmosphere and Hypothesized Ocean: A Reanalysis of the Voyager 1 Radio-Occultation and IRIS 7.7 mm Data," *Icarus* 79:328-349, 1989.

53. G.F. Lindal, G.E. Wood, H.B. Hotz, D.N. Sweetnam, V.R. Eshelman, and G.L. Tyler, "The Atmosphere of Titan: An Analysis of the Voyager 1 Radio-Occultation Measurements," *Icarus* 53:348-363, 1983.

54. V.G. Kunde, A.C. Aikin, R.A. Hanel, D.E. Jennings, W.C. Maguire, and R.E. Samuelson, "C_4H_2, HC_3N, and C_2N_2 in Titan's Atmosphere," *Nature* 292:686-688, 1981.

55. D.M. Hunten, M.G. Tomasko, F.M. Flasar, R.E. Samuelson, D.F. Strobel, and D.J. Stevenson, "Titan," in *Saturn*, T. Gehrels and M.S. Matthews (eds.), University of Arizona Press, Tucson, 1984, pp. 671-759.

56. A. Coustenis and B. Bezard, "Titan's Atmosphere from Voyager Infrared Observations. IV Latitudinal Variations of Temperature and Composition," *Icarus* 115:126-140, 1995.

57. R.D. Lorenz, M.T. Lemmon, P.H. Smith, and G.W. Lockwood, "Seasonal Change on Titan Observed with the Hubble Space Telescope WFPC-2," *Icarus* 142:391-401, 1999.

58. P. Coll, D. Coscia, N. Smith, M.-C. Gazeau, S.I. Ramirez, G. Cernogora, G. Israel, and F. Raulin, "Experimental Laboratory Simulation of Titan's Atmosphere: Aerosols and Gas Phase," *Planet. Space Sci.* 47:1331-1340, 1999.

59. C.P. McKay, J.B. Pollack, and R. Courtin, "The Greenhouse and Antigreenhouse Effects on Titan," *Science* 253:1118-1121, 1991.

60. P.H. Smith, M.T. Lemmon, R.D. Lorenz, L.A. Sromovsky, J.J. Caldwell, and M.D. Allison, "Titan's Surface, Revealed by HST Imaging," *Icarus* 119:336-349, 1996.

61. B.N. Khare, C. Sagan, E.T. Arakawa, F. Suits, T.A. Callcott, and M.W. Williams, "Optical Constants of Organic Tholins Produced in a Simulated Titanian Atmosphere: From Soft X-ray to Microwave Frequencies," *Icarus* 60:127-137, 1984.

62. C.P. McKay, A. Coustenis, R.E. Samuelson, M.T. Lemmon, R.D. Lorenz, M. Cabane, P. Rannou, and P. Drossart, "Physical Properties of the Organic Aerosols and Clouds on Titan," *Planet. Space Sci.* 49:79-99, 2001.

63. CETEX, "Development of International Efforts to Avoid Contamination by Extraterrestrial Exploration," *Science* 128:887-889, 1958.

64. CETEX, "Contamination by Extraterrestrial Exploration," *Nature* 183:925-928, 1959.

65. See, for example, G.A. Derbyshire, "Resumé of Some Earlier Extraterrestrial Contamination Activities," in *A Review of Space Research,* National Academy of Sciences, Washington, D.C, 1962, p. 11.

66. See, for example, C.R. Phillips, *The Planetary Quarantine Program: Origins and Achievements, 1956-1973,* NASA SP-4902, U.S. Government Printing Office, Washington, D.C., 1974.

67. National Aeronautics and Space Administration (NASA), *Biological Contamination Control for Outbound and Inbound Planetary Spacecraft,* NPD 8020.7E, NASA Headquarters, Washington, D.C, 1999.

68. National Aeronautics and Space Administration (NASA), *Planetary Protection Provisions for Robotic Extraterrestrial Missions,* NPG 8020.12B, NASA Headquarters, Washington, D.C., 1999.

69. National Aeronautics and Space Administration (NASA), *NASA Standard Procedures for the Microbial Examination of Space Hardware,* NPG 5340.1C, NASA Headquarters, Washington, D.C., 1999.

70. National Aeronautics and Space Administration (NASA), *Biological Contamination Control for Outbound and Inbound Planetary Spacecraft,* NPD 8020.7E, NASA Headquarters, Washington, D.C, 1999.

71. National Aeronautics and Space Administration (NASA), *Biological Contamination Control for Outbound and Inbound Planetary Spacecraft,* NPD 8020.7E, NASA Headquarters, Washington, D.C, 1999.

72. National Aeronautics and Space Administration (NASA), *Biological Contamination Control for Outbound and Inbound Planetary Spacecraft,* NPD 8020.7E, NASA Headquarters, Washington, D.C, 1999.

73. United Nations, "Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies," Article IX, U.N. Doc. A/RES/2222/(XXI), 25 Jan 1967; *TIAS* No. 6347, 1967.

74. C.R. Phillips, *The Planetary Quarantine Program: Origins and Achievements, 1956-1973,* NASA SP-4902, U.S. Government Printing Office, Washington, D.C., 1974.

75. D.L. DeVincenzi, P.D. Stabekis, and J.B. Barengoltz, "A Proposed New Policy for Planetary Protection," *Adv. Space Research* 3(8):13- 21, 1983.

76. J.D. Rummel, P.D. Stabekis, D.L. DeVincenzi, and J.B. Barengoltz, "COSPAR's Planetary Protection Policy: A Consolidated Draft, *Adv. Space Res.,* in press.

77. Space Studies Board, National Research Council, *Preventing the Forward Contamination of Europa,* National Academy Press, Washington, D.C., 2000.

78. See, for example, M. Enserink, "Biological Invaders Sweep In," *Science* 285:1834-1836, 1999.

79. Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations,* National Academy Press, Washington, D.C., 1997.

80. Space Studies Board, National Research Council, *Biological Contamination of Mars: Issues and Recommendations,* National Academy Press, Washington, D.C., 1992.

81. Space Science Board, National Research Council, *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan,* National Academy Press, Washington, D.C., 1978.

82. Space Studies Board, National Research Council, *Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies,* National Academy Press, Washington, D.C., 1998.

83. Space Studies Board, National Research Council, *Mars Sample Return: Issues and Recommendations,* National Academy Press, Washington, D.C., 1997.

84. See, for example, E. Hargrove, *Beyond Spaceship Earth: Environmental Ethics and the Solar System,* Sierra Club, Berkeley, California, 1987.

Session 3: Detecting Extant Life

X-RAY MICROSCOPY AND THE DETECTION OF LIFE

Chris Jacobsen
Department of Physics and Astronomy
State University of New York, Stony Brook

Abstract

The microscopic investigation of possibly life-containing specimens can be greatly aided by looking not just at the morphology of a feature of interest, but at its chemistry as well. Soft x-ray microscopes are well suited to this task. The basic ideas of soft x-ray microscopes, and their application to organic material detection from sample masses as small as 10^{-17} g, are briefly outlined.

Introduction

In the search for life elsewhere in the solar system, we have some expectations about what life might look like: It will likely involve carbon chemistry, and a spatial segregation of organic compounds in a way that simple chemical processes would not permit. Instrumentation for bulk chemical measurements on robotic probes may well provide more than enough of a signature to convince the scientific community of life elsewhere, but further proof (and proof that might be especially convincing to the general public) could be provided if we could actually see something in a sample returned to Earth that we could somehow recognize as representing life, either with something that was recently living or in a fossil record.

However, seeing something that looks familiar may not be enough. For example, early reports of the imaging of DNA in scanning tunneling microscopes (STMs) used highly ordered pyrolitic graphite (HOPG) substrate, and subsequent investigations showed that at least some of the features reported were not those of DNA at all but of artifacts on the HOPG surface.[1] Similar care must be applied to the interpretation of scanning electron microscope (SEM) images of intriguing features; ideally, one would like to know not just what is the *morphology* of the object under examination, but what is its *composition*. Better yet, when looking at the composition, one would like to go beyond a map of the concentration of *elements* in the specimen, to know of their *chemical binding states*.

How does one map the chemistry of a specimen? Simply recording the x-ray fluorescence signal in an energy-dispersive detector in an SEM is not sufficient; the intrinsic energy resolution for detecting x-rays of an energy E (in electron volts) in a silicon detector is about $\sqrt{(3.5E)}$, giving an energy width of about 30 eV for carbon K x-rays. Since chemical binding energies are in the range of 1-5 eV, one will have no ability to determine the chemical binding state of carbon in such a system. However, there are several microscopes that can be used to look at chemical binding states of carbon compounds:

- Secondary ion mass spectroscopy microscopes can provide 50-nm spatial resolution mapping of molecular masses, but because the specimen is eroded in the microscope, one can examine a microscopic feature only once.
- Infrared microspectroscopy provides very good chemical state mapping of organic molecules based on their well-defined vibrational and rotational states; it does so at about 10-μm resolution.
- Visible-light microscopes can be used to obtain some information about the chemistry of nonfluorescing compounds at a spatial resolution of 200 to 1000 nm.
- Electron energy loss spectroscopy allows one to look at chemical binding states of 100-nm thin section specimens, with about 0.5-eV energy resolution in most instruments and a spatial resolution of 5 nm or better.
- X-ray microscopy allows one to look at somewhat thicker sections, with an improved energy resolution of about 0.1 eV and a spatial resolution of 30-50 nm.

While each of these tools has its own set of capabilities and limitations, x-ray microscopy is especially well suited to the examination of possible microbial life if samples can be returned to Earth.

Soft X-ray Microscopy

We outline some of the characteristics of soft x-ray microscopes. More complete discussions can be found elsewhere.[2–5]

Photons in the energy range of about 100 to 1000 eV are often called "soft" x-rays (though usage of the term varies a bit). In this energy range, x-rays have a wavelength of a few nanometers, so that the potential is there for very high spatial resolution in microscopes. More importantly, soft x-rays are well suited to providing high spatial resolution images of organic specimens. By operating at photon energies above ~289 eV, one has enough energy to remove inner shell electrons from carbon atoms by ionization. However, if the photon energy is kept below ~540 eV, one will *not* have enough energy to remove inner shell electrons from oxygen. A consequence is that one can image hydrated, organic specimens with very high concentration by operating in the "water window" energy range between about 289 and 540 eV.[6,7] Furthermore, by operating at energies slightly below the ionization threshold of carbon, the inner shell electron can be excited to an unoccupied molecular orbital. This gives rise to pre-edge absorption resonances that go by the name of x-ray absorption near-edge structure (XANES) or near-edge x-ray absorption fine structure (NEXAFS). This same effect forms the basis for energy loss near-edge structure (ELNES) in electron energy loss spectroscopy (EELS), except that in EELS the near-edge signal lies upon a large background of various plural inelastic scattering events. The intrinsically higher signal-to-noise ratio of XANES spectroscopy means that one can generally obtain better chemical state mapping information with less radiation dose to the specimen.[8,9]

While x-ray illumination of a specimen can be used to generate photoelectrons from the outermost 100-nm specimen layer that are then imaged with electron optics,[10] transmission imaging is more commonly used for chemical state mapping of features located not just on a specimen surface. Most soft x-ray transmission microscopes use Fresnel zone plates as the focusing optic. These zone plates operate as circular diffraction gratings, and their Rayleigh spatial resolution can be as good as 1.22 times the outermost zone width. The best zone plates now available have an outermost zone width of 20-30 nm,[11–13] giving a Rayleigh resolution of 25 to 35 nm. When used in scanning transmission x-ray microscopes with grating monochromators and undulator radiation,[14,15] this spatial resolution can be combined with spectroscopy at 0.1 to 0.2-eV energy resolution,[16,17] thereby matching the intrinsic width of XANES resonances. With this approach of soft x-ray spectromicroscopy (Figure 1), one can determine the bonding state of organic materials at 1 percent or greater concentration in a $50 \times 50 \times 200$ nm^3 volume, or a feature mass of only 10^{-17} g.

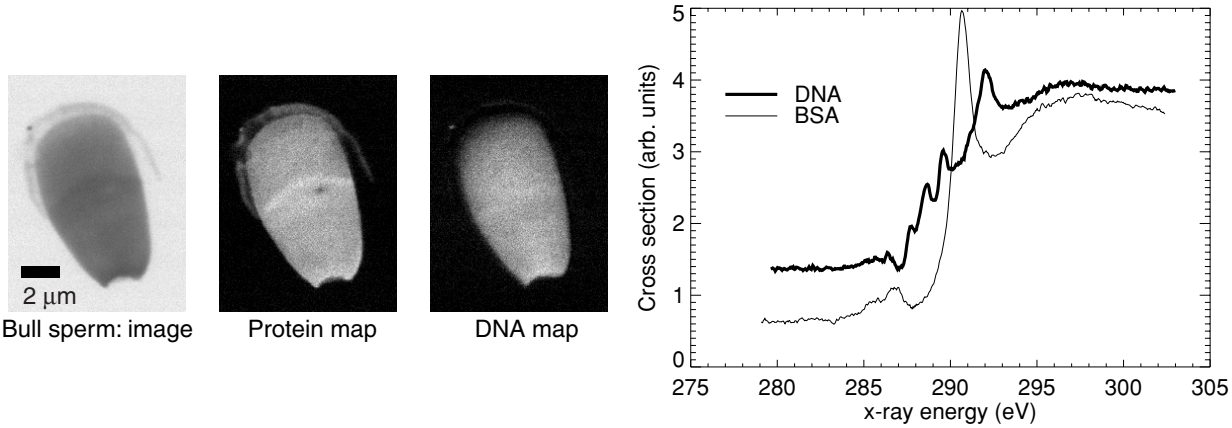


FIGURE 1. X-ray spectromicroscopy in biology. The image on the left was one of six taken at specific photon energies in the carbon XANES spectral region (absorption spectra of a protein standard, bovine serum albumin [BSA], and of DNA are shown on the right). From these images, maps of protein and DNA distributions in bull sperm were obtained as part of a study of protamine binding. SOURCE: X. Zhang, R. Balhorn, J. Mazrimas, and J. Kirz, "Mapping and Measuring DNA to Protein Ratios in Mammalian Sperm Head by XANES Imaging," *Journal Struct. Biol.* 116:335-344, 1996.

With these microscopes, one can in favorable circumstances take a single image of an initially living specimen in fully hydrated conditions; however, the radiation dose delivered to the specimen as part of the imaging process is on the order of 10^6 Gy, which produces immediate changes in a room-temperature specimen.[18] The solution is to examine the specimen at liquid nitrogen temperatures, where the diffusion of irradiation-produced free radicals is essentially stopped; in this case, specimens can withstand radiation doses of about 10^{10} Gy before mass loss is observed.[19,20] When the specimen is stable enough to allow the acquisition of multiple images, one can rotate the specimen within the depth of focus of a zone plate and obtain tomographic reconstructions of the three-dimensional nature of micrometer-size specimens.[21-23]

X-ray Microscopy for Life Detection

Soft x-ray microscopes are being used for studies in biology[24,25] and polymer science,[26,27] among other fields.[28] In Figure 2, we show the examination of a thin section of the ALH84001 meteorite where one can determine that the ratio of organic carbon to carbonates is significantly higher in the "rim" region. Soft x-ray microscopy studies on astrobiology specimens are only beginning,[29,30] but the combination of high spatial resolution and chemical state sensitivity should prove quite powerful. In organic geochemistry, Cody et al. have carried out investigations on the diagenesis of organic matter through geological time, including wood, fossilized plants and wood, and coal.[31] X-ray microscopy has been able to reveal that signatures of life, including the presence of carbohydrates and recognizable cell walls, can be unambiguously identified even in partially mineralized fossils in a way that other techniques cannot address. We therefore conclude that x-ray microscopy may prove useful in the study of returned samples to aid the search for life outside of Earth.

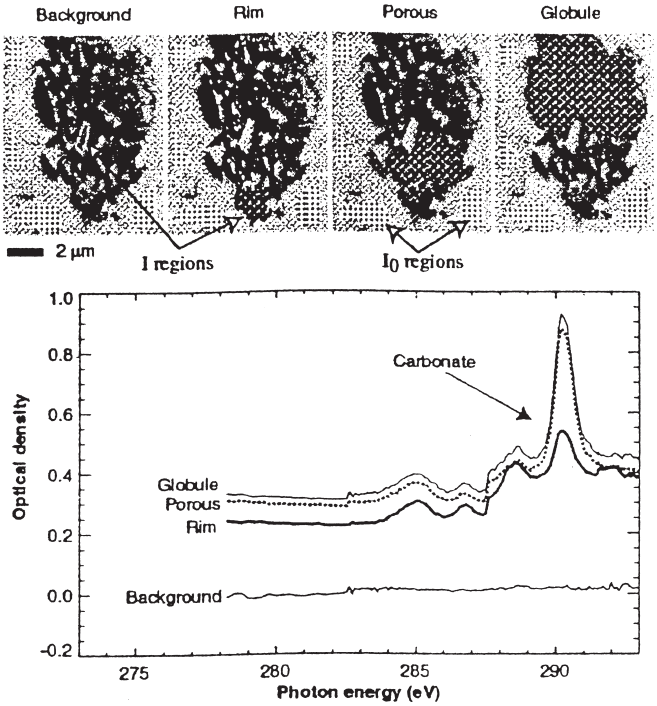


FIGURE 2. Illustration of the use of soft x-ray spectromicroscopy for the study of a thin-section sample of the ALH84001 meteorite. The image shown was taken at a photon energy of 284.0 eV with a pixel size of 48 nm. Different analysis regions were selected (two are indicated by arrows) to highlight a background region within the specimen; the carbonaceous-rich rim, which is rich in polycyclic aromatic hydrocarbons (PAHs); and the porous and globule regions of the meteor chip. The corresponding absorption spectra indicate that the rim has a higher ratio of organic material to carbonate. SOURCE: C. Jacobsen, S. Wirick, G. Flynn, and C. Zimba, "Soft X-ray Spectroscopy from Image Sequences with Sub-100 nm Spatial Resolution," *Journal Microsc.* 197:173-184, 2000.

CHARACTERIZING THE INTACT MICROBE-MINERAL INTERFACE

William W. Barker and Jillian F. Banfield
Department of Geology and Geophysics
University of Wisconsin, Madison

Abstract

A predictive model for mineralogical and textural biosignatures has been developed from a descriptive model of geomicrobial influences on silicate mineral weathering by lithobiontic microbial communities. Zone 1, represented by the upper thallus of lichens, is devoid of substratum-derived minerals. In the lower thallus, physical disruption of the mineral substratum by microbial communities delineates Zone 2 (direct biochemilithic). Complex mixtures of organic polymers and secondary phases coat extensively corroded mineral surfaces. Mineral weathering reactions in Zone 3 (indirect biochemilithic) occur within spaces too small for microbes, resemble physiochemical processes, and are accelerated by soluble organic compounds. Physiochemical weathering and unaltered primary silicates characterize Zone 4. Textures defined by detrital and authigenic minerals trapped in extracellular polymers, mineralization of cell walls and sheaths, and the presence of biominerals may generate recognizable mineralogical biosignatures within Zones 1 and 2. Distinctive etch patterns of mineral surfaces may comprise mineralogical biosignatures within Zones 1, 2, and 3. Sample preparation techniques designed to preserve the morphology, antigenicity, and mineralogy of these highly hydrated and complex samples for high-resolution electron microscopy are an important tool for evaluating these materials.

Introduction

An ideal biosignature should be widespread, easily recognized, and preservable in the planetologic record. Despite the inherent necessity for biosignatures to be non-"Earth centric," important clues may be extracted through examination of elemental, isotopic, mineralogical, and textural heterogeneities that arise due to interactions between living entities and their mineralogical environment. In the foreseeable future, time, resources, and engineering constraints will limit the search for extraterrestrial life to sites within our own solar system and to the near surface of these targets, where it is likely that organic material is scarce and the bulk of the material is mineralogical in nature. A reasonable biosignatures program must likewise address these realities.

Herein we describe the microbe-mineral environment and potential mineralogical biosignatures in the context of a model based upon electron microscopic characterization of the intact organic-inorganic interface between silicate mineral assemblages and lithobiontic microbial communities, and laboratory studies. Interactions in the rhizosphere, soils, and sediments share similarities with those at the lichen-mineral interface, so data gathered on microbe-mineral interactions in the context of mineralogical biosignature formation may apply widely.

Zone Model of Mineralogical Biosignature Formation

The lichen mineral interface, comprised of an extremely complex microbial community in contact with a limited mineral assemblage grading from fresh to deeply weathered, is an ideal microcosm for studying processes that ultimately result in mineralogical biosignatures. Based on several years of high-resolution electron microscopic characterization of the intact microbe-mineral interface and supporting laboratory investigations, we developed a descriptive model (Figure 1) of biogeochemical weathering, which we have here adapted to predict the potential for biosignature formation.[32]

In Zones 1 and 2 of lichens (which for the purposes of geomicro- or astrobiology are best considered biofilms), mineral particles derived from either the air (Zone 1) or the underlying rock (Zone 2) are in intimate contact with a diverse microbial community. Minerals range from almost unaltered, cleavage-bound fragments to highly corroded grains displaying etch pits on external surfaces and extensive internal porosity. In all cases, mineral fragments are coated and bound together by films composed of complex mixtures of organic polymers and secondary minerals (Figure 2).

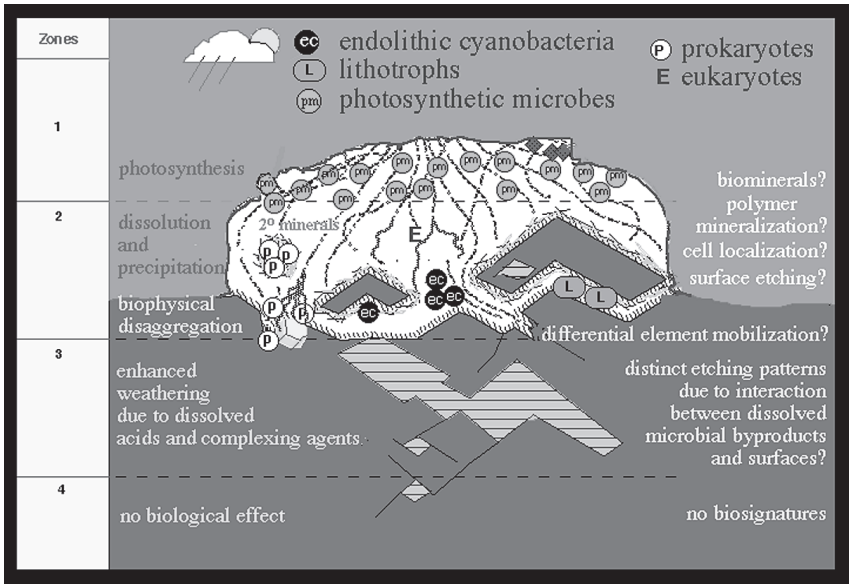


FIGURE 1. A zone model of mineralogical biosignatures.

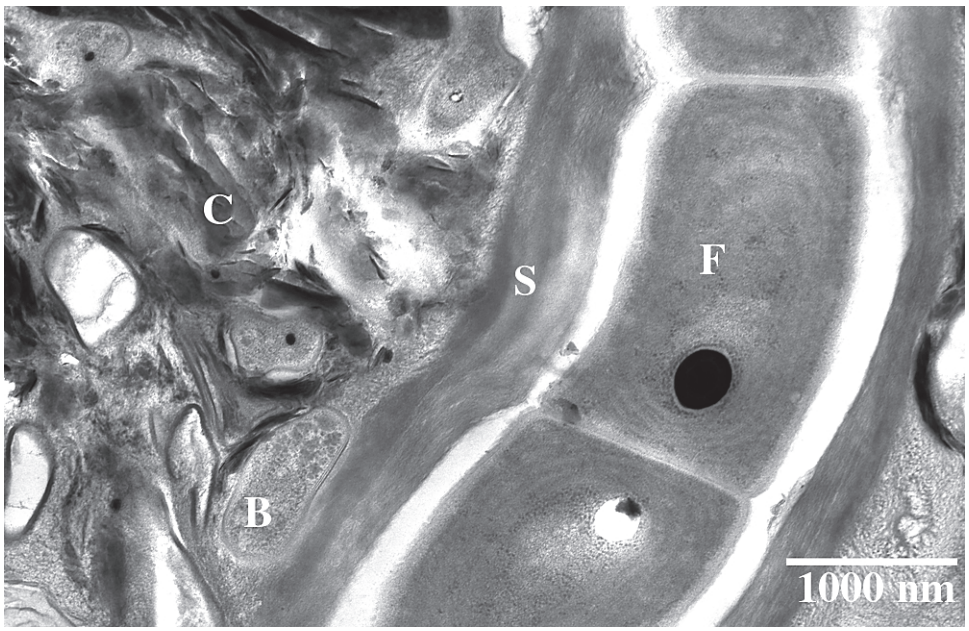


FIGURE 2. A filamentous cyanobacterium (F) inside a thick sheath (S), coccoid cells (B), and a complex mixture of nanocrystalline clays and oxyhydroxide minerals bound in extracellular polymers (C) define the complex geochemical environment of biosignature formation. High-pressure cryofixed, freeze substituted zero loss energy-filtered transmission electron microscopy micrograph of ultramicrotomed thin section.

While surface-parallel layers of adsorbed cations and water on mineral surfaces probably provide nucleation sites for clays, textures and distribution of secondary minerals also are controlled in large part by extracellular organic polymers. In addition to trapping and binding allochthonous mineral particles, these act as substrates for precipitation and affect crystallization of clays on mineral surfaces and in the polysaccharide capsules surrounding microbial cells.

Zone 3 occurs within the intact substratum beneath the lichen community and grades upward into the "direct biochemilithic zone." However, the small size of openings precludes microbial colonization, so it is defined based upon the absence of direct contact between high molecular weight microbial polymers and mineral surfaces. While silicate mineral reactions in this zone resemble those seen in strictly physiochemical weathering, they differ from the dissolution and recrystallization mechanisms seen in Zones 1 and 2. Comparison with nearby uncolonized surfaces, which show no evidence of reaction, indicates appreciable enhancement of weathering due to downward percolation of solutions containing dissolved low molecular weight organic products, especially acids. These findings have been quantified though experimental studies.[33] Consequently, we term this the "indirect biochemilithic" zone.

A fourth "physiochemilithic" zone results from abiotic weathering. It may be found at distance from the microbe-mineral interface (as in Figure 1) or in environments where microorganisms are not present. This zone represents a standard against which mineralogical biosignatures must be measured, in part to ensure that non-biological explanations cannot be provided for chemical and textural patterns detected.

Possible biosignatures applicable to our model identified through characterization and experimental studies of microbe-mineral interactions include the following:

1. Entrained or authigenic reaction products (e.g., clays) in extracellular polymers;
2. Mineralization of cell walls and sheaths; and
3. Crystallographically controlled etching of mineral surfaces.

Recommended Analytical Protocols

A research program in which qualitative findings from field studies of natural materials are quantitatively pursued by laboratory experiments is recommended.

Characterization of complex mixtures of nanocrystalline minerals, highly hydrated extracellular polymers of variable composition and structure, and a diverse microbial assemblage is required to understand microbe-mineral interactions leading to formation and preservation of mineralogical biosignatures. The size of the components and the scale of the heterogeneities dictate the use of high-resolution analytical scanning and transmission electron microscopy in combination with biological cytochemical methods. Specimen preparation techniques must rigorously avoid changes in colloidal chemistry, hydration state, and mineralogy, all of which drastically affect organic-mineral adsorption and, hence, textural relationships. Additionally, specimen preparation must provide superior ultrastructural and antigenic preservation while minimizing changes in elemental concentrations.

Many important characterization techniques will provide new information about processes occurring at the microbe-mineral interface. For example, elemental and valence state mapping by X-ray methods[34] and surface characterization by atomic force microscopy[35] appear promising.

Ultrarapid cryoimmobilization is a superior specimen preparation technique for both scanning electron microscopy (SEM) and transmission electron microscopy because it minimizes dehydration artifacts. Furthermore, fixation is instantaneous (as opposed to the tens of minutes to hours required for more conventional chemical fixation methods) and provides superior ultrastructural and antigenic preservation. The goal of all cryofixation techniques is to freeze the water component rapidly and prevent sample damage from ice crystal nucleation and growth resulting in vitreous ice.[36,37] High-pressure cryofixation offers the potential for achieving this in large volumes, in some cases up to 1 mm^3. The ability to produce vitreous ice conditions in samples of these dimensions makes this technique particularly useful for preserving samples containing intact microbial biofilm communities on colonized minerals. Once cryoimmobilized, samples can be stored indefinitely under liquid nitrogen and subjected to a wide variety of preparation and analytical techniques (Figure 3).

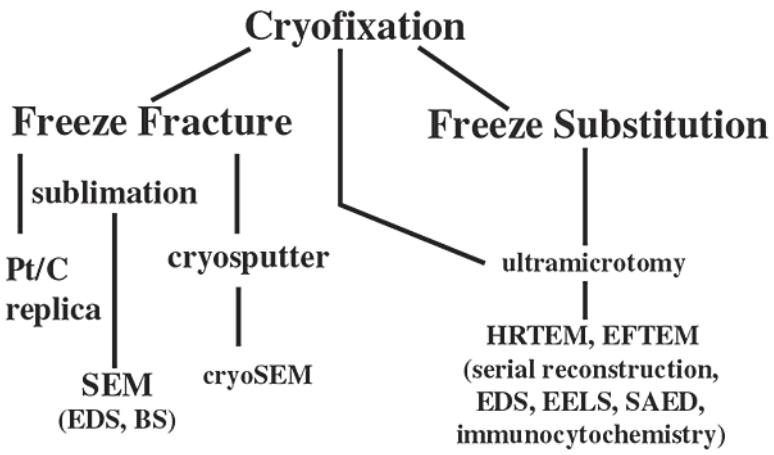


FIGURE 3. Recommended analytical flow chart for samples containing mixtures of microbes and minerals.

Given the aforementioned constraints imposed by geobiological samples, freeze substitution appears to be an extremely useful preparative method for mineralogical biosignatures research. Resultant epoxy-embedded samples are amenable to standard ultramicrotomy. Thin sections can be examined at high resolution with immunocytochemical methods and microanalytical and structural techniques appropriate for mineralogical investigation.

Freeze fracture and examination of partially freeze dried samples in a cryostage-equipped field emission gun SEM is a useful correlative technique for examining the hydrated textures and structures of extracellular polymers, expansible clays, and microorganisms. These data are necessary to better understand formation of biosignatures as a consequence of biologically induced precipitation, dissolution, enzymatic redox reactions, uptake and redistribution of nutrients and toxins, and elemental mobility arising from complexation of metal ions by organics.

Acknowledgments

This research is supported by the Department of Energy Basic Energy Sciences, the National Science Foundation, the National Aeronautics and Space Administration, and NASA's Astrobiology Institute.

SINGLE-POLYMER MODEL DETECTION USING NANOPORES

Amit Meller
Rowland Institute at Harvard
Daniel Branton
Department of Molecular and Cellular Biology
Harvard University

Abstract

The transmembrane channel created by α-hemolysin can be used to detect single linear charged molecules. Detection relies on drawing the linear molecule into the channel, thus reducing or blocking the ionic current that moves through the open pore. Translocation of the charged macromolecule through the channel is driven by a bias that is applied across the channel-containing membrane. This detection method does not rely on specific interactions between the protein pore and the translocating molecule. Rather, it is based on modulating the open pore cross section and depends on the "bulkiness" of the linear molecule. Information about the exact chemical composition of the molecules is not required to perform the measurements. Therefore, this method may lend itself to general use with a broad spectrum of uncharacterized materials. We have shown that different types of DNA molecules can be discriminated based on the blockade level and duration they produce when forced to translocate through our pore. Although the basic mechanism responsible for the translocation process and the resulting blockade level is not fully understood, we think that these measurements constitute the first step toward a fast and low-cost polymer characterization method.

Introduction

The genetic code of the form of life we are familiar with is stored in DNA molecules. By analogy to computers, we can think of DNA as the "media" used for code storage. When we speak about new life forms we may ask for alternative or analogue media to the familiar DNA. Although the existence of "alternatives" remains unknown, we can nonetheless conceive suitable tools to examine unfamiliar genetic material. When developing these tools one would face two fundamental requirements: First, since we may encounter situations where only small samples of the material under test will be available, our detection method should be able to resolve single molecules. Second, we would require a generic detection method that does not rely on particular enzymatic reactions but rather relies on more general properties of the molecule such as its bulkiness or local density. For example, the common modern techniques used for DNA sequencing make use of a particular set of nucleotides and rely on specific interaction between these molecules and enzymes.[38] This chemistry may or may not be compatible with the new genetic material we will need to probe.

We have recently developed a novel single-molecule DNA or RNA detection method that does not rely on specific interactions or chemistry. We have shown that DNA and RNA molecules can be probed by monitoring the ionic current blockade they produce as they are drawn through a narrow pore.[39,40] We embed a single α-hemolysin pore from *Staphylococcus aureus* in a lipid bilayer that separates two small containers. The inner dimension of the self-assembled α-hemolysin channel is comparable with the typical cross section of polynucleotides. The pore allows free motion of small electrolytes that are present in the buffer solution. Charged molecules such as DNA or RNA are drawn through the channel under the influence of an external electric field (see Figure 1a). When a single polynucleotide molecule enters the pores it blocks most of the otherwise unperturbed ionic current, thus signaling its presence in the pore.

Our method makes use of sensitive ionic current measurements. We use a commercial patch-clamp electrometer to apply a constant electric field across the membrane and measure the resulting current. When a DNA molecule enters our pore the current drops from its initial ("open pore") value of about 116 pA (at 120 mV applied voltage, KCl concentration of 1 M, and 22.0°C) to about 14 pA (see Figure 1b). The current is restored to its initial value when the molecule exits from the pore. We define the translocation time duration, t_D , as the time that the signal stays at its lower state. We have found that t_D is proportional to the linear length of the probed molecule.

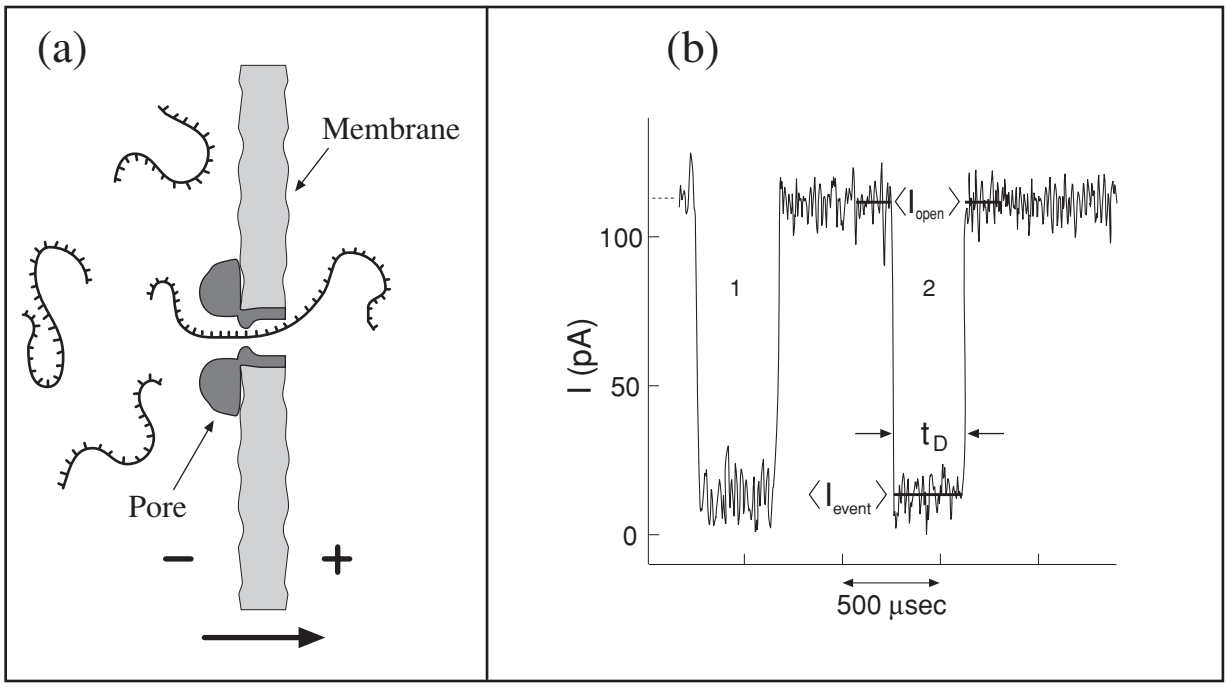


FIGURE 1. (a) Schematic view of the nanopore detection method. The nanopore is embedded in a membrane separating two small containers filled with electrolyte solution. Charged polymers such as DNA are drawn through the pore under the influence of an electric field. The arrow indicates the translocation direction. (b) Typical current trace showing two DNA translocation events when a 120-mV gradient is applied across a membrane containing the α-hemolysin channel. For each event, we measured the translocation duration, t_T, and the normalized blockade level defined as

$$I_B = \langle I_{Event} \rangle / \langle I_{Open} \rangle .$$

The fractional blockade level, I_B, is defined as the ratio of the average current at the translocation period to the average open pore current. We have found that t_D and I_B may be used to discriminate different types of DNA molecule "on the fly."

Polynucleotides Discrimination

To illustrate the capability of our system to discriminate single polynucleotides we characterized the blockade signals produced as homopolymers containing cytosines ($poly(dC)_{100}$) or adenines ($poly(dA)_{100}$) translocated through an α-hemolysin channel. Each DNA molecule was characterized by the duration of the blockade it produced, t_D, and the average blockade current, I_B. These parameters were plotted on an event diagram in which each point represents a single translocation event (Figure 2a and the corresponding histograms on Figures 2b and 2c). The most prominent features of these plots are the following:

1. The events corresponding to the two polymers, each cluster in well-separated regions; less than 1 percent of the $poly(dA)_{100}$ events fall in the $poly(dC)_{100}$ region and vice versa. Thus, discrimination between the two polymer types is readily achieved.
2. The $poly(dA)_{100}$ events separate into two groups. So too do the $poly(dC)_{100}$ events, albeit the separation into two groups is not as clear for $poly(dC)_{100}$ as it is for $poly(dA)_{100}$. The two groups are evident as two peaks in the current histograms for each polymer type (Figure 2b).

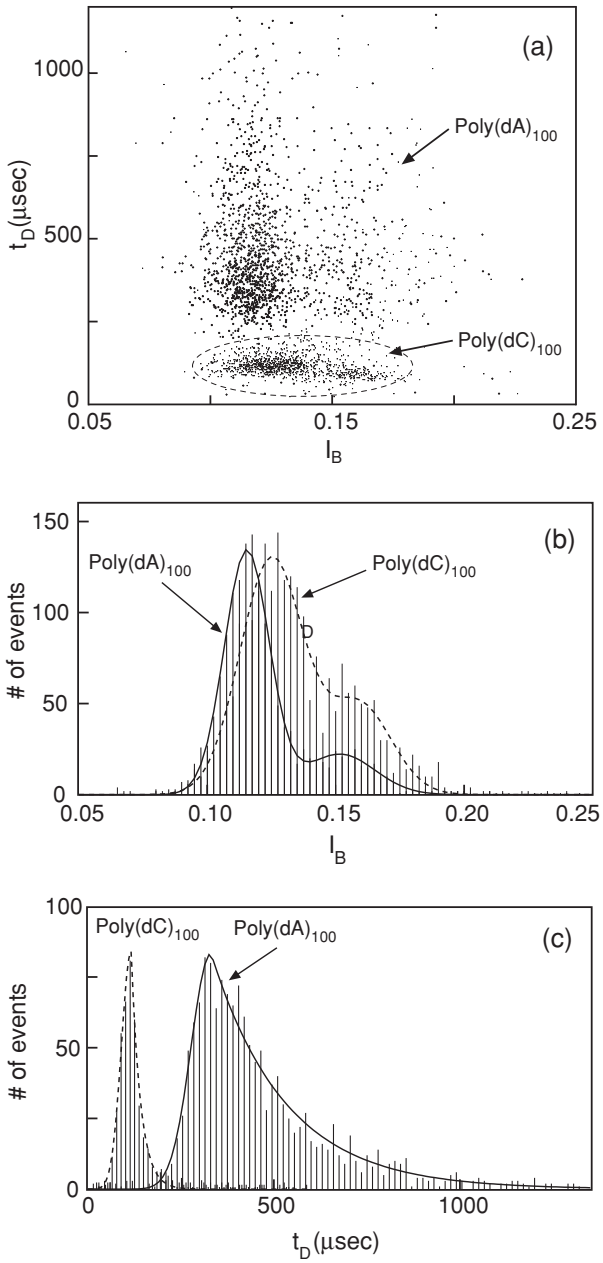


FIGURE 2. (a) Event diagram showing translocation duration versus blockade level for poly(dA)$_{100}$ (heavy markers) and poly(dC)$_{100}$ (light markers). The two polymers were examined separately. The poly(dC)$_{100}$ events were circled by a dashed line for clarity. Each point on this diagram represents the translocation of a single molecule that was characterized by its translocation duration, t_D, and blockade current, I_B. (b) Current histogram projected from the above event diagram for the poly(dA)$_{100}$ events (solid line) and the poly(dC)$_{100}$ events (dashed line). The two peaks corresponding to the two groups of events are denoted by I_{P1} and I_{P2}. The lines are fits of the data to a sum of two Gaussian distributions. (c) Duration histogram projected from (a) for the first group of events. Note the well-defined peak locations of 120 μsec for the poly(dC)$_{100}$ and 330 μsec for the poly(dA)$_{100}$ events. At times longer than the peak time, t_P, the distribution decays exponentially. The longer exponential decay corresponding to the poly(dA)$_{100}$ events reflects their larger temporal dispersion compared with the poly(dC)$_{100}$ events.

3. Histograms of group 1 translocation durations exhibit a clear peak which we define as t_P. The separation between the peaks corresponding to the two polymer types is large: 330 μsec for poly(dA)$_{100}$ and 120 μsec for poly(dC)$_{100}$.

Mixed polymers containing adenines and cytosines as well as thymines were tested in a similar way. Together the I_P, t_P, and τ_T values unambiguously characterizes each of the polymer types.[41] They can provide a simple tool for rapidly discriminating between the different populations. To demonstrate discrimination between individual polymer molecules, albeit for the best case reported here, we mixed a sample of poly(dA)$_{100}$ with a sample of poly(dC)$_{100}$ and performed translocation experiments similar to those described above.

A simple computer algorithm that made use of a probability distribution function derived from Figure 2c was used to estimate the probability that each successive event is a molecule of poly(dA)$_{100}$ or poly(dC)$_{100}$ (Figure 3). As expected, more than 98 percent of the events are assigned to either poly(dA) or poly(dC) with probabilities larger than .90. Preliminary experiments show that algorithms that take into account the other parameters will make it possible to discriminate between other types of polynucleotides mixtures.

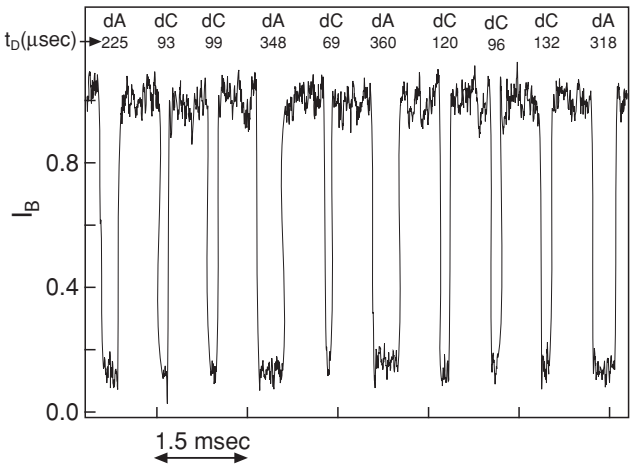


FIGURE 3. Representative current trace showing 10 events recorded from a mixture of equal molar concentration of poly(dA)$_{100}$ and poly(dC)$_{100}$. The time between events is truncated. The individual events are identified, on the basis of t_D alone, as traversal of either the poly(dA)$_{100}$ or the poly(dC)$_{100}$ molecule (Meller et al.[42]), in real time.

We have found that temperature has a strong, nonlinear, effect on the translocation duration. At low temperatures the translocation process is slowed down dramatically. Although further investigation is needed to fully understand this behavior, this finding explains why unambiguous detection and characterization of much shorter DNA molecules (down to 4-mers)[43] is possible at low temperatures. The ratio between the translocation duration times of different polymer types is also extended at lower temperatures, allowing easier discrimination between several polymer types in the same sample.

Probing Foreign Groups on DNA Molecules

As discussed earlier, the electrical current and translocation time measurements are expected to be sensitive to the bulkiness of the probed molecule. This feature may be used to detect "foreign" or irregular groups attached on individual DNA molecules. To test this idea, we prepared 50-base-long single-stranded DNA molecules that were "decorated" with two bulk groups: the first at position 25 from the molecule's 5'-end and the second at position 37. Figure 4 shows two typical current traces corresponding to the translocation of two such molecules. In each of the

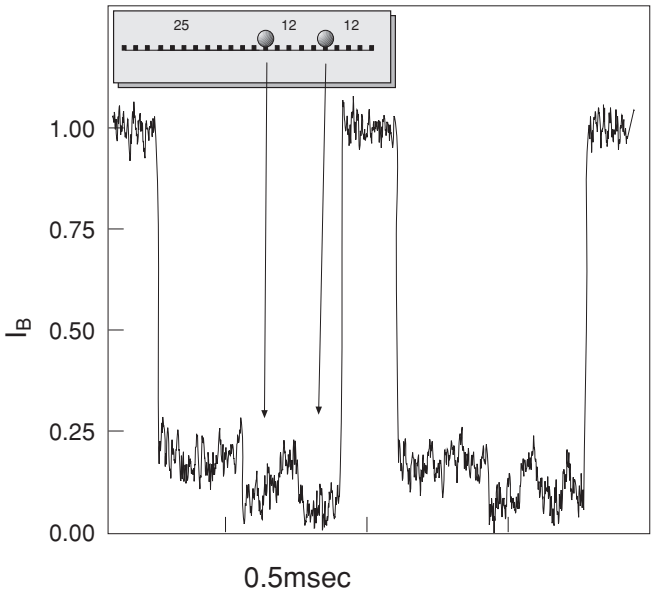


FIGURE 4. Typical current traces recorded from 50-mer DNA molecules each labeled with two bulk groups at positions 25 and 37 from the 5'-end. In this experiment, more than 85 percent of the events showed a clear two dips in the current from the initial plateau at around 18 percent, corresponding to the additional blockades caused by the extra groups.

traces the current initially drops to about 18 percent of the open pore current, followed by an additional two drops to about 5 percent corresponding to the location of the two bulk groups on the molecules. Between the two drops, the blockade current goes up to the initial blocked level of about 18 percent. Out of about 700 molecules in this experiment, more than 85 percent showed this pattern suggesting a relatively high fidelity of our detection method.

Conclusions

A nanopore makes it possible to record the coincident observation of several independent parameters (e.g., I_B and t_D) upon traversal of a single molecule. Experimental work suggests that a nanopore can discriminate between several different polynucleotides of similar length on the basis of three well-defined statistical parameters: (1) the current peak, I_P; (2) the translocation duration peak, t_P; and (3) the temporal dispersion of individual events, represented by τ_T. Furthermore, in a favorable case, measurement of just one parameter, t_D, makes it possible to discriminate between individual polynucleotides on a molecule-by-molecule basis.

We have shown that the current trace can be further modulated by the addition of bulk groups on the tested polymers. Our detection method is not limited by the requirement of having specific interaction between the tested molecules and the nanopore. Thus, other types of charged polymers such as DNA analogues can be tested. Although our present detection resolution is limited by the given geometry of the α-hemolysin pore, our results provide an initial proof of the underlying concepts. Attempts to make artificial silicone-based nanopores with a controlled geometry are already under way. We expect that these types of nanopores will provide the basis for a more robust nanopore device capable of detecting any polymer-based life form.

Acknowledgments

This research was supported by Defense Advanced Research Projects Agency (DARPA) award N65236-98-1-5407. We would like to acknowledge Dr. Charles R. Cantor for financial support (DARPA contract number N65236-98-1-5410) and the Rowland Institute at Harvard.

EXTANT LIFE DETECTION USING STABLE ISOTOPES AND PROTEIN-CHIP TECHNOLOGY

Marilyn L. Fogel
Geophysical Laboratory
Carnegie Institution of Washington

Abstract

Stable isotopes are used in geological studies to distinguish processes that have occurred under the influence of biological as opposed to geological processes. They are therefore a potentially valuable signature of the past or present existence of life in extraterrestrial samples. To reliably use stable isotopes as tools for finding the signatures of life on other planetary bodies, it is important to know whether biological isotopic signatures survive on geologic time scales. Crucial for linking the isotopic patterns seen in ancient Earth rocks to those in modern ecosystems is a systematic study of the alterations in the primary isotopic signal as organic matter enters the geologic record. ProteinChip analysis is a promising approach to providing the type of data needed to link the biochemical inventory with the biogeochemical record on Earth, and its development is described here.

Introduction

Stable isotopes at the natural abundance levels in living organisms or geological materials are among the most effective means for studying biochemical and ecosystem processes on Earth. The methodology, background principles, and applications are well established; however, this is a very active area of research with new methodology, broader principles, and new applications emerging at a logarithmic pace. Almost all Earth science departments have stable isotope ratio mass spectrometers, the necessary analytical equipment for undertaking studies in rocks, carbonate fossils, or other geological materials. In recent years, owing to the development and marketing of stable isotope instrumentation that can be largely automated, the technology is becoming an integral part of most ecological and biological studies. Many of Earth's major ecosystems have been studied isotopically, although as in any field, golden opportunities for discovery still remain (Table 1). For example, while igneous rock and the

TABLE 1. Applications of Stable Isotopes for Studying Biological Processes

Carbon	$^{13}C/^{12}C$	Photosynthesis Methanogenesis Central metabolism Biochemical fractions
Nitrogen	$^{15}N/^{14}N$	Biochemical fractions Nitrogen fixation Denitrification Ammonification
Oxygen	$^{18}O/^{16}O$	Photosynthesis Environmental conditions
Hydrogen	D/H	Photosynthesis Biochemical fractions Methanogenesis Hydrological cycle
Sulfur	$^{33, 34, 36}S/^{32}S$	Sulfate reduction Sulfur oxidation Redox conditions Biochemical fractions

leaves of plants have been studied for almost 50 years, there is almost nothing known about the isotope systematics of the Archae or the Fungi. Research into the isotopic behavior in microbes and their remains in the fossil record is one of the active areas of research in astrobiology.

In contrast to the richness in studies of stable isotopes in living organisms, an inventory of the proteins and enzymes responsible for isotopic partitioning is at an early stage of a new pulse of active growth. Gel electrophoresis, the separation of molecules based on molecular weight and charge by applying an electrical field to a gel containing a mixture of proteins, has been the principal tool for protein biochemical studies. Two-dimensional gel electrophoresis involves combining two different electrical fields in subsequent analyses to completely separate the complex array of molecules in living organisms. The analyses are difficult to quantify exactly; they are time consuming; and most importantly, the final results and comparisons of one protein pattern relative to another rely on converting the pattern on a gel to a digital image. Thus, it is difficult to compare very complex protein patterns from one organism to the next to determine which, if any, of the proteins are unique. New technology has emerged that is capable of rapidly determining the protein inventory, or proteomics, of a biological material sensitively, quantitatively, and quickly. Studies on genomics, the inventory of an organism's complete genetic code, are the impetus for studies on the genetically expressed products, the proteins.

The goal of the workshop was to describe Earth-based methods for detecting life that might be adapted to search for life elsewhere in the universe. Stable isotopes are commonly used in geological studies to distinguish processes that have occurred under the influence of biological versus geological processes. The extraterrestrial materials that we have in hand today include meteorites and Moon rocks, which have been extensively examined for stable isotope systematics and compared to those of rocks on Earth. For detecting extant or extinct life on Earth, interpretations of stable isotope analyses are based on what we do know about biochemistry versus geochemistry versus astrochemistry (Tables 2 and 3). Beyond the general classification of bio-, geo-, and astro-, there is the distinction of ecology, which is one of the strongest signatures of extant life. Living organisms never live in isolation and they almost always leave some trace of their activity on the environment. An effective strategy for studying the biosignatures of extant life on any planetary body would be a combination of detailed sampling of individual specimens with broader spatial and temporal coverage to detect gradients driven by biological reactions or fluctuations indicative of metabolic activity.

TABLE 2. Isotopic Fractionation Comparisons

BIOCHEMICAL PROCESSES	GEOCHEMICAL PROCESSES
Large isotope fractionations	Smaller isotope fractionations
Out of equilibrium	Equilibrium
Associated with	Associated with
Specific biochemicals	Graphite or reduced C
Cellular remains	Absence of cellular evidence
Regulated	Random

TABLE 3. Isotopic Fractionation Comparisons Between Biochemical and Astrochemical Processes

BIOCHEMICAL	ASTROCHEMICAL
Fractionations based on kinetic principles	Fractionations based on many principles
Moderate temperatures	Radical temperatures
Minimum –50°C	Extreme lows 50 K
Maximum 200°C	Extreme highs 2000 K
Higher molecular weight biochemicals	Smaller, random polymers
Moderate range of isotopic compositions	Extreme range in isotopic compositions

Isotopic measurements are determined on increasingly smaller amounts of material on more compact and sensitive instruments. Compound-specific isotopic analysis, for example, allows us to interrogate the isotopic composition of just a few nanograms of almost any individual molecule that can be processed through a gas chromatograph. On the alternate side, almost any material, rock fragment, soil, sediment, or fossil can be reacted in high-temperature furnaces interfaced to isotope ratio mass spectrometers for analyzing as little as 2-5 µg of C, N, O, or H. As the isotopic ratio is generated, elemental data can be acquired as well, which is one of the most common applications of stable isotope technology for studying modern ecosystem processes. Living organisms have elemental ratios of C:N:P and %H and %O levels that are consistent with the distributions of the major biochemicals in the organism. For example, any organism such as a bacterium contains 50 percent of its dry biomass in the form of proteins, composed of nitrogen-rich amino acids. Plant material on the other hand is principally composed of cellulose, which contains no nitrogen. The ratio of C:N in bacteria is 3, but in plants it is 25-60. Thus, a measurement of elemental abundance (nature of organism) linked to a stable isotope measurement (biological process) can be used to assemble a quick snapshot of extant life.

While it is important to study the stable isotope compositions and fractionations in living organisms as tools for finding signatures of life on other planetary bodies, it is equally important to know whether biological isotopic signals survive on geological time scales. We can surmise from the genetic record that many of the biological processes that occur today were important on early Earth. In fact, projections of the origins of life and/or specific biochemical reactions (e.g., photosynthesis) have been based principally on the stable isotope record. Compound-specific isotopic analyses of lipids at the Precambrian-Cambrian boundary have provided an even more complex, if not controversial, picture of the ecology of the ocean at that time.[44] One of the critical elements for linking the isotopic patterns in these ancient rocks to modern ecosystems, therefore, is a systematic study of the alterations in the primary isotopic signal as organic matter enters the geological record. Ziegler and Fogel have shown that heterotrophic bacteria living on photosynthetically derived protein alter the compound-specific isotopic profile during metabolism (Figure 1).[45] The change in the photosynthetic isotopic biosignature to the microbial isotopic biosignature at the compound-specific level of analysis is a reminder that a broader suite of isotopic measurements (e.g., bulk isotopes and compound-specific isotopes) on planetary samples is important.

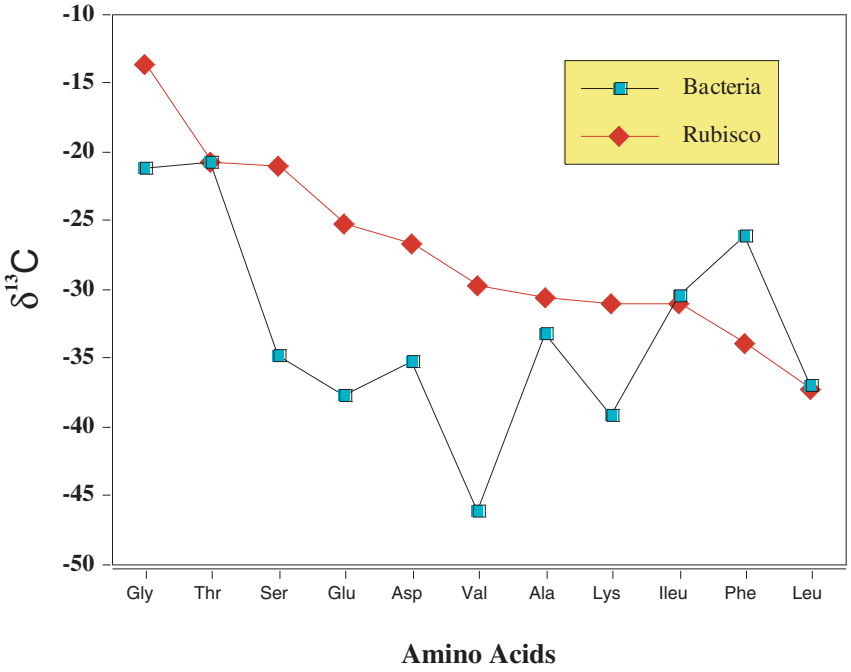


FIGURE 1. Carbon isotopic compositions of the photosynthetic protein, Rubisco, and the heterotrophic microorganisms that consumed the protein. Note that a majority of the compound-specific signals have changed in the amino acids of the bacteria.

Compound-specific isotopic analyses, therefore, can go only so far in providing diagnostic, specific signals of biological activity. A second step is to examine isotopic biosignatures in specific high molecular weight biochemicals, for example, cellular membranes, specific proteins, or isolated mRNA. Biological molecules at this level relate directly to certain types of organisms and their metabolic processes. Very sensitive techniques for identifying these biochemical molecules in biological and environmental samples are being developed.[46,47] Characterization of the protein inventory by chip technology might provide the type of data needed to link the biochemical inventory with the biogeochemical record.

The basic principle of ProteinChip analysis (Ciphergen Biosystems) is the development of specially modified surfaces that serve as "bait" for trapping organic molecules out of complex mixtures (Figure 2). Once molecules are selectively bound to the surface of the chip, they are desorbed and analyzed by time-of-flight mass spectrometry, which is capable of resolving 1 dalton in molecular weight in a molecule that may be as large as 100,000 daltons. The molecular weights of molecules that are desorbed by laser energy are then stored digitally, and the patterns of their molecular weights can be compared to what is expected from genome analyses. Each protein coded for by a gene has a specific molecular weight, based on the linear structure of the amino acids of the protein. Often, after the genetic information is translated into a specific protein, further biochemical reactions modify the protein either for increased catalysis or for stability. These small but important changes in protein biochemistry, posttranslational modifications, cannot be detected purely by genomic analysis.

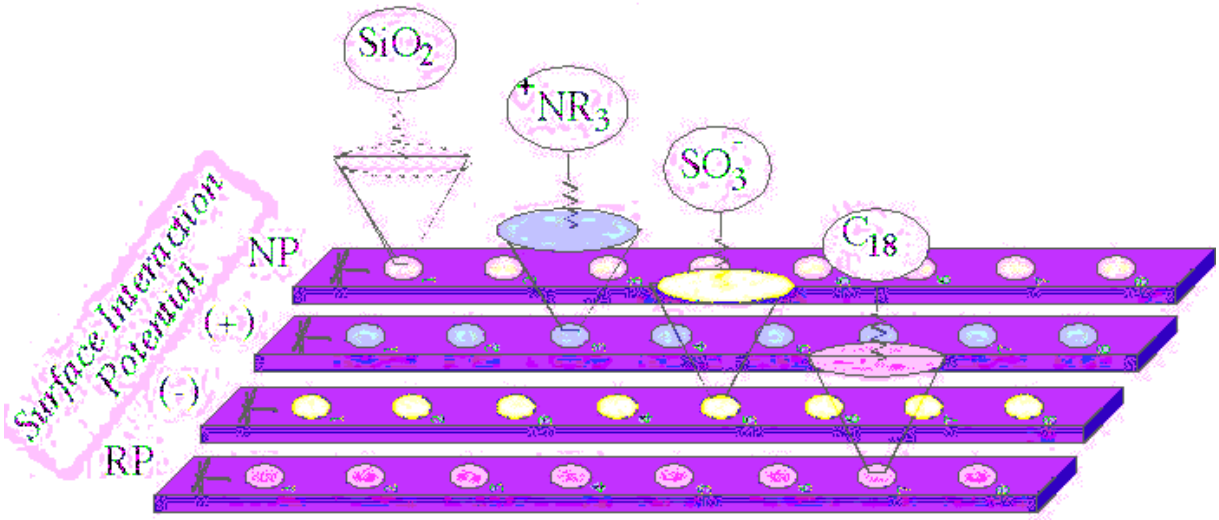


FIGURE 2. Diagram of surface enhancements of the ProteinChip system designed by Ciphergen Biosystems.

Advantages of using the ProteinChip technology include the need for only a few microliters of sample in liquid. The instrumentation is capable of detecting femtomoles of organic matter, theoretically a single molecule. Because the molecules are captured on an enhanced surface, rather than in a matrix, biochemical manipulations can be performed on the molecules on the chip's surface. For example, microbial cell walls have been shown to be highly stable components that are important for deriving a principal component of the ocean's dissolved carbon

pool.[48] These analyses are based on monomeric structural signatures of amino acids and specific sugar analyses. ProteinChip analyses of these samples give the intact molecular weight distribution, a complex pattern that can be related to the presence of microbial cell wall structures (Figure 3). To confirm the presence of cell wall material, lysozyme—the enzyme that breaks down the peptidoglycan cell wall—could be used on the surface of the chip to break the higher molecular weight materials down into smaller fragments.

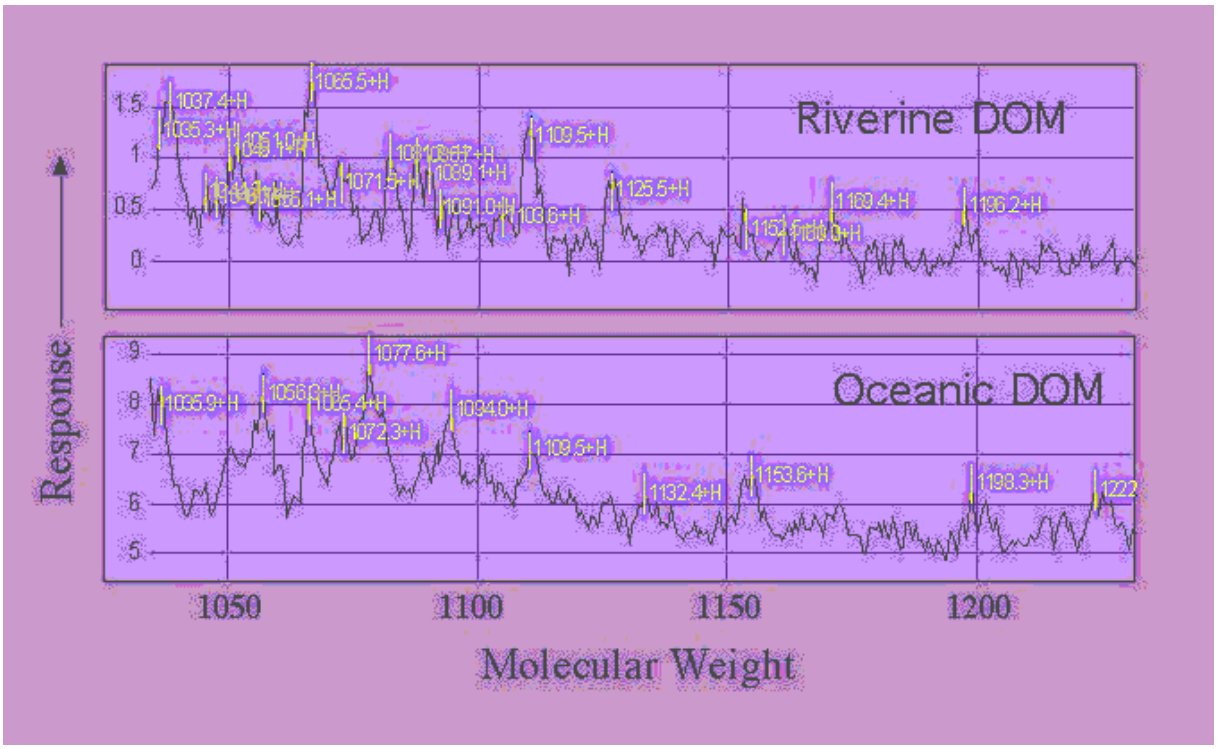


FIGURE 3. Analysis of dissolved organic matter (DOM) by the ProteinChip instrument. The samples are from two very different locations, a riverine environment and the deep ocean. There were 14 peaks in these samples that had identical molecular weights. The material has resisted chemical identification by other conventional analyses, such as gas chromatography-mass spectrometry. On this graph, it can be seen that there are numerous molecules in the molecular weight region greater than 1,000 and less than 1,200. Peptidoglycan units, the backbones of microbial cell walls, have molecular weights in this range. Many of the peaks correspond to degraded fragments of the original peptidoglycan molecule.

Currently, this technology is being used to look for biomarkers in cancer research or to detect the action of a drug treatment on a patient's metabolism. The search for one or two diagnostic molecules in a field of others is accomplished by comparing the spectral data from one tissue (e.g., precancerous) with that from another (e.g., cancerous). The application of this technology to the field of astrobiology is just beginning. The proteins of microorganisms grown in cultures will be studied and compared with environmental samples in which these organisms are known to occur. It should be possible by comparison of the complex spectra to find molecules that are similar in cultures and in the environment, and to relate these molecules to a specific protein structure. The integrative goal will be to connect the dots between specific protein molecules and stable isotopic analyses of these compounds. The chip technology is not limited to the study of well-defined proteins. With modification to the surface environment, perhaps the molecules absorbing the laser energy, and the laser intensity, it should be possible to examine a wider range of organic compounds.

Summary

Stable isotope analyses are advancing to include a wider range of isotopes at smaller and smaller scales and amounts. The instrumentation is increasingly automated and becoming miniaturized. The challenges to the field in the next decade include adapting the technique to spaceflight and to other chromatographic links (e.g., routine liquid chromatography-isotope ratio monitoring). The community is at the start of introducing compound-specific analyses of molecules other than those containing carbon,[49] which should open new avenues for isotopic biosignature development. Because stable isotope technology is so widespread in the community, it will be an important tool for detecting biological signatures in any returned planetary sample. ProteinChip technology is a wide-open field for microbial profiling,[50] environmental sampling, and geochemical analysis. The technology is changing rapidly as new innovations in nanotechnology are developed and incorporated. Future research in stable isotopes at the molecular level, coupled to molecular fingerprinting of biochemical molecules in the geological record, should provide a new level of biosignature for the search for extant or extinct life on other planets.

IRON BIOMINERALS AS BIOMARKERS

Joseph L. Kirschvink
Division of Geological and Planetary Sciences
California Institute of Technology

Abstract

Because iron is an essential trace element in virtually all living organisms, the low solubility of Fe^{3+} in most aerobic environments on Earth has driven the evolution of a wide variety of iron acquisition and storage mechanisms. Many of these involve precipitation of iron in the form of well-ordered biominerals, including ferrihydrite, magnetite, goethite, and lepidocrocite. Of these, biogenic magnetite in the magnetotactic bacteria shows one of the clearest fingerprints of natural selection, particularly adapting to perfect its role as a "biological bar magnet." Magnetofossils, the fossil remains of bacterial magnetosomes, have been studied extensively for the past 20 years, primarily because of their importance to the field of paleomagnetism. In sedimentary materials from Earth, magnetofossils have been accepted without controversy as unambiguous biomarkers. Their presence in materials returned from Mars or Europa would provide strong support for life in those places.

Introduction

Chemical energy in the form of oxidation-reduction pairs is a central component of all life on Earth, and organisms have been surprisingly adept at exploiting energy-yielding reactions.[51] Frequently, the redox reactions utilized by microorganisms result in the precipitation of inorganic mineral phases. Examples include elemental sulfur, manganese oxides, and various iron oxides.[52] Often these mineral phases prove to be the most enduring traces of past microbial life, leaving unambiguous biosignatures. Iron is one of the major elements on planetary surfaces (including Earth) and particularly Mars (~20 atomic percent). The geochemical cycle of iron, and particularly the derived iron phases, is strongly coupled to the chemistry of biogenic elements, H, C, O, and S. As one of the best oxidation-reduction agents available, it is not surprising that the Fe^{2+}/Fe^{3+} cycle has a central role in some of the most primitive metabolic pathways known (e.g., the cytochrome system). As Rudyard Kipling noted, "Iron, cold iron, is master of them all!"

On the Archaean Earth, geochemical evidence supports strongly the idea that the bulk of the oceans and atmosphere were reducing environments.[53] Under these conditions, Fe^{2+} remains in solution and is easily available for biological use. However, after the Paleoproterozoic Snowball Earth event about 2.4 billion years ago,[54,55] all geological evidence points to the presence of more oxygen-rich conditions at the surface.[56] At this time, iron must have become a limiting resource, forcing the evolution of acquisition and storage mechanisms. For a motile bacterium capable of moving across the redox boundary in a vertically stratified system, natural selection could favor the gathering of iron in the anaerobic zone, and storing it for use when it is needed to function in an aerobic environment. As noted next, this need for iron storage probably resulted in the first bioprecipitation of the mineral magnetite (Fe_3O_4).

Magnetite Biomineralization

Most iron in living tissues is stored in the form of the mineral ferrihydrite (basically, a hydrated mineral with a hematite-like structure) in the core of the iron storage protein ferritin.[57] However, aggregations of ferric iron, in the presence of phospholipids and in moderately reducing conditions, can reduce spontaneously to magnetite.[58] In the presence of a local or planetary magnetic field with a strength comparable to that of Earth, natural selection for magnetotaxis provides an obvious mechanism for perfecting magnetite's role as a biological bar magnet.[59-61] In the process, it also yields a definitive set of biomarkers.

Magnetotactic bacteria are the simplest organisms known to contain biogenic magnetite,[62,63] a typical example of which is shown in Figure 1. They precipitate individual submicron-sized magnetite crystals within an

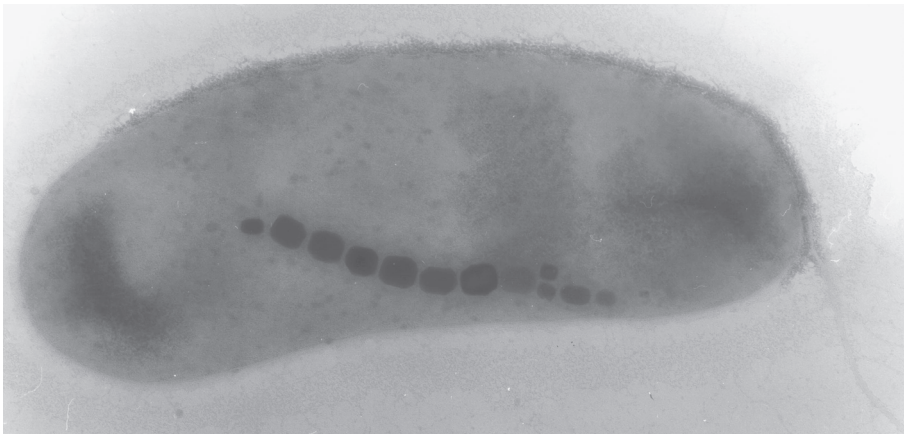


FIGURE 1. A typical magnetotactic bacterium. The individual crystals are ~50 nm in size and composed of pure magnetite (Fe_3O_4).

intracellular phospholipid membrane vacuole, forming structures termed "magnetosomes."[64,65] The presence of lipid-bilayer membranes demonstrates that magnetosomes are proper organelles, and their alignment in linear chains will maximize the net magnetic moment of the individual cells. These are "Nature's biological compasses," and natural selection has perfected the size, shape, composition, crystallographic orientation, and ultrastructure of individual crystals within the cells in order to enhance their magnetic properties.[66] Chains of these magnetosomes act as simple compass needles that passively torque the bacterial cells into alignment with Earth's magnetic field and allow them to seek the microaerophilic zone at the mud-water interface of most natural aqueous environments. These bacteria swim to the magnetic north in the Northern Hemisphere,[67] to the magnetic south in the Southern Hemisphere,[68,69] and both ways on the geomagnetic equator.[70,71] Magnetosomes have also been found in eukaryotic magnetotactic algae, with each cell containing several thousand crystals.[72] Figure 2 illustrates in schematic

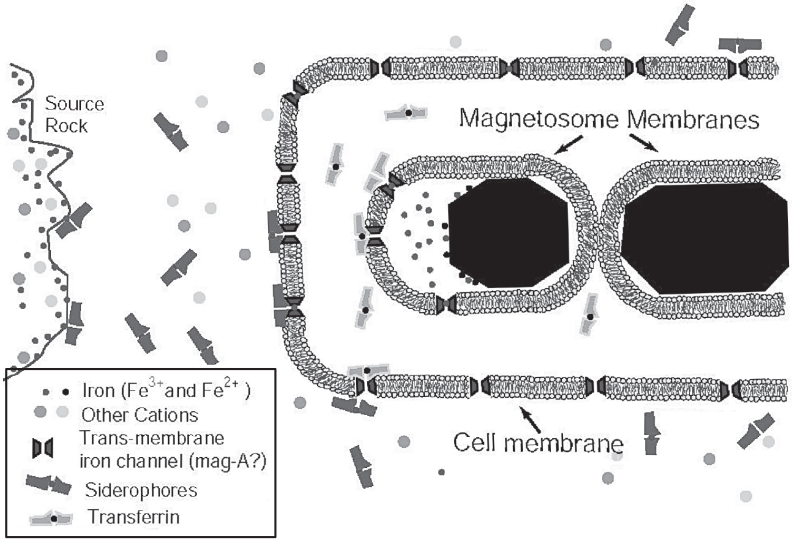


FIGURE 2. Schematic illustration of the typical iron uptake and purification process in a magnetotactic bacterium. Iron in the environment is bound selectively by a variety of siderophores, transported actively into the cell via specialized transmembrane iron receptors (often accompanied by oxidation-reduction activity), and eventually dumped into a preformed magnetosome membrane. The shape of the membrane vesicle limits diffusion to specific surfaces of the growing crystallites, allowing the particles to assume specific shapes, as well as producing a sharp size distribution.

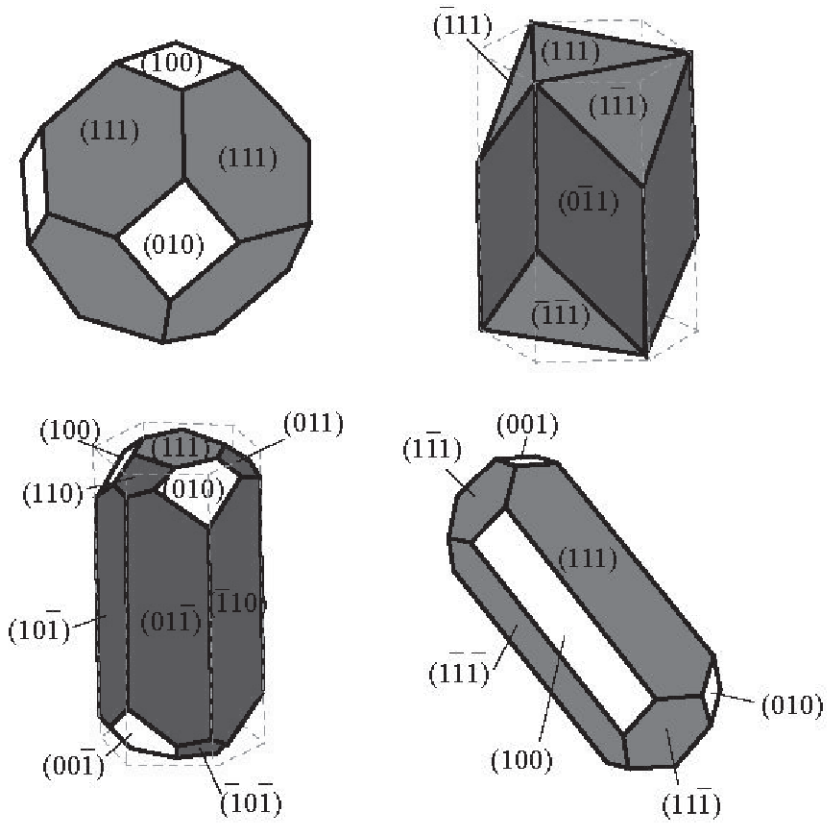


FIGURE 3. Typical morphologies found in natural populations of magnetotactic bacteria. Only the cubo-octahedral form shown on the upper left is commonly found in natural systems. Elongation of the other forms involves breaking of the cubic crystal symmetry of magnetite. For nanometer-size crystals, the surface free energy is quite high compared with the energy of formation, and the minimum energy configuration favors an equant crystal. Elongation of these particles involves breaking symmetry rules. In the upper right particle (similar to strain MV-1 and the ALH84001 prismatic fraction), the particle is elongated along only one of four possible (111) directions, which are all chemically equivalent. The magnetosome membrane (Figure 2) stops diffusion to the (011) and equivalent faces by simply blocking ionic access to them. (Adapted from S. Mann, N.H.C. Sparks, and V.J. Wade, "Crystallochemical Control of Iron Oxide Biominerals," in *Iron Biomineralization,* R.B. Frankel and R.P. Blakemore (eds.), Plenum Press, New York, 1991, p. 435.)

form the process by which these bacteria form magnetosomes, and Figure 3 illustrates typical magnetosome morphologies.

Inorganic magnetites are usually small octahedral crystals, often with lattice dislocations, chemical impurities, and other crystal defects. In contrast, magnetite crystals formed within these magnetosome vesicles have five main features that serve to distinguish them from magnetites formed through geological processes: (1) High-resolution transmission electron microscopy (HRTEM) studies reveal that bacterial magnetites are nearly perfect crystals, which (2) have unusual crystal morphologies. They (3) are usually elongate in the (111) crystal direction,[73-76] (4) are chemically quite pure Fe_3O_4, and (5) are of just the right size and shape to be single domains, requiring restricted anisotropic width-length ratios. The elongation of biogenic crystals in the (111) direction serves to stabilize the magnetic moment of the particle and presumably is the result of natural selection for their magnetic properties.[77,78] It may also be the passive effect of the growth of individual crystallites in the strong

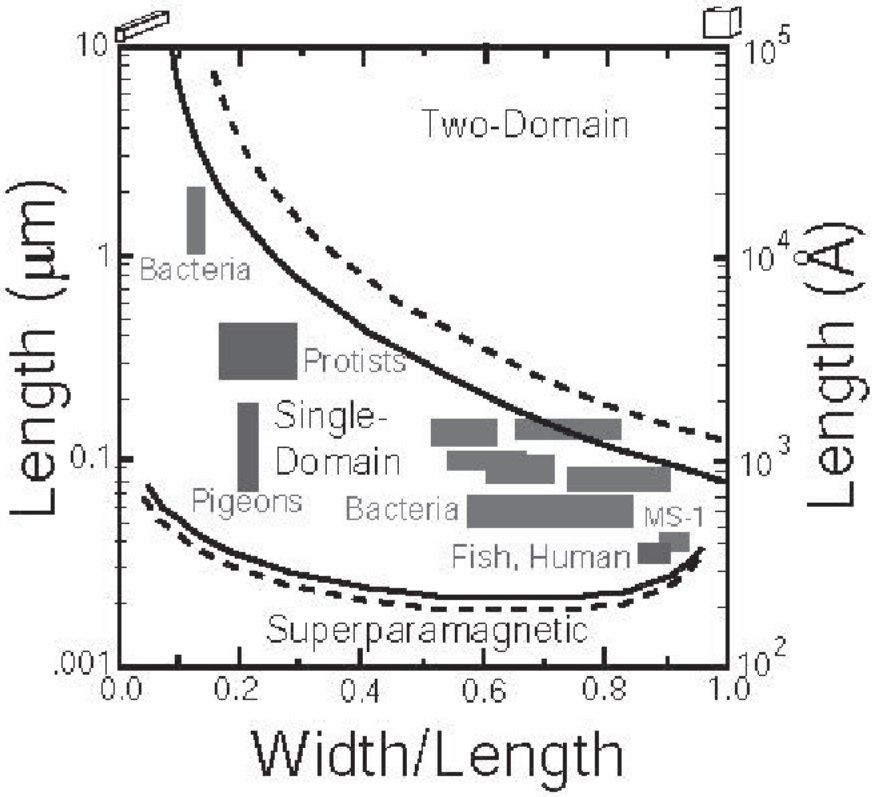


FIGURE 4. Experimentally and theoretically determined size and shape requirements for the production of magnetic single-domain particles in pure magnetite parallelipipeds. Particles in the single-domain field are uniformly and permanently magnetized, perfect little bar magnets. In smaller particles, thermal agitation will cause the magnetic moment of the crystals to wander relative to the particle, yielding "superparamagnetic" behavior. Larger particles will break down into regions of multiple domains. The moments will cancel, reducing the magnetization of the particle. Interparticle interactions will slightly stabilize the single-domain field, as indicated by the dashed lines. Natural selection for magnetotaxis or magnetoreception will yield single-domain crystals.

magnetic field at the end of a magnetosome.[79] As shown in Figure 4, bacterial magnetite crystals are restricted to a size range from 35 to about 500 nm, with shapes that confine them to the single-domain magnetic stability field.[80,81] Inorganic magnetites tend to have log-normal size distributions that often spill up into the multidomain size region; this arises from fundamental inorganic growth principles.[82] Bacterial magnetites tend to be rather pure iron oxide, with no detectable titanium, chromium, or aluminum, which are often present in geologically produced magnetite. This is presumably due to the biopurification processes produced by the iron-specific binding and transport proteins shown in Figure 2. An additional feature is the alignment of the crystals into linear chains, which can often be preserved in the fossil record.[83,84] The Venn diagram of Figure 5 summarizes these features.

In higher animals, an obvious function for magnetite biomineralization is its role in magnetoreception.[85-87] Magnetoreception is now well established in virtually all major groups of animals,[88] and specialized cells containing single-domain chains of magnetite are the best candidates for the receptor cells.[89,90] In the brown trout, Walker et al. have shown elegantly that magnetically sensitive nerves in the ophthalmic branch of the trigeminal nerve connect to specialized, trilobed cells in the olfactory laminae that contain magnetite crystals.[91] More recently, they have used magnetic force microscopy (MFM) and confocal techniques both to image the magnetic field of these receptors and to identify the neural processes that connect the magnetoreceptor cells to the nervous system.[92]

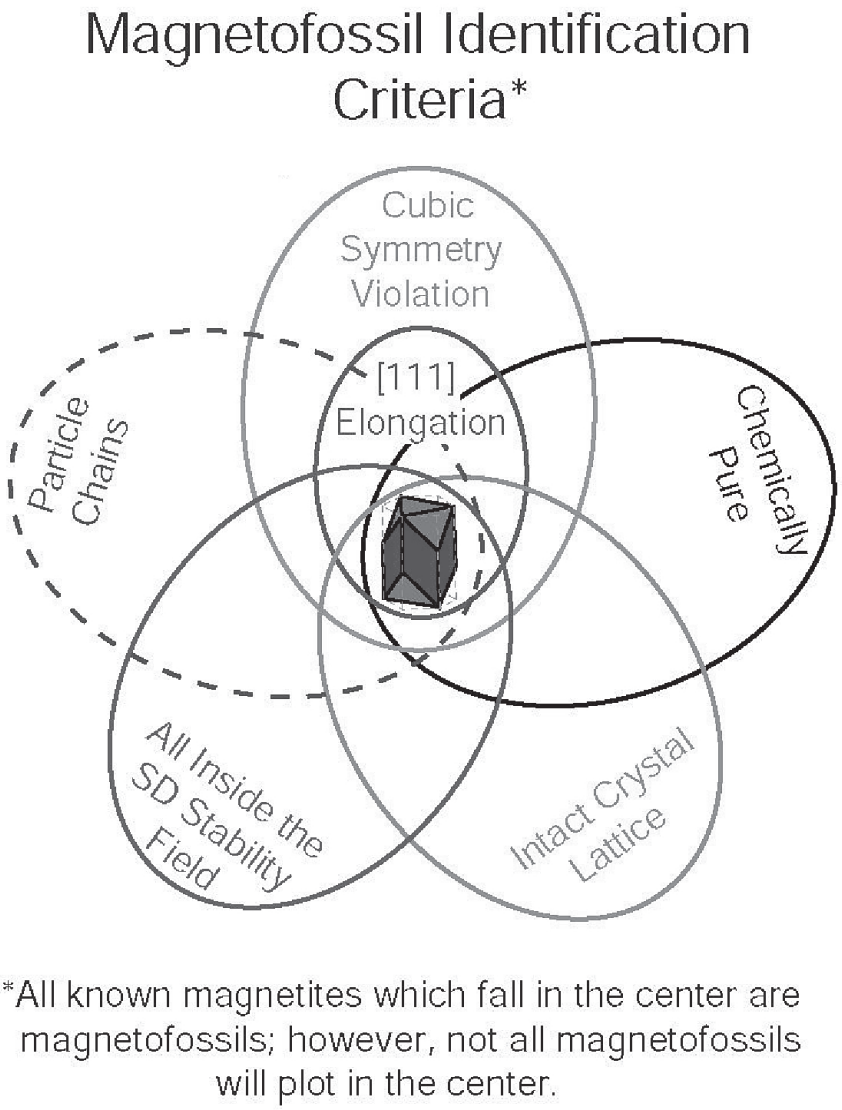


FIGURE 5. Venn diagram summary of the criteria used for identification of intracellularly produced biogenic magnetite. These criteria include unusual crystal morphology (e.g., elongated hexagonal prismatic magnetite with faceted ends produced by magnetotactic bacteria strain MV-1); chemically pure composition; an intact crystal lattice that is relatively free of defects (though it may occasionally have (111) twinned crystals); restricted width-length ratios and volumes that place the crystals in the single-domain stability field; and occurrence in chains that maximize the magnetic moments of the magnetite crystals. Although bacterial magnetite crystals produced by some common strains (e.g., equant cubo-octahedra crystals of *Magnetosprillum magnetotacticum*) fall outside the central area of the diagram, no inorganically produced population of magnetite crystals has yet been found that inhabits this central region. With the exception of particles in chains, elongated prismatic magnetite crystals in ALH84001 meet all other criteria and thus fall within the central region of this diagram.

Similarly, behavioral work with honeybees and birds has shown that brief magnetic pulses are able to alter the magnetic responses, confirming that a ferromagnetic material such as magnetite is indeed part of the magnetic sensory system.[93–99]

Magnetofossils as Biomarkers

The distinctive features of magnetosomes outlined in Figure 5 have enabled bacterially precipitated magnetites (dubbed "magnetofossils"[100]) to be identified in earthly sediments up to 2 billion years old.[101] Because of natural selection, these bacterial magnetofossils are as clearly and uniquely biogenic as are the teeth or skulls from a vertebrate. There has never been a controversy with their recognition in earthly sediments, despite a 20-year history of investigation.[102-120] Many of the bacterial magnetite crystals are simply not found anywhere outside of the biosphere. Chemists working in the ferrite industry (which makes magnetic particles for recording tape, disk drives, etc.) have failed consistently for the past 50 years to produce synthetic magnetite crystals with a similar set of properties. They have failed for very good reason: they cannot duplicate the microenvironment of the bacterial magnetosome membrane. They cannot contain magnetite to stay within its single-domain stability field (Figure 4) and produce the (111) elongated morphology, which is rather peculiar for a cubic mineral. These properties are the end result of natural selection for magnetic properties of the magnetite crystals, and they are a unique biological fingerprint.

Discussion: Martian Magnetofossils in ALH84001 Carbonates?

Among their original arguments for ancient life on Mars, McKay et al. noted the similarity between some of the fine-grained magnetite in the carbonate blebs and those of the magnetotactic bacteria.[121] Unlike the other lines of evidence cited in support of their claim for ancient martian life, subsequent examination (by HRTEM) of the ALH84001 magnetites have strengthened the magnetofossil hypothesis considerably. In an extraordinarily thorough paper, Thomas-Keprta et al. summarize the crystallographic measurements on nearly 600 magnetite particles extracted from the carbonate globules, comparing them with similar measurements from biogenic and inorganic magnetites.[122] Approximately 27 percent of the ALH84001 crystals have an elongated, chemically pure hexagonal prismatic morphology that is *identical* to bacterial strain MV-1. These particles have simply not been found anywhere outside of the biosphere. Since the carbonate blebs that contain the magnetite crystals are ~4.0 billion years old,[123] these putative magnetofossils predate the oldest terrestrial fossils by 500 million years. Their presence in the ALH84001 carbonates is therefore as solid evidence as any fossil materials could provide for the past existence of life on Mars.

Weiss et al. have shown that ALH84001 traveled from Mars to Earth without ever experiencing temperatures as high as 40°C.[124] Hence, martian life would have had ample opportunity to reach Earth through a natural process. Magnetotactic bacteria may have evolved first on Mars at least 4 billion years ago and only established themselves on Earth via the process of meteorite seeding (Panspermia) when suitably oxidizing conditions were produced after the Paleoproterozoic snowball event.[125] This line of reasoning argues that the genes that control magnetite biomineralization in animals could be of martian origin.

Acknowledgments

This work was supported in part by the NASA Astrobiology Institute, and Tim Raub made helpful comments on the manuscript.

TIME-OF-FLIGHT MASS SPECTROMETERS: MINIATURIZED INSTRUMENTS WITH A BIOLOGICAL MASS RANGE

Robert J. Cotter
Middle Atlantic Mass Spectrometry Laboratory
Johns Hopkins University School of Medicine

Abstract

While many types of mass spectrometers are used for the analysis of molecules from living species, time-of-flight (TOF) mass spectrometers are unique in their ability to provide high mass range when miniaturized. Thus there is considerable interest in their use for the detection of biomarkers, both those that might be used to identify biological threat agents and those that would indicate the presence of life.

Introduction

Mass spectrometers have been used for a number of years to determine the chemical structures of simple (and volatile) organic compounds. In recent years, new ionization, mass-analysis, and other instrumental techniques have made it possible to analyze more complex biological structures, including peptides and proteins, complex carbohydrates, glycolipids, and DNA or RNA. In particular, two techniques: matrix-assisted laser desorption and ionization (MALDI) and electrospray ionization (ESI) provide the capability for ionizing these complex and generally nonvolatile molecules. And the resulting ions are then analyzed on time-of-flight, quadrupole ion trap (IT), and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers that have very high mass range and sensitivity.

Mass spectrometers have been found in space applications where they have been used to measure atmospheric species and small organic compounds. A double-focusing (electric and magnetic sector) mass spectrometer developed by A.L. Nier for the Viking I mission to Mars launched on August 20, 1975, was used to measure the concentration of species in the Mars atmosphere and had a mass range of 49 daltons. In addition, a gas chromatograph-mass spectrometer (GC-MS) developed by Klaus Biemann, with a mass range from 12 to 200 daltons, was used on the Viking lander to measure volatile species on the martian surface. The Cassini Mission to Saturn includes an ion and neutral mass spectrometer (INMS) composed of a quadrupole mass analyzer. Recently, Richard Zare and others have used multiphoton ionization and TOF mass spectrometry to selectively ionize and mass-analyze polyaromatic hydrocarbons (PAHs) from an interior fracture surface of the ALH84001 martian meteorite located in Antarctica. Also, Luann Becker and coworkers have analyzed trapped noble gases in fullerenes found in the 4.6-billion-year-old Allende meteorite.

Nearly every type of mass analyzer has been miniaturized. Recently, Jorge Diaz at the University of Minnesota has developed a subminiature double-focusing mass spectrometer with crossed electric and magnetic fields and a radius of curvature of 2 cm. The instrument has a mass range of 103 daltons and a mass resolution of one part in 106. At the Jet Propulsion Laboratory, Mahadeva P. Sinha has developed a miniaturized sector mass spectrometer with a focal plane detector, while at the Lawrence Livermore National Laboratories, Dan Dietrich and Mari Prieto have developed a miniaturized (15-inch) FT-ICR mass spectrometer. Ion traps are generally small mass analyzers; however, R. Graham Cooks and others at Purdue University have developed ion traps with a simplified 2.5-mm-diameter cylindrical ion trap geometry. These traps can then be assembled in arrays, with different portions of the mass range assigned to each of the traps. J. Michael Ramsey and William B. Whitten at Oak Ridge National Laboratories have developed ion traps with a radius of 0.5 mm, while Ferran Scientific in San Diego, California, has introduced bundled quadrupole mass filters known as *micropole arrays*.

Time-of-flight mass spectrometers have also been miniaturized. Kore technology offers an electron impact (EI) ionization instrument in a suitcase for detecting and identifying volatile and semivolatile organics. The Applied Physics Laboratory of the Johns Hopkins University, in collaboration with the Defense Advanced Research Projects Agency (DARPA), is developing tiny TOF mass spectrometers for the detection of biological

agents. Our laboratory has been involved in that project as well, developing a high-order kinetic energy focusing TOF mass analyzer known as the endcap reflectron, which is also being developed by Science & Engineering Services.

The Time-of-flight Mass Spectrometer

In the TOF mass spectrometer ions are formed in a short source region (Figure 1), accelerated by the electric field formed by the voltage *V* to a constant energy and injected into a longer drift region (*D*). The time of flight of the ion across this drift region is a function of the ion mass:

$$t = (m/2eV)^{1/2}D.$$

Mass resolution is generally improved using a reflectron, a device that corrects for the spread in ion velocities due to difference in initial kinetic energy by reversing the direction of the ion motion in the drift region back toward the ion source. Most of these provide first- or second-order energy correction, which is sufficient in instruments of the size used in the laboratory (1 meter or greater) to provide mass resolutions of 10,000 or more. Our own approach to miniaturization has been to develop a high-order reflectron in which a quadratic reflecting field is achieved by capping a grounded cylinder with a reflection electrode at high voltage (Figure 2).

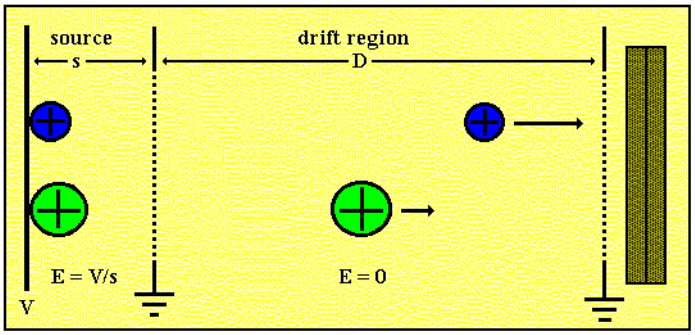


FIGURE 1. Diagram of a TOF mass spectrometer.

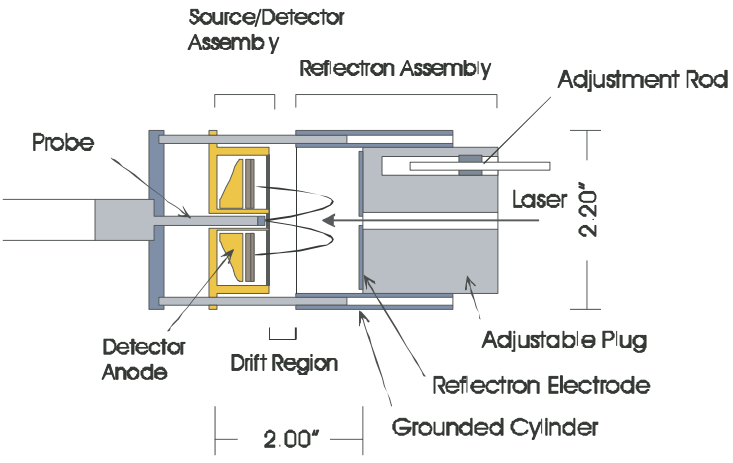


FIGURE 2. Diagram of endcap reflectron TOF mass spectrometer.

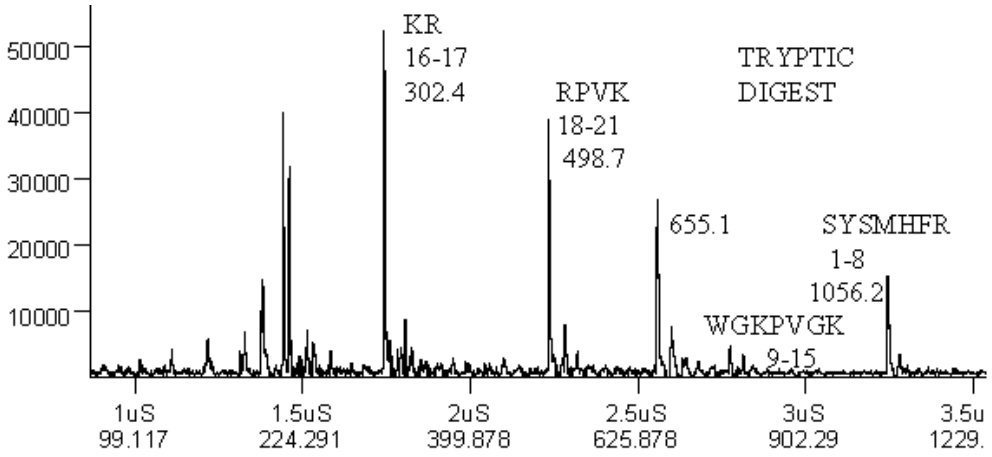


FIGURE 3. Mass spectrum of the tryptic peptides from a protein digest.

This instrument can be used to identify proteins from the mass spectra of their tryptic digests and comparison of the masses observed with protein or genomic databases (Figure 3).

Conclusions for Life Detection

At the University of Maryland, Catherine Fenselau and Plamen Demirev have obtained mass spectra for microorganisms, including bacterial spores lysed with a corona plasma discharge. The masses observed in the range of 500 to 10,000 daltons are generally those that can be identified with peptides in the bacteria. Rather than utilizing pattern recognition techniques, which require prior mass spectral investigation of each specific microorganism, these masses are compared directly with the protein database, which generally yields the protein source. This approach, which they have termed phyloproteomics, is a robust technique for identification of organisms that does not depend upon reproducible growth conditions or mass spectra. One should expect that MALDI time-of-flight mass spectrometers would provide similar biomarkers in both terrestrial and extraterrestrial scenarios, in which it would be possible to rapidly assess whether these are of known origin or are similar to known species.

LIFE DETECTION USING MOLECULAR METHODS

David A. Stahl
Department of Civil and Environmental Engineering
University of Washington

Abstract

Most molecular detection methods have not been designed to clearly resolve living from dead cellular material. Many diagnostic biopolymers—for example, DNA—may persist for very long periods of time within dead cells or following release from the cell. This contribution considers several basic features of the living cell that could serve as a basis to develop more definitive molecular tests of viability, independent of culture-based criteria. Molecular signatures that may serve this need include the transcriptional and translational machinery, the adenylate pool, and cell-membrane structure.

Introduction

The ultimate measure of life is documentation of the capacity for replication. Life detection has traditionally relied upon cultivation, and this remains the principal criterion presently used by NASA. However, recent advances in molecular biology and molecular systematics have revealed that most environmental populations of microorganisms resist cultivation. Therefore, available culture-based techniques are inadequate.

From a planetary protection mandate, current and future life detection needs can be assigned to two general categories:

1. Life detection—quantification of viable microorganisms and evaluation of physiological status; and
2. Identification—taxonomic and functional group assignment.

The higher priority is given to the first category, since the absence of life negates concerns of possible survival and propagation. However, estimations of survival and propagation require information provided by the second category. Thus, this brief overview is divided into two sections. The first section addresses available methods for life detection; the second addresses methods of identification. In addition to basic methodological approaches, some consideration is given to available sample size and sample collection since the abundance of organisms on spacecraft components is expected to be low. Although these are key issues that must be considered in the implementation of a comprehensive microbiological interrogation of a spacecraft, they are not elaborated here. Another area not explicitly addressed is detection of specialized microbial resting states, such as endospores.

Life Detection: Molecular Features Relevant to Life Detection

Intact Transcriptional and Translational Systems

Ribosomal RNA and mRNA Content It is well recognized that DNA-based techniques may not distinguish between living and dead materials. DNA is known to persist in some noncellular contexts, and DNA bound to surfaces such as clay is resistant to degradation.[126] However, other biopolymers such as RNA and phospholipids are much less stable and generally are rapidly degraded following cell death.[127,128] For example, reverse transcriptase polymerase chain reaction (RT-PCR) has been used to confirm that microbial populations detected at the ribosomal DNA level are metabolically active.[129] A major advantage of an rRNA-based measure of viability is the universal distribution of these biopolymers and the availability of PCR primers for highly efficient amplification from virtually any source organism.

Recent studies of *Escherichia coli* suggest that the loss of mRNAs for key housekeeping genes (e.g., rpoH, groEL, and tufA) could provide a sensitive measure of viability. All mRNAs comprising this study set were

undetectable by RT-PCR within 2 to 16 hours of heat killing.[130] However, the mechanism of killing, ethanol versus heat, also influenced mRNA stability. All mRNAs remained detectable in ethanol-killed cells for the entire 16-hour post-kill incubation period. The rRNAs were detected at the end of 16 hours' incubation in both ethanol- and heat-inactivated cells. Since PCR-based techniques are extremely sensitive—in principle detecting a single target molecule—rRNA- and mRNA-based viability testing may be a fruitful area for detection development. However, more complete characterization of rRNA and mRNA stability in relationship to different killing agents and post-killing incubation conditions is essential.

ATP and Energy Charge The presence of adenosine 5'-triphosphate (ATP) is one of the most general markers of life and has frequently been used in environmental studies to map the distribution of active microbiota. Chemiluminescent-based detection of ATP using luciferase is the most sensitive assay currently available; most luminometers can detect as little as 1 picomole of ATP. A recent study evaluated a commercial kit (Clean-Trace, Biotrace Ltd.) for detecting microorganisms on wet or dry surfaces.[131] The detection limit was approximately 10^4 colony-forming units (CFUs) per 100 cm^2 on surfaces artificially contaminated with *E. coli* or *Staphylococcus aureus*. A second study using this method to evaluate microbial contamination of milk reported similar detection limits. ATP content among different microbial species was found to be $0.8 \pm 0.1 \times 10^{-18}$ mol/CFU in coliforms; $12.0 \pm 8.1 \times 10^{-18}$ mol/CFU in *S. aureus*; $35.2 \pm 16.9 \times 10^{-18}$ mol/CFU in *S. thermophilus*; and $42.5 \pm 1.3 \times 10^{-18}$ in *Streptococcus* group D.[132]

A related measure of cellular energy status is energy charge, as defined by D.E. Atkinson:[133]

$$\text{Energy Charge} = \{[\text{ATP}] + [\text{ADP}]/2\}/\{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]\}.$$

Energy charge reflects the relative number of high-energy phosphate bonds (anhydride-bound phosphate groups) in the adenylate pool. The energy charge of bacteria under normal conditions varies between 0.87 and 0.95 irrespective of growth rate. Energy charge decreases slowly with starvation, and values below about 0.5 are thought to be incompatible with life. Although ATP content and energy charge are attractive physiological parameters for assessing viability, current detection formats provide relatively low sensitivity.

Intact Membrane Structure

Phospholipid Fatty Acid Analysis The presence of an intact membrane is a well-established measure of viability. Viable microbes have intact membranes containing phospholipids. Cellular enzymes hydrolyze (release) the phosphate group from phospholipids within minutes to hours of cell death.[134] Therefore, the determination of the total phospholipid ester-linked fatty acids (PLFAs) has been reported to provide a quantitative measure of the viable or potentially viable biomass. A study of subsurface sediment showed the viable biomass determined by PLFA was equivalent (but with a much smaller standard deviation) to that estimated by intercellular ATP, cell wall muramic acid, and very carefully done acridine orange direct counts (AODC).[135] Reported detection limits are in the range 10 to 100 bacteria.

Viability Staining Microscopy alone is insufficient to assess the viability of a cell. However, a variety of fluorescent and fluorogenic stains provide a mechanism to evaluate viability when used in combination with epifluorescence microscopy or flow cytometry. There are two complementary types of stains used to achieve discrimination. The first type are lipid-soluble fluorogenic compounds that freely diffuse into most cells and become fluorescent following cleavage by nonspecific intracellular esterases. The structure of the fluorescent product retards subsequent loss from cells with intact membranes. The other class of dyes has high affinity for nucleic acid but will not penetrate intact cell membranes. Therefore, only nonviable cells are stained. One recent study evaluated several dyes (fluorescein diacetate [FDA] derivatives carboxyfluorescein diacetate, 2',7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester, and calcein acetoxymethyl ester, and ChemChrome B) for the assessment of microbial viability.[136] None of these dyes stained all organisms in the study set. ChemChrome B stained the greatest variety of Gram-negative and Gram-positive organisms, whereas FDA derivatives were reported to preferentially stain Gram-negative organisms. A commercial product based on this general

screening (BacLight, Molecular Probes) is now being evaluated at the Jet Propulsion Laboratory.[137] In its current format, samples are collected from spacecraft surfaces by swapping, resuspended in buffer, and stained with the BacLight reagents. Stained samples are then filtered and quantified using epifluorescence microscopy. Reported technical problems include low efficiency of cell removal from surfaces and high error associated with quantifying extremely low cell numbers microscopically.

Molecular Identification—Taxonomic and Functional Group Assignment

Molecular Systematics—The Foundation for Comprehensive Detection Strategies

The conceptual foundation for molecular systematics was formally introduced in 1965 by E. Zuckerkandl and L. Pauling.[138] Biopolymers (DNA, RNA, or polypeptides) were classified according to the degree to which they reflected the genealogy of an organism. The central model of molecular evolution is one of random evolutionary changes occurring at a stochastically constant rate. This model was introduced in the form of the "molecular clock," which requires a constant rate of change in the sequence of a common biopolymer, the "molecular chronometer." Useful chronometers must display certain properties including the following:

1. Molecular clock-like behavior;
2. Range—rates of change must be commensurate with the spectrum of evolutionary distances measured; and
3. Size—the molecule must be large enough to provide an adequate amount of information.

A good molecular chronometer should also fulfill a number of additional criteria, including universal distribution, functional constancy, and the absence of lateral transfer.

The ease by which nucleic acid sequence can be determined has resulted in its near routine use in microbial systematics and increasing application to direct microbial detection. Genes so far employed in general phylogenetic studies include the ribosomal RNAs, ATPases, protein elongation factors, gyrB,[139,140] dnaK, and cytochromes.[141,142] Among all available, the ribosomal RNAs (rRNAs) remain the target of choice. The establishment of a robust microbial phylogeny based on comparative sequencing of the rRNAs provided the first consistent taxonomic description of microorganisms[143] and was an essential prelude to the unambiguous characterization of environmental populations (Pace et al.,[144] Stahl,[145] Stahl and Amann,[146] Ward et al.,[147] Hugenholtz et al.[148]).

Three types of rRNA are common to the ribosomes of prokaryotes and eukaryotes, the 16S (and 16S-like), the 23S (and 23S-like), and the 5S rRNAs. Since the larger rRNAs of many eukaryotes and some prokaryotes differ significantly in size, the terms 16S-like and 23S-like have been used to refer to the two larger rRNAs. Certain attributes of the rRNAs favor their use as molecular chronometers and their use in direct detection. Although the genes that code for the rRNAs are among the most highly conserved,[149] sequence conservation is not uniform along the length of these genes. Some regions of sequence are highly conserved and others show intermediate levels of sequence conservation. Regions that vary sufficiently slowly allow inference of relationships between members of the three domains (Bacteria, Eucarya, and Archaea),[150] whereas the most variable regions provide for discrimination between organisms of approximate genus and species rank differences. Regional variation in sequence conservation has provided the basis for designing nucleic acid probes of varying specificity; group- and species-specific oligonucleotide probes have been used for direct assessment of population presence and abundance.[151] The most highly conserved regions have also been used as general PCR priming sites for the selective amplification of virtually any rRNA. Well over 10,700 16S-like rRNA sequences are now available in the Ribosomal Database Project (RDP) database.[152,153] Thus, the 16S-like rRNA is now the most encompassing of available molecular frameworks for identification and detection of microorganisms.

Identification of Diagnostic Nucleic Acid Sequences in Environmental Samples

There are three basic methods now used to recover nucleic acid sequence information from environmental samples:

1. PCR amplification, cloning, and sequencing;
2. DNA probe hybridization; and
3. Restriction enzyme digestion.

The PCR-based methods can use either DNA or RNA as template, the latter requiring initial use of reverse transcriptase to generate cDNA from an rRNA template. As noted above, the use of rRNA as a template may serve to provide a more direct measure of viable population numbers, although this has yet to be rigorously evaluated. The PCR-based methods are of exquisite sensitivity. Less than a single microorganism can be detected by RT-PCR using rRNA as a template (a single organism generally contains more than a thousand copies of each rRNA species). However, its sensitivity also makes this method very prone to artifacts of laboratory or reagent contamination.

Sequencing Methods As discussed above, for a variety of technical and practical considerations (size, information content, ease of sequencing), the 16S-like rRNA has become the standard measure for identification.[154,155] The now well-established techniques of PCR amplification, DNA cloning, and nucleic acid sequencing have become general tools for identification of microorganisms in the environment based on rRNA sequence relationship. They are not elaborated upon here.

Terminal Restriction Fragment-Length Polymorphism Terminal restriction fragment length polymorphism (T-RFLP) is a refined fingerprinting technique. It has been most productively used to resolve amplified rRNA gene products by virtue of sequence differences detected following digestion with restriction enzymes.[156] The general steps include PCR amplification of a conserved target sequence (most commonly a region of the 16S-like rRNA gene) followed by restriction enzyme digestion and gel fractionation of the resulting fragments. Thus, the method incorporates two levels of target recognition—the first provided by selective PCR amplification and the second by virtue of presence and location of restriction enzyme recognition sequences.

Following recovery of DNA or RNA from an environmental sample, the 16S-like rRNA sequences are amplified using one of the many well-characterized PCR primer sets. However, in this method one of the two PCR primers is fluorescent labeled at the 5'-end. This results in PCR amplification products that are tagged with a fluorescent dye at only one terminus. Following restriction enzyme digestion (usually with a tetrameric restriction enzyme) the restricted products are resolved using an automated DNA sequencer or a capillary electrophoresis system equipped with laser-induced fluorescence detector. Only the fluorescently tagged terminal fragments are detected and quantified. This is an extremely sensitive technique (as low as 100 attomole of dye can be detected), and fragment lengths between 37 and 937 can be accurately assigned (±1-2 base pairs) by comparison to a set of size markers labeled with a different fluorescent dye and incorporated in each sample prior to electrophoresis.

As a whole, each T-RFLP pattern can be regarded as a fingerprint of population structure that provides information on population diversity (each terminal fragment and associated restriction site sequence define different "ribotypes") and a semiquantitative estimate of relative abundance (peak area). T-RFLP provides an advantage over most molecular fingerprinting methods in that phylogenetic inference of predominant terminal fragment lengths within a community can be made once an appropriate database for the given sample is constructed.[157] Thus, comparison of T-RFLP fingerprints derived from both the rRNA gene and the rRNA of an environmental sample serves to confirm that microbial populations detected at the rDNA level are metabolically active and also provides some information of relative activity.[158] T-RFLP has also been extensively applied to examine variation among different functional genes in different environmental settings. Thus, this general approach might be used for highly sensitive detection of viable and commonly occurring assembly-room contaminants.

DNA Probes There are two basic categories of DNA probes, group-specific (phylogenetic or taxonomic) and functional. Group-specific probes generally target conserved biopolymers that can be used to infer phylogenetic relationships among the host organisms.[159,160] Today, the most widely used target molecule is the small subunit rRNA (16S-like rRNA), and probes have been designed to target phylogenetic groups of varying evolutionary diversity ("phylotypes"). Phylogenetic probes therefore have the potential to provide explicit quantification of

microbial contamination at different levels of resolution. This approach could be used for the detection of very general classes of assembly area microbial contaminants.

Quantification of Populations or Single Cells There are two basic formats for using phylogenetic probes to study the environmental distribution of microorganisms. These are hybridization to total rRNA recovered from environmental samples and hybridization to fixed whole cells for subsequent microscopic visualization and enumeration. The latter technique, fluorescent in situ hybridization (FISH), uses fluorescent dye-labeled probes for microscopic detection of cells that hybridize to the probe. Both methods are well developed and in routine application.[161-163] Both methods avoid the biases associated with PCR amplification by taking advantage of the natural amplification of the rRNAs. There are generally thousands of copies of each rRNA per cell, although the number for slow-growing cells is not well defined. However, both detection formats are much less sensitive than PCR-based methods. The lower limit of detection for radio-labeled probes is approximately 10^3-10^4 cells,[164] and although a single cell can be observed microscopically, enumeration of low-abundance samples is limited by the image analysis requirements previously discussed. The efficiency of sample recovery and cell lysis or permeabilization are also poorly defined variables.

Functional Probes An important distinction is made between probes designed to identify phylogenetic or taxonomic groups, and probes designed to monitor specific metabolic functions. There has been considerable development of probes targeting genes encoding specific enzymes to evaluate specific chemical transformations or potential activity of environmental populations. These "functional" gene probes should have application in determining whether spacecraft contaminants harbor microbial phenotypes more likely to proliferate in nonterrestrial habitats (e.g., autotrophs). Some examples of traits, and corresponding genes, sufficiently conserved to be easily identified in environmental samples include genes for nitrogen fixation,[165] Ni-Fe hydrogenase,[166] CO_2 fixation, and the dissimilatory sulfite reductase.[167,168]

Other Technical Issues

The following technical issues are key considerations in detection. However, since relatively little explicit or comparative data is available, they are only briefly listed here:

- Time and cost of analysis;
- Confidence levels;
- Signal to noise—the ability to "see" life in a complex background matrix;
- Sensitivity;
- Specificity;
- Analytical framework;
- Destructive sampling versus replication;
- In situ analyses versus the requirement for sample collection;
- Efficiency of sample collection and possible associated loss of viability; and
- Witness plates-active versus passive collection.

A Possible Role for Improved Culture- and Activity-Based Methods

The NASA Standard Assay is based on standard culture-based enumeration of cells and spores. However, the current protocol requires extended incubation periods and may miss more than 99 percent of microorganisms. Although this and other standard culture-based methods are now recognized to be inadequate, there may be merit in exploring alternative culture-based methods to either enumerate or measure physiological activity. For example, a recent study of low-abundance ice-entrained bacteria (200-300 cells/ml) showed that at least some of the assemblage was viable as measured by respiration of ^{14}C-labeled acetate and glucose.[169]

A ROBOTIC-PCR DETECTOR FOR DNA-BASED LIFE ON OTHER PLANETS

Gary Ruvkun
Harvard Medical School, Department of Molecular Biology, Massachusetts General Hospital
Michael Finney
MJ Research, 590 Lincoln St., Waltham, Massachusetts
Walter Gilbert
Department of Molecular and Cellular Biology, Harvard University
George M. Church
Department of Genetics, Harvard Medical School

Abstract

Key genetic components such as the ribosomal RNA genes are conserved in all living organisms on Earth. The polymerase chain reaction (PCR) with DNA primers corresponding to conserved elements in the ribosomal RNA genes is now used to amplify minute quantities of DNA from extreme Earth environment samples, allowing the detection and classification of life in those samples without need to isolate, culture, or grow the organisms in any way. This is currently our most sensitive detector of life on Earth. These same primers can be used to search for life on Mars that is related to life on Earth. Life on Mars would be ancestrally related to life on Earth if there was exchange of viable organisms between Mars and Earth, or more generally, common ancestry from a source outside of the solar system, something we call Panarchaea. If microbial life exists on Mars currently and is more abundant than about one cell per gram at the landing site, PCR is sensitive enough to detect the signature of life. This signature is the amplification of a 500 base-pair-long DNA fragment that can be sequenced in situ on Mars using simple gene array technology to determine its phylogenetic placement. PCR technology is very mature. A low power consumption, very light, robotic PCR thermal cycler could be sent to the surface of Mars to sample the soil for microbial life.

Even though life has so far only been found on Earth, most strategies for the detection of life on other planets and moons assume independent evolution on those planets. General life detection strategies involve the detection of structural and informational polymers, of microscopic or macroscopic structures, or of chemical signatures of metabolism. It is worth noting, however, that these are not the tools currently used by molecular biologists to search for the most divergent life on Earth. First, the rate of metabolism for many of the most divergent organisms, and the numbers of individual organisms in many niches, is so low that such detection tests currently fail. For the detection of the most diverse microbial life on Earth, there is a much more sensitive and simple DNA-based search strategy that is essentially definitive.

The exploration of the diversity of life on Earth has revealed key genetic components that are conserved in all free-living organisms. The most conserved of these genes are the ribosomal RNA genes, one class of which is called the 16S or 18S RNA genes.[170] Ribosomal RNAs are the main structural and catalytic components of the ribosome, the molecular machine that translates RNA into proteins. Because of this universality, all organisms are thought to have inherited their ribosomal RNA gene (as well as hundreds of other genes that have drifted more significantly during the past 3 billion years) from a common ancestor. The root of that tree has been hypothesized to be an archaeal-like hyperthermophile.[171]

Within the approximately 1500 nucleotides of the ribosomal RNA gene, there are multiple 20-nucleotide segments that are exactly conserved between organisms as disparate as the single-celled extremophile Archaeae that live in Earth's crust and humans, or corn.[172] These conserved elements in the ribosomal genes are our most sensitive detectors of life on Earth and are being used to drastically expand the known universe of life. The reason they are so sensitive is that the PCR with DNA primers corresponding to these absolutely conserved elements can be used to amplify any DNA species, without the need to isolate, culture, or grow the organsims in any way.

The polymerase chain reaction amplifies the number of copies of a specific region of DNA in order to produce enough DNA, for example, for DNA sequence analysis. In PCR, one must know the sequences that flank a given region, but one need not know the DNA sequence in between. In addition to the range of organisms that can be detected, the PCR approach has the added advantages of extreme sensitivity and almost trivial ease. PCR can often detect a single DNA double helix in a clean sample in a two-hour analysis. The biochemical processing of the sample can be as crude as a cheek swab from humans to agitation of a soil sample. The technology of PCR involves adding stable 20-nucleotide-long DNA primers, a stable enzyme and nucleotide monomers that function at 370 Kelvin, and a simple heat pump that thermally cycles 40 times in two hours to amplify DNA from a few strands to billions of identical DNA fragments. In the case of the ribosomal genes, the DNA primer 5' GTGCCAGCAG CCGCGGTAAT, which corresponds to nucleotides 601 to 621 of a ribosomal gene, and GGTGGTCCTG ACCTCGGAC 5', which corresponds to the base pairing complement of nucleotides 1213 to 1233, are added to a soil extract. These DNA primers pair with their complement on each DNA strand, even if there are only a few DNA molecules in a sample. When DNA polymerase and mononucleotides are added, that DNA strand is duplicated. If one repeats the thermal cycle with all the same components in the same tube, there will be eight strands. Twenty cycles produce one million copies of the original sequences.

```
5'   GTGCCAGCAGCCGCGGTAAT      -------→
601  GTGCCAGCAGCCGCGGTAATTCCAGCT ...600 nt. GGGCACCACCAGGAGTGGAGCCTG 1233
                                       ←------      GGTGGTCCTGACCTCGGAC 5'
```

This PCR technology is standard in small labs all over the world. Currently, hundreds of research groups are using ribosomal RNA PCR primers to amplify samples isolated from a wide range of environments—for example, from marine water samples or extreme environments.[173] In this way, vast numbers of new archaeal and bacterial species have been found in extreme temperature or in dehydrated or radioactive environments as well as in the most typical ocean sample. Interestingly, most of the life that is detected in these samples cannot be cultured, suggesting either very slow growth rates or very particular growth conditions not met in the laboratory. This also suggests that metabolic detection of life can be very problematic since the growth rates can be slow or nil in simple culture conditions.

Since universal PCR primers from the ribosomal RNA gene can detect essentially all living organisms on Earth, these same primers can be used to search for life on Mars that is related to life on Earth. If such microbial life exists on Mars currently and is more abundant than about one cell per gram at the landing site, PCR is sensitive enough to detect the signature of life—amplification of DNA with particular DNA sequence features using these DNA primers.

There are two conditions under which life on Mars should resemble life on Earth to the point of sharing ribosomal RNA sequences: exchange between Mars and Earth or, more generally, common ancestry from another source, perhaps even outside of the solar system. In either case, organisms (or perhaps the hardier spores that many microbes form) would have to survive the passage. Ultraviolet radiation damage appears to be easily shielded for example, by simple embedding in a rock, though survival for millions of years has not been addressed.[174,175]

The ability of a crustal sample to escape Mars and land on Earth without sterilizing heating is demonstrated by ALH84001 martian meteorite. While the ALH84001 meteorite evidence for fossils is debated, this rock may not have been heated above the survival temperature of modern spores,[176] though this too is debated.[177] The flux of such meteoritic exchange between Mars and Earth would be much more intense at the heavy bombardment stage of the solar system, as well as in the period of sporadic collisions towards the end of that era. Thus, life in the solar system may have evolved on Earth, Mars, or another planet and exchanged between those planets.

There is also a reasonable argument for life on Earth coming from outside the solar system, something we call Panarchaea. The fossil record of Earth shows that within a few hundred million years of the cessation of the bombardment phase (at 4.0 billion to 4.2 billion years ago), living organisms morphologically similar to contemporary stromatolites, a mat composed of billions of bacteria, had already become abundant (at 3.6 billion years ago).[178] This observation is usually marshaled as evidence that the evolution of life from simple molecules to

simple organisms was rapid. In that 300 million to 500 million years, life had to evolve from simple organic molecules to a bacteria-like organism with DNA-based genetic material, a very complex ribosome, and many other "core" genetic components now shared between the disparate branches on the tree of life.[179]

A different interpretation of the appearance of bacteria-like organisms within a short period of time after the end of bombardment is that it may represent the time it took for preexisting microbes to colonize the virgin chemical gradients and reduced carbon substrates in Earth's crust. If one accepts that evolution to microbes can occur in 500 million years, then there may have been planets with microbial life that evolved during the 8 billion years of galactic evolution that preceded the birth of the solar system. Since planets are thought to be common and the habitable zone for life has broadened to much of the solar system, life may have evolved on many planets. Once life evolved in any planetary system, ejection of that life by meteorite and comet impacts or by chaotic planetary orbit pertubation and ejection could allow exchange within and between solar systems.[180] Of course, if extraterrestrial life seeded Earth, Mars might have been seeded by the same source.

The ability of microbial life to survive such long transits in vacuum and high radiation strains credulity, though protection could be afforded if the ejecta was as large as a planet or asteroid.[181] However, the improbability of the arrival of life on Earth from another source must be compared to the similarly improbable scenario that the isotopic record in ancient rocks implies: that life evolved on Earth within 500 million years from primordial soup to full-fledged microbes.

A strength of the Panarcheal argument is that it makes clear predictions of what extraterrestrial life should look like. For both the case of exchange between Mars and Earth and the general case of exchange between planetary systems, life on Mars should share ribosomal RNA genes with DNA sequence homology to earthly ribosomal genes, and PCR will be able to detect them.

Life on Mars related to life on Earth may have flourished a billion years ago, when water was more plentiful. PCR has been used to detect DNA in samples thousands of years old but not a million or a billion years old—we do not expect to detect microbial fossils with this procedure. But microbial life may still flourish in the very low temperature and low hydration of martian soils. Microbes are very adaptable: we find them growing in the extreme cold and dryness of Antarctica, as well as the extreme heat and pressure of Earth's crust.[182] Martian microbes may have solved the problem of life at 150 to 210 K with near zero water. In addition, the signs of massive recent water flows on Mars suggest more hospitable microbial niches in the not too distant past.[183] Also, evidence for recent martian volcanism, suggests that temperatures may rise below the martian surface, especially near the sites of the most recent volcanism.[184] And even if life flourishes only in isolated oases away from any landing site, the dust storms evident on Mars may blow recently alive or living microbial cells and/or spores into the landing site and be detectable with a technique as sensitive as PCR.

The technology to amplify and detect the ribosomal RNA signature of life on Mars is simple and can be engineered into a lightweight and reliable automated form. PCR thermal cyclers involve very simple heating and cooling that can use just a few watts. The technology is very mature, with thousands of solid-state thermal cycling machines, which cost from $1,000 to $10,000 each, installed in small laboratories all over the world. Many of these machines are robotically controlled in genome centers for high-throughput genome sequencing. PCR can thus be done with very little weight, low power consumption, and very forgiving technology.

The soil sample handling capabilities of a future Mars lander could deliver samples to a suspension and dilution station. Filtration would be used to sieve out microbial-sized particles (microbial size on Earth is remarkably uniform, across vast phylogenetic distances). A simple extraction procedure can release the contents of any cells and remove contaminants. Samples would then be mixed with reagents including PCR primers corresponding to the universal elements of ribosomal genes and thermally cycled. Bona fide ribosomal RNA genes would be expected to generate a PCR product of a size in a narrow range. The DNA fragment from the PCR reaction would be probed to a gene array bearing 100,000 small oligonucleotides representing the DNA sequence space for the ribosomal RNA gene, to remotely determine the DNA sequence. In this way, any organism detected on Mars, could be placed in or outside of known microbial phylogeny.

The phylogenetic placement of the detected ribosomal RNA gene is important for a quite different reason. PCR is so powerful that contamination is a critical issue. A ribosomal RNA gene sequence corresponding to a known earthly clade (such as humans or any of the common bacteria that live on the skin) would be rejected.

Another key to ruling out contamination will be dilution of the soil samples followed by PCR analysis; any true martian organism should be found in proportion to the input soil amount. There are also a number of well-established procedures, such as chemical and ultraviolet treatments to eliminate DNA contamination.

The fluid handling components to handle the soil samples and transit to the PCR module could be microfluidic and weigh less than 100 grams. The thermal cycler for amplification could weigh just a few hundred grams. In principle, the DNA sequence analysis chip and charge coupled device electronics to read the chip scanner could be similar in size to a portable CD player. Such a micromicrobial analysis machine would also encourage useful advances in portable terrestrial DNA analyses as spinoffs.

Acknowledgment

We thank Jonathan Lunine for helpful comments on the manuscript.

REFERENCES FOR PAPERS IN SESSION 3

1. C.R. Clemmer and T.P. Beebe, "Graphite: A Mimic for DNA and Other Biolmolecules in Scanning Tunneling Microscope Studies," *Science* 251:640-642, 1991.

2. See, for example, A.G. Michette, *Optical Systems for Soft X Rays,* Plenum Press, New York, 1986.

3. See, for example, J. Kirz, C. Jacobsen, and M. Howells, "Soft X-ray Microscopes and Their Biological Applications, *Q. Rev. Biophys.* 28:33-130,1995.

4. See, for example, J. Thieme, G. Schmahl, E. Umbach, and D. Rudolph (eds.), *X-ray Microscopy and Spectromicroscopy,* Springer-Verlag, Berlin, 1998.

5. See, for example, W. Meyer-Ilse, D.T. Attwood, and A. Warwick (eds.), *X-ray Microscopy: Proceedings of the Sixth International Conference, Berkeley, California, 2-6 August 1999,* American Institute of Physics (AIP), Melville, New York, 2000.

6. H. Wolter, "Spiegelsysteme Streifenden Einfalls als Abbildende Optiken für Röntgenstrahlen," *Ann. Phys.* 10:94-114, 286, 1952.

7. D. Sayre, J. Kirz, R. Feder, D.M. Kim, and E.D. Spiller, "Potential Operating Region for Ultrasoft X-ray Microscopy of Biological Materials," *Science* 196:1339-1340, 1977.

8. M. Isaacson and M. Utlaut, "A Comparison of Electron and Photon Beams for Determining Micro-chemical Environment," *Optik* 50:213-234, 1978.

9. E.G. Rightor, A.P. Hitchcock, and H. Ade, "Spectromicroscopy of Polyethylene terephthalate: Comparison of Spectra and Radiation Damage Rates in X-ray Absorption and Electron Energy Loss," *Journal of Physical Chemistry B* 101:1950-1960, 1997.

10. See, for example, G. De Stasio, D. Dunham, B.P. Tonner, D. Mercanti, M.T. Ciotti, P. Perfetti, and G. Margaritondo, "Application of Photoelectron Spectromicroscopy to a Systematic Study of Toxic and Natural Elements in Neurons," *Journal of Synchrotron Radiation* 2:106-112, 1995.

11. S. Spector, C. Jacobsen, and D. Tennant, "Process Optimization for Production of Sub-20 nm Soft X-ray Zone Plates," *Journal of Vacuum Science and Technology B* 15:2872-2876, 1997.

12. E. Anderson and D. Kern, "Nanofabrication of Zone Plate Lenses for High-resolution X-ray Microscopy," in *X-ray Microscopy III: Proceedings of the Third International Conference, London, September 3-7, 1990,* A.G. Michette, G.R. Morrison, and C.J. Buckley (eds.), Springer-Verlag, New York, 1992.

13. D. Weiss, M. Peuker, and G. Schneider, "Radiation-enhanced Network Formation in Copolymer Galvanoforms for Diffractive Nickel X-ray Optics with High Aspect Ratios," *Applied Physics Letters* 72:1805-1807, 1998.

14. C. Jacobsen, S. Williams, E. Anderson, M.T. Browne, C.J. Buckley, D. Kern, J. Kirz, M. Rivers, and X. Zhang, "Diffraction-limited Imaging in a Scanning Transmission X-ray Microscope," *Optics Communications* 86:351-364, 1991.

15. T. Warwick, H. Ade, S. Cerasari, J. Denlinger, K. Franck, A. Garcia, S. Hayakawa, A. Hitchcock, J. Kikuma, S. Klingler, J. Kortright, G. Morisson, M. Moronne, E. Rightor, E. Rotenberg, S. Seal, H.-J. Shin, W.F. Steele, and B.P. Tonner, "Development of Scanning X-ray Microscopes for Materials Science Spectromicroscopy at the Advanced Light Source," *Journal of Synchrotron Radiation* 5:1090-1092, 1998.

16. X. Zhang, H. Ade, C. Jacobsen, J. Kirz, S. Lindaas, S. Williams, and S. Wirick, "Micro-XANES: Chemical Contrast in the Scanning Transmission X-ray Microscope," *Nuclear Instruments and Methods in Physics Research* A 347:431-435, 1994.

17. C. Jacobsen, S. Wirick, G. Flynn, and C. Zimba, "Soft X-ray Spectroscopy from Image Sequences with Sub-100 nm Spatial Resolution," *Journal Microsc.* 197:173-184, 2000.

18. See, for example, J. Kirz, C. Jacobsen, and M. Howells, "Soft X-ray Microscopes and Their Biological Applications, *Q. Rev. Biophys.* 28:33-130, 1995.

19. G. Schneider, "Cryo X-ray Microscopy with High Spatial Resolution in Amplitude and Phase Contrast," *Ultramicroscopy* 75:85-104, 1998.

20. J. Maser, A. Osanna, Y. Wang, C. Jacobsen, J. Kirz, S. Spector, B. Winn, and D. Tennant, "Soft X-ray Microscopy with a Cryo Scanning Transmission X-ray Microscope: I. Instrumentation, Imaging and Spectroscopy," *Journal Microsc.* 197:68-79, 2000.

21. W.S. Haddad, I. McNulty, and J.E. Trebes, "Ultrahigh-resolution X-ray Tomography," *Science* 266:1213-1215, 1994.

22. J. Lehr, "3D X-ray Microscopy: Tomographic Imaging of Mineral Sheaths of Bacteria *Leptothrix ochracea* with the Göttingen X-ray Microscope at BESSY," *Optik* 104:166-170, 1997.

23. Y. Wang, C. Jacobsen, J. Maser, and A. Osanna, "Soft X-ray Microscopy with a Cryo Scanning Transmission X-ray Microscope: II. Tomography," *Journal Microsc.* 197:80-93, 2000.

24. See, for example, Figure 1 from X. Zhang, R. Balhorn, J. Mazrimas, and J. Kirz, "Mapping and Measuring DNA to Protein Ratios in Mammalian Sperm Head by XANES Imaging," *Journal Struct. Biol.* 116:335-344, 1996.

25. J. Kirz, C. Jacobsen, and M. Howells, "Soft X-ray Microscopes and Their Biological Applications, *Q. Rev. Biophys.* 28:33-130, 1995.

26. H. Ade, X. Zhang, and S.H. Cameron, "Chemical Contrast in X-ray Microscopy and Spatially Resolved XANES Spectroscopy of Organic Specimens," *Science* 258:972-975, 1992.

27. H. Ade, "Compositional and Orientational Characterization of Polymeric Systems with X-ray Microscopy," *Trends in Polymer Science* 5:58-66, 1997.

28. For further examples see, for example, W. Meyer-Ilse, D.T. Attwood, and A. Warwick (eds.), *X-ray Microscopy: Proceedings of the Sixth International Conference, Berkeley, California, 2-6 August 1999,* American Institute of Physics (AIP), Melville, New York, 2000.

29. C. Jacobsen, S. Wirick, G. Flynn, and C. Zimba, "Soft X-ray Spectroscopy from Image Sequences with Sub-100 nm Spatial Resolution," *Journal Microsc.* 197:173-184, 2000.

30. G.J. Flynn, L.P. Keller, C. Jacobsen, and S. Wirick, "Carbon and Potassium Mapping and Carbon Bonding State Measurements on Interplanetary Dust," *Meteoritics and Planetary Science* 33(supplement):A50-51, 1998.

31. G. Cody, R.E. Botto, H. Ade, and S. Wirick, "Soft X-ray Microscopy and Microanalysis: Applications in Organic Geochemistry," in *X-ray Microbeam Technology and Applications,* W. Yun (ed.), International Society for Optical Engineering (SPIE), Bellingham, Washington, 1995.

32. W.W. Barker and J.F. Banfield, "Zones of Chemical and Physical Interaction at Interfaces Between Microbial Communities and Minerals: A Model," *Geomicro. Journal* 15:223-244, 1998.

33. S.A. Welch, W.W. Barker, and J.F. Banfield, "Microbial Extracellular Polysaccharides and Plagioclase Dissolution," *Geochim. Cosmochim. Acta* 63:1405-1409, 1999.

34. S.C.B. Myneni, J.T. Brown, G.A. Martinez, and W. Meyer-Ilse, "Imaging of Humic Substance Macromolecular Structures in Water and Soils," *Science* 286:1335-1337, 1999.

35. H.H. Teng and P.M. Dove, "Surface Site-specific Interactions of Aspartate with Calcite During Dissolution: Implications for Biomineralization," *Am. Mineral.* 82:878-887, 1997.

36. See P. Echlin, *Low-Temperature Microscopy and Analysis,* Plenum Press, New York, 1992, 539 pp.

37. See R.A. Steinbrecht and K. Zierold, *Cryotechniques in Biological Electron Microscopy,* Springer-Verlag, New York, 1987, 297 pp.

38. R.C. Cantor and L.C. Smith, *Genomics: The Science and Technology Behind the Human Genome Project,* John Wiley & Sons, New York, 1999.

39. J.J. Kasianowicz, E. Brandin, D. Branton, and D.W. Deamer, "Characterization of Individual Polynucleotide Molecules Using a Membrane Channel," *Proc. Natl. Acad. Sci. USA* 93:13770-13773, 1996.

40. M. Akeson, D. Branton, J.J. Kasianowicz, E. Brandin, and D.W. Deamer, "Microsecond Time-scale Discrimination Among Polycytidylic Acid, Polyadenylic Acid, and Polyuridylic Acid as Homopolymers or as Segments Within Single RNA Molecules," *Biophys. J.* 77:3227-3233, 1999.

41. A. Meller, L. Nivon, E. Brandin, J. Golovchenko, and D. Branton, "Rapid Nanopore Discrimination Between Single Polynucleotide Molecules," *Proc. Natl. Acad. Sci USA.* 97:1079-1084, 2000.

42. A. Meller, L. Nivon, E. Brandin, J. Golovchenko, and D. Branton, "Rapid Nanopore Discrimination Between Single Polynucleotide Molecules," *Proc. Natl. Acad. Sci USA* 97:1079-1084, 2000.

43. A. Meller, L. Nivon, and D. Branton, "Voltage-driven DNA Translocations Through a Nanopore," *Phys. Rev. Lett.* 86:3435-3438, 2001.

44. G.A. Logan, J.M. Hayes, G.B. Hieshima, and R.E. Summons, "Terminal Proterozoic Reorganization of Biogeochemical Cycles," *Nature* 376:53-56, 1995.

45. S. Ziegler and M.L. Fogel, "Seasonal and Diel Determinants of the Isotopic Composition of Organic Matter in a Freshwater Wetland," *American Chemical Society Abstract* 221, GEOC 61, 2001.

46. See paper by D. Stahl, Session 3, in this appendix.

47. See paper by J.M. Muldowan, Session 4, in this appendix.

48. M.D. McCarthy, J.I. Hedges, and R. Benner, "Bacterial Origin of a Major Fraction of Dissolved Organic Nitrogen in the Sea," *Science* 281:231-233, 1998.

49. M.L. Fogel and N. Tuross, "Transformation of Plant Biochemicals to Geological Macromolecules During Early Diagenesis," *Oecologia* 120:336-346, 1999.

50. See paper by R.J. Cotter, Session 3, in this appendix.

51. E.J. Gaidos, K.H. Nealson, and J.L. Kirschvink, "Life in Ice-covered Oceans," *Science* 284:1631-1633, 1999.

52. H.A. Lowenstam and S. Weiner, *On Biomineralization,* Oxford University Press, Oxford, U.K., 1989, p. 324.

53. J.F. Kasting, "Earth's Early Atmosphere," *Science* 259:920-926, 1993.

54. D.A. Evans, N.J. Beukes, and J.L. Kirschvink, "Low-latitude Glaciation in the Paleoproterozoic," *Nature* 386(6622):262-266, 1997.

55. J.L. Kirschvink, E.J. Gaidos, L.E. Bertani, N.J. Beukes, J. Gutzmer, L.N. Maepa, and R.E. Steinberger, "Paleoproterozoic Snowball Earth: Extreme Climatic and Geochemical Global Change and Its Biological Consequences," *Proc. Natl. Acad. Sci. USA* 97:1400-1405, 2000.

56. R. Rye and H.D. Holland, "Paleosols and the Evolution of Atmospheric Oxygen: A Critical Review," *Amer. Journal Sci.* 298(8):621-672, 1998.

57. P.M. Harrison, "The Structure and Function of Ferritin," *Biochem. Ed.* 14:154-162, 1986.

58. S. Mann, J. Hannington, and R. Williams, "Phospholipid Vesicles as a Model System for Biomineralization," *Nature* 324:565-568, 1986.

59. J.L. Kirschvink and H.A. Lowenstam, "Mineralization and Magnetization of Chiton Teeth: Paleomagnetic, Sedimentologic, and Biologic Implications of Organic Magnetite," *Earth Planet. Sci. Lett.* 44:193-204, 1979.

60. J.L. Kirschvink, "Paleomagnetic Evidence for Fossil Biogenic Magnetite in Western Crete," *Earth Planet. Sci. Lett.* 59:388-392, 1982.

61. H. Vali and J.L. Kirschvink, "Observations of Magnetosome Organization, Surface Structure, and Iron Biomineralization of Undescribed Magnetic Bacteria: Evolutionary Speculations," in *Iron Biomineralization,* R.P. Frankel and R.P. Blakemore (eds.), Plenum Press, New York, 1991, pp. 97-115.

62. R.P. Blakemore, "Magnetotactic Bacteria," *Science* 190:377-379, 1975.

63. R.B. Frankel, R.P. Blakemore, and R.S. Wolfe, "Magnetite in Freshwater Magnetotactic Bacteria," *Science* 203:1355-1356, 1979.

64. H. Vali and J.L. Kirschvink, "Observations of Magnetosome Organization, Surface Structure, and Iron Biomineralization of Undescribed Magnetic Bacteria: Evolutionary Speculations," in *Iron Biomineralization,* R.P. Frankel and R.P. Blakemore (eds.), Plenum Press, New York, 1991, pp. 97-115.

65. Y.A. Gorby, T.J. Beveridge, and R.P. Blakemore, "Characterization of the Bacterial Magnetosome Membrane," *Journal Bacteriol.* 170:834-841, 1988.

66. R. Blakemore, "Magnetotactic Bacteria," *Annu. Rev. Microbiol.* 36:217-238, 1982.

67. R.P. Blakemore, "Magnetotactic Bacteria," *Science* 190:377-379, 1975.

68. J.L. Kirschvink, "South-seeking Magnetic Bacteria," *Journal of Experimental Biology* 86:345-347, 1980.

69. R.P. Blakemore, R.B. Frankel, and A.J. Kalmijn, "South-seeking Magnetotactic Bacteria in the Southern Hemisphere," *Nature* 286:384-385, 1980.

70. R.B. Frankel, R.P. Blakemore, F.F. Torres de Araujo, E.M.S. Esquivel, and J. Danon, "Magnetotactic Bacteria at the Geomagnetic Equator," *Science* 212:1269-1270, 1981.

71. S.-B.R. Chang and J.L. Kirschvink, "Magnetofossils, the Magnetization of Sediments, and the Evolution of Magnetite Biomineralization," *Annu. Rev. Earth Planet. Sci.* 17:169-195, 1989.

72. F.F. Torres de Araujo, M.A. Pires, R.B. Frankel, and C.E.M. Bicudo, "Magnetite and Magnetotaxis in Algae," *Biophys. Journal* 50:375-378, 1985.

73. H. Vali and J.L. Kirschvink, "Observations of Magnetosome Organization, Surface Structure, and Iron Biomineralization of Undescribed Magnetic Bacteria: Evolutionary Speculations," in *Iron Biomineralization,* R.P. Frankel and R.P. Blakemore (eds.), Plenum Press, New York, 1991, pp. 97-115.

74. S. Mann, R.B. Frankel, and R.P. Blakemore, "Structure, Morphology and Crystal Growth of Bacterial Magnetite," *Nature* 310:405-407, 1984.

75. S. Mann, T.T. Moench, and R.J.P. Williams, "A High-resolution Electron Microscopic Investigation of Bacterial Magnetite: Implications for Crystal Growth," *Proceedings of the Royal Society of London B* 221:385-393, 1984.

76. S. Mann, "Structure, Morphology, and Crystal Growth of Bacterial Magnetite," in *Magnetite Biomineralization and Magnetoreception in Animals: A New Biomagnetism,* J.L. Kirschvink, D.S. Jones, and B.J. McFadden (eds.), Plenum Press, New York, 1985, pp. 311-332.

77. H. Vali and J.L. Kirschvink, "Observations of Magnetosome Organization, Surface Structure, and Iron Biomineralization of Undescribed Magnetic Bacteria: Evolutionary Speculations," in *Iron Biomineralization,* R.P. Frankel and R.P. Blakemore (eds.), Plenum Press, New York, 1991, pp. 97-115.

78. J.L. Kirschvink, "Magnetite Biomineralization and Geomagnetic Sensitivity in Higher Animals: An Update and Recommendations for Future Study," *Bioelectromagnetics* 10:239-259, 1989.

79. J.L. Kirschvink, "On the Magnetostatic Control of Crystal Orientation and Iron Accumulation in Magnetosomes," *Automedica* 14:257-269, 1992.

80. R.F. Butler and S.K. Banerjee, "Theoretical Single-domain Size Range in Magnetite and Titanomagnetite," *Journal of Geophysical Research* 80:4049-4058, 1975.

81. J.C. Diaz-Ricci and J.L. Kirschvink, "Magnetic Domain State and Coercivity Predictions for Biogenic Greigite (Fe3O4): A Comparison of Theory with Magnetosome Observations," *Journal of Geophysical Research* 97:17309-17315, 1992.

82. K.L. Thomas-Keprta et al., "Elongated Prismatic Magnetite Crystals in ALH84001 Carbonate Globules: Potential Martian Magnetofossils," *Geochim. Cosmochim. Acta* 64:6049-6081, 2000.

83. J.L. Kirschvink and S.-B.R. Chang, "Ultra Fine-grained Magnetite in Deep-sea Sediments: Possible Bacterial Magnetofossils," *Geology* 12:559-562, 1984.

84. N. Petersen, T. von Dobeneck, and H. Vali, "Fossil Bacterial Magnetite in Deep-sea Sediments from the South Atlantic Ocean," *Nature* 320:611-615, 1986.

85. J.L. Gould, J.L. Kirschvink, and K.S. Deffeyes, "Bees Have Magnetic Remanence," *Science* 201:1026-1028, 1978.

86. C. Walcott, J.L. Gould, and J.L. Kirschvink, "Pigeons Have Magnets," *Science* 205:1027-1029, 1979.

87. J.L. Kirschvink and J.L. Gould, "Biogenic Magnetite as a Basis for Magnetic Field Detection in Animals," *Biosystems* 13:181-201, 1981.

88. R. Wiltschko and W. Wiltschko, "Magnetic Orientation in Animals," *Zoophysiology*, Vol. 33, Springer, Berlin, 1995, p. 297.

89. M.M. Walker, C.E. Diebel, and C.V. Haugh, "Structure and Function of the Vertebrate Magnetic Sense," *Nature* 390:371-376, 1997.

90. J.L. Kirschvink, "Magnetoreception: Homing in on Vertebrates," *Nature* 390:339-340, 1997.

91. M.M. Walker, C.E. Diebel, and C.V. Haugh, "Structure and Function of the Vertebrate Magnetic Sense," *Nature* 390:371-376, 1997.

92. C.E. Diebel, R. Proksch, C.R. Green, P. Neilson, and M.M. Walker, "Magnetite Defines a Vertebrate Magnetoreceptor," *Nature* 406:299-302, 2000.

93. J.L. Kirschvink and A. Kobayashi-Kirschvink, "Is Geomagnetic Sensitivity Real? Replication of the Walker-Bitterman Conditioning Experiment in Honey Bees," *American Zoologist* 31:169-185, 1991.

94. J.L. Kirschvink, S. Padmanabha, C.K. Boyce, and J. Oglesby, "Measurement of the Threshold Sensitivity of Honeybees to Weak, Extremely Low Frequency Magnetic Fields," *Journal of Experimental Biology* 200:1363-1368, 1997.

95. R.C. Beason, R. Wiltschko, and W. Wiltschko, "Pigeon Homing: Effects of Magnetic Pulses on Initial Orientation," *Auk* 114(3):405-415, 1997.

96. U. Munro, J.A. Munro, J.B. Phillips, and W. Wiltschko, "Effect of Wavelength of Light and Pulse Magnetization on Different Magnetoreception Systems in a Migratory Bird," *Australian Journal of Zoology* 45(2):189-198, 1997.

97. U. Munro, J.A. Munro, J.B. Phillips, R. Wiltschko, and W. Wiltschko, "Evidence for a Magnetite-based Navigational Map in Birds," *Naturwissenschaften* 84(1):26-28, 1997.

98. W. Wiltschko, U. Munro, R.C. Beason, H. Ford, and R. Wiltschko, "A Magnetic Pulse Leads to a Temporary Deflection in the Orientation of Migratory Birds," *Experientia* 50:697-700, 1994.

99. W. Wiltschko and R. Wiltschko, "Migratory Orientation of European Robins Is Affected by the Wavelength of Light as Well as by a Magnetic Pulse," *Journal of Comparative Physiology* 177(3):363-369, 1995.

100. J.L. Kirschvink and S.-B.R. Chang, "Ultra Fine-grained Magnetite in Deep-sea Sediments: Possible Bacterial Magnetofossils," *Geology* 12:559-562, 1984.

101. S.-B.R. Chang and J.L. Kirschvink, "Magnetofossils, the Magnetization of Sediments, and the Evolution of Magnetite Biomineralization," *Annu. Rev. Earth Planet. Sci.* 17:169-195, 1989.

102. J.L. Kirschvink and H.A. Lowenstam, "Mineralization and Magnetization of Chiton Teeth: Paleomagnetic, Sedimentologic, and Biologic Implications of Organic Magnetite," *Earth Planet. Sci. Lett.* 44:193-204, 1979.

103. S.-B.R. Chang and J.L. Kirschvink, "Magnetofossils, the Magnetization of Sediments, and the Evolution of Magnetite Biomineralization," *Annu. Rev. Earth Planet. Sci.* 17:169-195, 1989.

104. J.L. Kirschvink and S.-B.R. Chang, "Ultra Fine-grained Magnetite in Deep-sea Sediments: Possible Bacterial Magnetofossils," *Geology* 12:559-562, 1984.

105. H. Vali, O. Forster, G. Amarantidis, and N. Petersen, "Magnetotactic Bacteria and Their Magnetofossils in Sediments," *Earth Planet. Sci. Lett.* 86:389-400, 1987.

106. T. von Dobeneck, N. Petersen, and H. Vali, "Bakterielle Magnetofossilien," *Geowissenschaften in unser Zeit* 5:27-35, 1987.

107. H. Vali and J.L. Kirschvink, "Magnetofossil Dissolution in a Paleomagnetically Unstable Deep-sea Sediment," *Nature* 339:203-206, 1989.

108. B.M. Moskowitz, R.B. Frankel, and D.A. Bazylinski, "Rock Magnetic Criteria for the Detection of Biogenic Magnetite," *Earth Planet. Sci. Lett.* 120(3-4):283-300, 1993.

109. P.P. Hesse, "Evidence for Bacterial Paleoecological Origin of Mineral Magnetic Cycles in Oxic and Sub-oxic Tasman Sea Sediments," *Marine Geology* 117:1-17, 1994.

110. T. Yamazaki and H. Kawahata, "Organic Carbon Flux Controls the Morphology of Magnetofossils in Marine Sediments," *Geology* 16(12):1064-1066, 1998.

111. Z. Gibbs-Eggar, "Possible Evidence for Dissimilatory Bacterial Magnetite Dominating the Magnetic Properties of Recent Lake Sediments," *Earth Planet. Sci. Lett.* 168:1-6, 1999.

112. R.B. Frankel, J.P. Zhang, and D.A. Bazylinski, "Single Magnetic Domains in Magnetotactic Bacteria, *Journal of Geophysical Research* 103:30601-30604, 1998.

113. J.A. Peck and J.W. King, "Magnetofossils in the Sediment of Lake Baikal, Siberia," *Earth Planet. Sci. Lett.* 140(1-4):159-172, 1996.

114. P.P. Hesse, "Evidence for Bacterial Paleoecological Origin of Mineral Magnetic Cycles in Oxic and Sub-oxic Tasman Sea Sediments, *Marine Geology* 117:1-17, 1994.

115. J.F. Stolz, "Magnetosomes," *Journal of General Microbiology* 139:1663-1670, 1993.

116. T. Yamazaki, I. Katsura, and K. Marumo, "Origin of Stable Remanent Magnetization of Siliceous Sediments in the Central Equatorial Pacific," *Earth Planet. Sci. Lett.* 105(1-3):81-93, 1991.

117. J. Akai, T. Sato, and S. Okusa, "TEM Study on Biogenic Magnetite in Deep-sea Sediments from the Japan Sea and the Western Pacific Ocean," *Journal of Electron Microscopy* 40(2):110-117, 1991.

118. M. Farina, D.M.S. Esquivel, and H. Debarros, "Magnetic Iron-sulfur Crystals from a Magnetotactic Microorganism," *Nature* 343(6255):256-258, 1990.

119. S. Mann, N.H.C. Sparks, R.B. Frankel, D.A. Bazylinski, and H.W. Jannasch, "Biomineralization of Ferrimagnetic Greigite (Fe_3S_4) and Iron Pyrite (FeS_2) in a Magnetotactic Bacterium," *Nature* 343:258-261, 1990.

120. D.A. Bazylinski, R.B. Frankel, and H.W. Jannasch, "Anaerobic Magnetite Production by a Marine, Magnetotactic Bacterium," *Nature* 334:518-519, 1988.

121. D.S. McKay, E.K. Gibson, K.L. Thomas-Krepta, H. Vali, C.S. Romanek, S.J. Clemett, X.D.F. Chillier, C.R. Maechling, and R.N. Zare, "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001," *Science* 273:924-930, 1996.

122. K.L. Thomas-Keprta et al., "Elongated Prismatic Magnetite Crystals in ALH84001 Carbonate Globules: Potential Martian Magnetofossils," *Geochim. Cosmochim. Acta* 64:4049-4081, 2000.

123. L.E. Borg, J.N. Connelly, L.E. Nyquist, C.-Y. Shih, H. Wiesmann, and Y. Reese, "The Age of the Carbonates in Martian Meteorite ALH84001," *Science* 286:90-94, 1999.

124. B.P. Weiss, J.L. Kirschvink, F.J. Baudenbacher, H. Vali, N.T. Peters, F.A. MacDonald, and J.P. Wikswo, "A Low Temperature Transfer of ALH84001 from Mars to Earth," *Science* 290:791-895, 2000.

125. J.L. Kirschvink, E.J. Gaidos, L.E. Bertani, N.J. Beukes, J. Gutzmer, L.N. Maepa, and R.E. Steinberger, "Paleoproterozoic Snowball Earth: Extreme Climatic and Geochemical Global Change and Its Biological Consequences," *Proc. Natl. Acad. Sci. USA* 97:1400-1405, 2000.

126. A.J. Alvarez, M. Khanna, G.A. Toranzos, and G. Stotzky, "Amplification of DNA Bound on Clay Minerals," *Mol. Ecol.* 7:775-778, 1998.

127. D.C. White, W.M. Davis, J.S. Nickels, J.D. King, and R.J. Bobbie, "Determination of the Sedimentary Microbial Biomass by Extractable Lipid Phosphate," *Oecologia* 40:51-62, 1979.

128. D.L. Balkwill, F.R. Leach, J.T. Wilson, J.F. McNabb, and D.C. White, "Equivalence of Microbial Biomass Measures Based on Membrane Lipid and Cell Wall Components, Adenosine Triphosphate, and Direct Counts in Subsurface Sediments," *Microbial Ecology* 16:73-84, 1988.

129. M.M. Moeseneder, J.M. Arrieta, G. Muyzer, C. Winter, and G.J. Herndl, "Optimization of Terminal-restriction Fragment Length Polymorphism Analysis for Complex Marine Bacterioplankton Communities and Comparison with Denaturing Gradient Gel Electrophoresis," *Appl. Environ. Microbiol.* 65:3518-3525, 1999.

130. G.E.C. Sheridan, C.I. Masters, J.A. Shallcross, and B.M. Mackey, "Detection of mRNA by Reverse Transcription PCR as an Indicator of Viability in *Escherichia coli* Cells," *Appl. Environ. Microbiol.* 64(4):1313-1318, 1998.

131. C.A. Davidson, C.J. Griffith, A.C. Peters, and L.M. Fielding, "Evaluation of Two Methods for Monitoring Surface Cleanliness—ATP Bioluminescence and Traditional Hygiene Swabbing," *Luminescence* 14(1):33-38, 1999.

132. V.G. Frundzhyan, L.Y. Brovko, V.S. Babunova, V.M. Kartashova, and N.N. Ugarova, "A Bioluminescence Assay of Total Bacterial Contamination of Fresh Milk," *Appl. Biochem. Microbiol.* 35:321-327, 1999.

133. D.E. Atkinson, "The Energy Charge of the Adenylate Pool as a Regulatory Parameter. Interactions with Feedback Modifiers," *Biochemistry* 7:4030-4034, 1968.

134. D.C. White, W.M. Davis, J.S. Nickels, J.D. King, and R.J. Bobbie, "Determination of the Sedimentary Microbial Biomass by Extractable Lipid Phosphate," *Oecologia* 40:51-62, 1979.

135. D.L. Balkwill, F.R. Leach, J.T. Wilson, J.F. McNabb, and D.C. White, "Equivalence of Microbial Biomass Measures Based on Membrane Lipid and Cell Wall Components, Adenosine Triphosphate, and Direct Counts in Subsurface Sediments," *Microbial Ecology* 16:73-84, 1988.

136. J.P. Diaper and C. Edwards, "The Use of Fluorogenic Esters to Detect Viable Bacteria by Flow-cytometry," *Journal of Applied Bacteriology* 77(2):221-228, 1994.

137. K. Venkateswaran, personal communication.

138. E. Zuckerkandl and L. Pauling, "Molecules as Documents of Evolutionary History," *Journal of Theoretical Biology* 8:357-366, 1965.

139. S. Yamamoto and S. Harayama, "PCR Amplification and Direct Sequencing of Gyrb Genes with Universal Primers and Their Application to the Detection and Taxonomic Analysis of *Pseudomonas putida* Strains," *Appl. Environ. Microbiol.* 61(3):1104-1109, 1995.

140. K. Venkateswaran, D.P. Moser, M.E. Dollhopf, D.P. Lies, D.A. Saffarini, B.J. MacGregor, D.B. Ringelberg, D.C. White, M. Nishijima, H. Sano, J. Burghardt, E. Stackebrandt, and K.H. Nealson, "Polyphasic Taxonomy of the Genus *Shewanella* and Description of *Shewanella oneidensis* sp. nov.," *International Journal of Systematic Bacteriology* 49:705-724, 1999.

141. D. Jones and N.R. Krieg, "Serology and Chemotaxonomy," in *Bergey's Manual of Systematic Bacteriology,* N.R. Krieg and J.G. Holt (eds.), Vol. 1, Williams & Wilkins, Baltimore, 1984, pp. 15-18.

142. M. Goodfellow and A.G. O'Donnell, "Roots of Bacterial Systematics," in *Handbook of New Bacterial Systematics,* M. Goodfellow and A.G. O'Donnell (eds.), Academic Press, London, 1993, pp. 3-54.

143. C.R. Woese, "Bacterial Evolution," *Microbiol. Rev.* 51:221-271, 1987.

144. N.R. Pace, D.A. Stahl, D.J. Lane, and G.J. Olsen, "The Analysis of Natural Microbial Populations by Ribosomal RNA Sequences," *Advances in Microbial Ecology* 9:1-55, 1986.

145. D.A. Stahl, "Evolution, Ecology and Diagnosis: Unity in Variety," *Bio/Technology* 4:623-628, 1986.

146. D.A. Stahl and R. Amann, "Development and Application of Nucleic Acid Probes in Bacterial Systematics," in *Sequencing and Hybridization Techniques in Bacterial Systematics,* E. Stackebrandt and M. Goodfellow (eds.), John Wiley and Sons, Chichester, England, 1991, pp. 205-248.

147. D.M. Ward, M.M. Bateson, R. Weller, and A.L. Ruff-Roberts, "Ribosomal RNA Analysis of Microorganisms as They Occur in Nature," *Advances in Microbial Ecology* 12:219-286, 1992.

148. P. Hugenholtz, B.M. Goebel, and N.R. Pace, "Impact of Culture-independent Studies on the Emerging Phylogenetic View of Bacterial Diversity," *Journal Bacteriol.* 180:4765-4774, 1988.

149. C.R. Woese, "Bacterial Evolution," *Microbiol. Rev.* 51:221-271, 1987.

150. C.R. Woese, "Bacterial Evolution," *Microbiol. Rev.* 51:221-271, 1987.

151. D.A. Stahl, "Application of Phylogenetically Based Hybridization Probes to Microbial Ecology," *Mol. Ecol.* 4:535-542, 1995.

152. See <http://rdp.cme.msu.edu>.

153. B.L. Maidak, J.R. Cole, T.G. Lilburn, C.T. Parker, P.R. Saxman, J.M. Stredwick, G.M. Garrity, B. Li, G.J. Olsen, S. Pramanik, T.M. Schmidt, and J.M. Tiedje, "The RDP (Ribosomal Database Project) Continues," *Nucleic Acids Research* 28(1):173-174, 2000.

154. B.L. Maidak, J.R. Cole, C.T. Parker, G.M. Garrity, N. Larsen, B. Li, T.G. Lilburn, M.J. McCaughey, G.J. Olsen, R. Overbeek, S. Pramanik, T.M. Schmidt, J.M. Tiedje, and C.R. Woese, "A New Version of the RDP (Ribosomal Database Project)," *Nucleic Acids Research* 27(1):171-173, 1999.

155. B.L. Maidak, J.R. Cole, T.G. Lilburn, C.T. Parker, P.R. Saxman, J.M. Stredwick, G.M. Garrity, B. Li, G.J. Olsen, S. Pramanik, T.M. Schmidt, and J.M. Tiedje, "The RDP (Ribosomal Database Project) Continues," *Nucleic Acids Research* 28(1):173-174, 2000.

156. W.-T. Liu, T.L. Marsh, H. Cheng, and L.J. Forney, "Characterization of Microbial Diversity by Determining Terminal Restriction Fragment Length Polymorphisms of 16S Ribosomal DNA," *Appl. Environ. Microbiol.* 63:4516-4522, 1997.

157. W.-T. Liu, T.L. Marsh, H. Cheng, and L.J. Forney, "Characterization of Microbial Diversity by Determining Terminal Restriction Fragment Length Polymorphisms of 16S Ribosomal DNA," *Appl. Environ. Microbiol.* 63:4516-4522, 1997.

158. M.M. Moeseneder, J.M. Arrieta, G. Muyzer, C. Winter, and G.J. Herndl, "Optimization of Terminal-restriction Fragment Length Polymorphism Analysis for Complex Marine Bacterioplankton Communities and Comparison with Denaturing Gradient Gel Electrophoresis," *Appl. Environ. Microbiol.* 65:3518-3525, 1999.

159. D.A. Stahl and R. Amann, "Development and Application of Nucleic Acid Probes in Bacterial Systematics," in *Sequencing and Hybridization Techniques in Bacterial Systematics,* E. Stackebrandt and M. Goodfellow (eds.), John Wiley and Sons, Chichester, U.K., 1991, pp. 205-248.

160. R.I. Amann, W. Ludwig, and K.-H. Schleifer, "Phylogenetic Identification and In Situ Detection of Individual Microbial Cells Without Cultivation," *Microbiol. Rev.* 59:143-169, 1995.

161. E.F. DeLong, G.S. Wickham, and N.R. Pace, "Phylogenetic Stains: Ribosomal RNA-based Probes for the Identification of Single Cells," *Science* 243:1360-1363, 1989.

162. R.I. Amann, W. Ludwig, and K.-H. Schleifer, "Phylogenetic Identification and In Situ Detection of Individual Microbial Cells Without Cultivation," *Microbiol. Rev.* 59:143-169, 1995.

163. D.A. Stahl, "Application of Phylogenetically Based Hybridization Probes to Microbial Ecology," *Mol. Ecol.* 4:535-542, 1995.

164. D.A. Stahl and R. Amann, "Development and Application of Nucleic Acid Probes in Bacterial Systematics," in *Sequencing and Hybridization Techniques in Bacterial Systematics,* E. Stackebrandt and M. Goodfellow (eds.), John Wiley and Sons, Chichester, England, 1991, pp. 205-248.

165. H. Hennecke, K. Kaluza, B. Thony, M. Fuhrmann, W. Ludwig, and E. Stackebrandt, "Concurrent Evolution of Nitrogenase Genes and 16S rRNA in *Rhizobium* Species and Other Nitrogen-fixing Bacteria," *Arch. Microbiol.* 142:342-348, 1985.

166. C. Wawer and G. Muyzer, "Genetic Diversity of Desulfovibrio spp. in Environmental Samples Analyzed by Denaturing Gradient Gel Electrophoresis of [NiFe] Hydrogenase Gene Fragments," *Appl. Environ. Microbiol.* 61:2203-2210, 1995.

167. M. Wagner, A.J. Roger, J.L. Flax, G.A. Brusseau, and D.A. Stahl, "Phylogeny of Dissimilatory Sulfite Reductases Supports an Early Origin of Sulfate Respiration," *Journal Bacteriol.* 180:2975-2982, 1998.

168. D. Minz, J.L. Flax, S.J. Green, G. Muyzer, Y. Cohen, M. Wagner, B.E. Rittmann, and D.A. Stahl, "Diversity of Sulfate-reducing Bacteria in Oxic and Anoxic Regions of a Microbial Mat Characterized by Comparative Analysis of Dissimilatory Sulfite Reductase Genes," *Appl. Environ. Microbiol.* 65:4666-4671, 1999.

169. D.M. Karl, D.F. Bird, K. Bjorkman, T. Houlihan, R. Shackelford, and L. Tupas, "Microorganisms in the Accreted Ice of Lake Vostok, Antarctica," *Science* 286:2144-2147, 1999.

170. N.R. Pace, B.C. Thomas, and C.R. Woese, "Probing RNA Structure, Function, and History by Comparative Analysis," in *The RNA World: The Nature of Modern RNA Suggests a Prebiotic RNA,* R.F. Gesteland, T.R. Cech, and J.F. Atkins (eds.), 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999, pp. 79-112.

171. N.R. Pace, "Origin of Life—Facing Up to the Physical Setting," *Cell* 65:531-533, 1991.

172. S.M. Barns, R.E. Fundyga, M.W. Jeffries, and N.R. Pace, "Remarkable Archaeal Diversity Detected in a Yellowstone National Park Hot Spring Environment," *Proc. Natl. Acad. Sci. USA* 91:1609-1613, 1994.

173. S.M. Barns, R.E. Fundyga, M.W. Jeffries, and N.R. Pace, "Remarkable Archaeal Diversity Detected in a Yellowstone National Park Hot Spring Environment," *Proc. Natl. Acad. Sci. USA* 91:1609-1613, 1994.

174. W.L. Nicholson, N. Munakata, G. Horneck, H.J. Melosh, and P. Setlow, "Resistance of Bacillus Endospores to Extreme Terrestrial and Extraterrestrial Environments," *Microbiol. Mol. Biol. Rev.* 64:548-572, 2000.

175. C. Mileikowsky, F.A. Cucinotta, J.W. Wilson, B. Gladman, G. Horneck, L. Lindegren, J. Melosh, H. Rickman, M. Valtonen, and J.Q. Zheng, "Natural Transfer of Viable Microbes in Space. 1. From Mars to Earth and Earth to Mars," *Icarus* 145:391-427, 2000.

176. J.W. Valley, J.M. Eiler, C.M. Graham, E.K. Gibson, C.S. Romanek, and E.M. Stolper, "Low-temperature Carbonate Concretions in the Martian Meteorite ALH84001: Evidence from Stable Isotopes and Mineralogy," *Science* 275:1633-1638, 1997.

177. E.R. Scott, A. Yamaguchi, and A.N. Krot, "Petrological Evidence for Shock Melting of Carbonates in the Martian Meteorite ALH84001," *Nature* 387:377-379, 1997.

178. S.J. Mojzsis, R. Krishnamurthy, and G. Arrhenius, "Before RNA and After: Geophysical and Geochemical Constraints on Molecular Evolution," in *The RNA World: The Nature of Modern RNA Suggests a Prebiotic RNA,* R.F. Gesteland, T.R. Cech, and J.F. Atkins (eds.), 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999, pp. 1-48.

179. K.S. Makarova, L. Aravind, M.Y. Galperin, N.V. Grishin, R.L. Tatusov, Y.I. Wolf, and E.V. Koonin, "Comparative Genomics of the Archaea (Euryarchaeota): Evolution of Conserved Protein Families, the Stable Core, and the Variable Shell," *Genome Research* 9:608-628, 1999.

180. P.D. Ward and D. Brownlee, *Rare Earth: Why Complex Life Is Uncommon in the Universe,* Copernicus, New York, 2000.

181. W.L. Nicholson, N. Munakata, G. Horneck, H.J. Melosh, and P. Setlow, "Resistance of Bacillus Endospores to Extreme Terrestrial and Extraterrestrial Environments," *Microbiol. Mol. Biol. Rev.* 64:548-572, 2000.

182. K. Pedersen, "Exploration of Deep Intraterrestrial Microbial Life: Current Perspectives," *FEMS Microbiol. Lett.* 185:9-16, 2000.

183. M.C. Malin and K.S. Edgett, "Evidence for Recent Groundwater Seepage and Surface Runoff on Mars," *Science* 288:2330-2335, 2000.

184. M.C. Malin, A. McEwen, M. Carr, L. Soderblom, P. Thomas, E. Danielson, P. James, J. Veverka, and W.K. Hartmann, "Evidence for Recent Volcanism on Mars from Crater Counts," *Nature* 397:586-589, 1999.

Session 4: Detecting Extinct Life

FORMATION AND PRESERVATION OF BONA FIDE MICROFOSSILS

Sherry L. Cady
Department of Geology
Portland State University

Abstract

The discovery of microfossil-like objects in the martian meteorite ALH84001 underscores the principal challenge facing astrobiologists in the search for fossilized evidence of microbial life beyond Earth: How can bona fide microfossils be distinguished from carbonaceous or mineral pseudofossils? The same challenge faces paleontologists searching for the earliest signs of life on Earth. Criteria for assessing whether ancient microfossil-like objects are remnants of microorganisms have been established,[1] and only permineralized and non-mineralized cellular remains are accepted as bona fide microfossils. Although the exact mechanism and precise chemistry of permineralization are not known, permineralized cells retain enough morphological fidelity to be recognizable and, by definition,[2] are carbonaceous (composed of complex organic biopolymers). As such, permineralized and nonmineralized cellular remains harbor multiple biosignatures that include cellular morphology. To detect and confirm the biogenicity of bona fide microfossils, it is necessary to use analytical instruments that not only reveal their presence, but also reveal the biochemical nature of their organic compounds. Whether permineralized and nonmineralized microfossils harbor definitive biosignatures depends upon the intrinsic characteristics of the microorganisms, the extrinsic characteristics of their environment, and the diagenetic transformations that alter the microfossils and the fine-grained mineral matrix in which they are preserved. Discussed here are ways in which these factors affect the formation and preservation of bona fide microfossils in mineralizing ecosystems—environments that produce the types of sedimentary deposits targeted in the search for life on Mars.[3]

———

Life itself arose as a self-perpetuating product of physical processes, and it is likely that the characteristics of Earth's earliest organisms—their size, shape, molecular composition, and catalytic properties—bore a close resemblance to the products of physical processes that gave rise to biology. For this reason, detecting the remnants of early life in terrestrial rocks is difficult. In martian or other extraterrestrial samples, it is doubly challenging.

—Andrew H. Knoll[4]

———

Introduction

Evidence of early microbial life on Earth is preserved in chemically precipitated deposits that form from mineralizing fluids (e.g., hydrothermal deposits, evaporites, carbonates, and silicified carbonates) and detrital sediments deposited by water (e.g., clays, volcanic ash, siliciclastics). Although fossil-bearing strata are sparse in ancient rocks of Precambrian age, a continuous fossil record exists from about 2 billion years ago to the present. This fossil record has demonstrated that three-dimensionally preserved permineralized microbial cells are found primarily in deposits that formed in mineralizing environments, whereas two-dimensional compressions of cells, flattened by compaction during burial, are found primarily in detrital sediments.

Our understanding of early life on Earth is based upon the various types of biosignatures extracted from permineralized and nonmineralized cells and cellular remains. Permineralized microfossils retain the morphology the microbial cells had at the time of fossilization, as well as degradation-resistant (i.e., recalcitrant) cellular components such as cell walls and extracellular sheaths. Nonmineralized cells, while compressed, also retain morphological features and biochemical signatures. The structure and stereochemistry of individual organic

compounds that comprise the recalcitrant biomolecules of permineralized and nonmineralized cells may provide biomarker compounds that can be used to distinguish some of the major groups of microorganisms.[5] The isotopic signatures of cellular remains may also reveal specific groups of organisms based on the degree to which they metabolically fractionated stable carbon isotopes.[6] Some permineralized cellular remains also preserve anomalous concentrations of trace elements that were concentrated in vivo[7] or post mortem. While the search for ancient microfossils has produced a relatively robust set of criteria for assessing the biogenicity of even the most ancient microfossil-like objects,[8-10] proving that purported microfossils represent fossilized microorganisms is extremely difficult and time consuming, and numerous claims of the microfossils in ancient rocks remain contentious.

In those cases where primary biomolecules (or their diagenetic equivalent) are not preserved with a microfossil (e.g., when complete mineral replacement occurs), proving the biogenicity of such objects is often impossible. Microfossil-like objects composed entirely of minerals that do not harbor characteristics indicative of having formed uniquely via the replacement of cellular remains must be interpreted with the utmost caution. Almost all controvernial objects purported to be early Precambrian microfossils are mineralic.[11,12] As noted by Buick,[13] unless compelling reasons for accepting noncarbonaceous objects as microfossils exist (i.e., the morphology of the object matches the morphology of a distinctive microbe, and it is unlike the morphology of any known nonbiogenic structure), such objects should be discounted as bona fide microfossils. Only when attributes that distinguish the biogenicity of such objects are demonstrated, and the purported microfossils are confidently distinguished from possible carbonaceous or mineralic non-biologically produced objects, can they be considered bona fide evidence of life.

Cherts, Important Paleobiological Repositories on Earth (and Mars?)

Although silica deposits harbor less than one-half of the ancient permineralized microorganisms, those preserved in primary and early diagenetic cherts display some of the most exquisite morphological fidelity.[14] Siliceous rocks known as cherts consist primarily of opaline, micro- and macrocrystalline silica varieties (e.g., opal-A, opal-CT, opal-C, microquartz, fibrous quartz varieties, and macroquartz). Cherts form in a range of environments via a number of different processes.[15] Primary chert deposits form where biogenically or nonbiogenically precipitated silica grains accumulate. Early diagenetic cherts form as a result of the replacement of preexisting sedimentary phases such as opals, carbonates, or evaporites. Regardless of the origin (biogenic or nonbiogenic) and nature (hydrated opaline or microcrystalline silica varieties) of the primary silica phase, all cherts, given enough time, will eventually recrystallize to quartz cherts. The inevitable transformation of all authigenic silica phases to quartz often masks the exact sequence of events that led to the formation of a quartz chert deposit—hence, the need to place the deposit within the context of its regional and local geology. The long crustal residence time of cherts reflects the thermodynamic stability of quartz at Earth's surface temperatures and pressures.

Few modern-day analogues exist for ancient primary Precambrian cherts, most of which precipitated nonbiogenically. The remains of silica-secreting eukaryotes (i.e., sponges, radiolarians, diatoms) provide the source of silica for most younger, Phanerozoic cherts. Modern chert-forming environments that can be considered analogues for Precambrian chert-forming environments include silica-depositing thermal spring ecosystems. As mineralizing environments, hydrothermal systems have an enhanced potential to preserve biosignatures indicative of their prolific heat-loving microbial communities.[16-18] Hydrothermal systems are habitats for a variety of thermophilic and hyperthermophilic microbial communities.[19-21] As shown by Cady and Farmer,[22] the microbial inhabitants of thermal springs and the sedimentary structures they produce are fossilized in metastable opaline silica by a variety of mechanisms.

Quartz cherts, including those precipitated from hydrothermal fluids, are likely to have been stable at the surface of Mars throughout its history. The predominantly monomineralic composition of cherts enhances the probability that such deposits on Mars could be detected via remote spectroscopy, provided the surface area of the deposit exceeds or approximates the pixel dimension of the spectrometer utilized. Primary hydrothermal cherts could have formed on Mars as surficial siliceous thermal spring deposits[23] and as shallow subsurface siliceous epithermal deposits.[24] Silica leached from near-surface silicate rocks can be redeposited at depth as silcretes,

forming extensive silica-rich soil horizons, or it can be redeposited along the edges of playa lakes and evaporative shallow-water basins forming primary or replacement cherts.[25] Targets in the search for life on Mars, therefore, include hydrothermal deposits, hardpan subsoils, evaporites, and paleolake basin deposits.[26] On Earth, these types of deposits are relicts of ecosystems that produced microfossils predominantly via the encrustation of cells by minerals precipitated in situ (i.e., authigenically).

If microbial life did emerge on Mars and thrived in any of the targeted mineralizing environments mentioned above, it would likely have been fossilized and some of its biosignatures preserved. Given the lack of extensive recrystallization of microfossil-like objects in the ancient carbonate globules found in the martian meteorite ALH84001,[27] it appears likely that cell-sized objects, whether biological or nonbiological in origin, would have been preserved with higher morphological fidelity in ancient Mars rocks than their counterparts preserved in Earth's Precambrian geological record.

Intrinsic Characteristics of Microorganisms and Fossilization

The intrinsic characteristics of microbes that affect fossilization have been studied at various structural levels with a relatively limited number of techniques. Differences in the distribution and composition of the various extracellular and cellular components, as well as the reactivity of the subcellular biomolecules within these components of the cells, can affect an organism's susceptibility to fossilization. Modern analogue and experimental studies that utilize various types of electron microscopy techniques have revealed the types of preservational biases that occur during the earliest stages of microbial fossilization.

The example shown in Figure 1 illustrates a type of preservational bias introduced during the silicification of

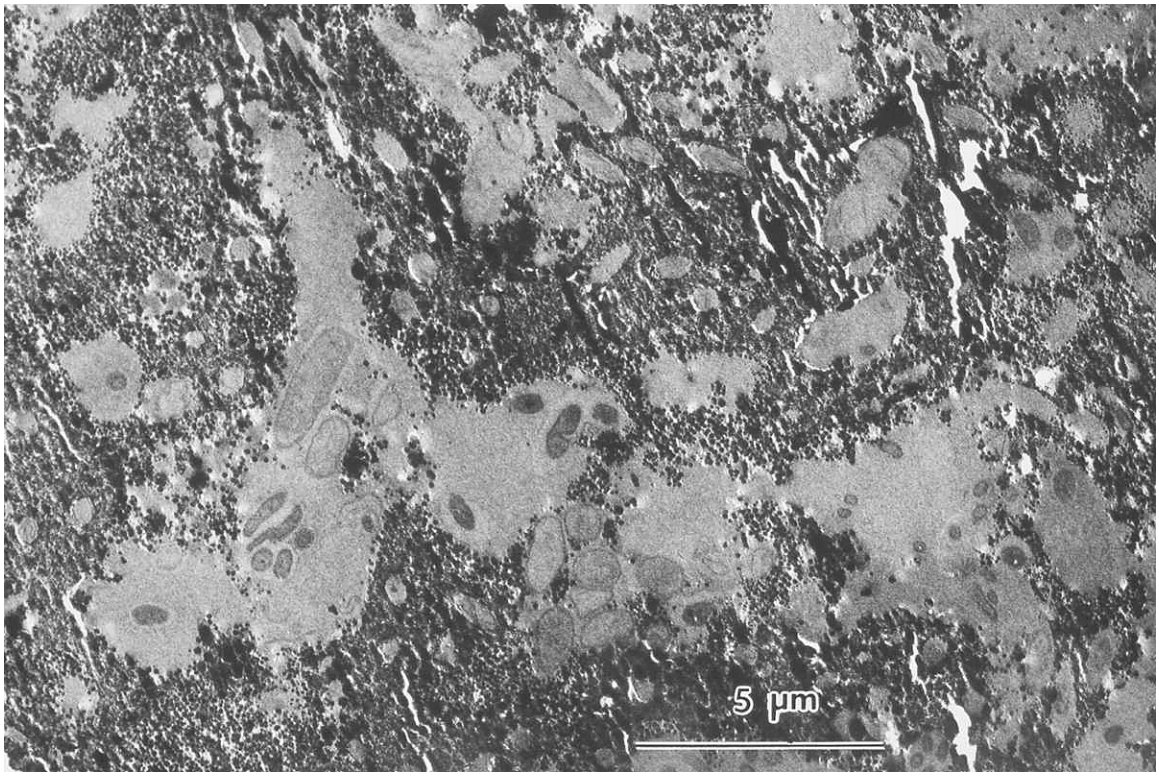


FIGURE 1. Transmission electron microscopy photomicrograph. Cross-sectional view of a high-temperature hot spring microbial community partially entombed within an opaline silica matrix (dark matrix). This image illustrates how differences in the susceptibility of various microbial taxa to fossilization could lead to biases in paleobiological information during the earliest stages of preservation.

a microbial biofilm at life's upper temperature limit. A biofilm containing a climax microbial community, which developed over several months on a substrate deployed by the author along the edge of a chert-forming hydrothermal spring located in Yellowstone National Park, was exposed subsequent to development to mineralizing fluid. Different microorganisms in the specimen, sectioned to electron transparency, are distinguished in the transmission electron photomicrograph on the basis of their ultrastructural characteristics. Note the variable amounts of extracellular matrix surrounding the different microorganisms. Some cells appear to be isolated within the opaline silica matrix, while other, more closely spaced cells, appear to form cohesive biofilm communities surrounded by opaline silica. It is clear that the proximity of opaline silica to the cell walls of the various organisms (one of the more robust cellular components during fossilization) differs as a function of the amount of extracellular matrix they produced. How such intrinsic differences in the amount and nature of the extracellular matrix of the different microorganisms affect their preservation cannot be predicted from such an image. Since postdepositional degradation processes will alter all cells to some degree, it is likely that the intrinsic differences displayed at this early stage of fossilization will lead to preservational biases.

Experimental silicification studies have revealed gross differences in the susceptibility of Gram-positive and Gram-negative bacteria. Gram positives silicify more rapidly than Gram negatives under controlled conditions.[28] Gram positive and negative refer to the way in which the cell wall of a bacterium reacts to the Gram staining procedure (the reaction being a function of the structure and composition of the cell wall). A polymeric peptidoglycan layer, located between the plasma membrane and the outer cell wall membrane, contains numerous carboxyl and phosphoryl groups that serve as metal cation-binding sites that promote mineral nucleation and growth. The thicker peptidoglycan layer in Gram-positive cell walls presumably contains a greater number of reactive sites for mineral nucleation. Based on these observations, Westall proposed that Gram-positive bacteria are more likely to be preserved in the geological record.[29]

Other experimental studies have focused on quantifying the type, density, and distribution of biomolecules that compose the cell walls and various extracellular components of a variety of microbial taxa.[30,31] Investigations to date indicate that the exposed reactive chemical groups on microbial cell walls interact ionically, as a function of pH, with solutes in the milieu surrounding the cell. The organometallic complexes that precipitate at reactive biomolecular sites provide additional sites for the sorption of metal and nonmetal ions. Fortin et al. recently reviewed the principal types of biomolecules known to alter the reactivity of microbial cell walls and extracellular layers (e.g., capsules, S-layers).[32]

When microbial cells attach to surfaces, they produce exopolymeric substances containing reactive biomolecules that can bind a variety of ions, including metals. Anomalous concentrations of trace metals localized by microbial cells and biofilm matrices have been proposed as possible biosignatures, the distribution of metal ions revealing the former presence of microbial cells.[33,34] Microbial taxa produce exopolymers under a range of environmental conditions, the composition of which can vary for various microbial species and for the same species under different environmental conditions.[35] Important topics for future research include studies to determine how environmental perturbations alter the function and composition of cellular and extracellular components, and how these changes affect the susceptibility of microorganisms to fossilization.

Extrinsic Environmental Factors and Microbial Fossilization

Distribution of Microorganisms

Most microorganisms immersed in aqueous fluids attach to surfaces and form biofilms that consist of distinct communities of cells immersed within a hydrated matrix of exopolymeric substances. Dynamic studies regarding biofilm architecture and composition, and the distribution of microorganisms within biofilms,[36] indicate that biofilms cannot be represented by a single, universal model. At the present time, with limited understanding of the complexities of microbial communities, models for biofilms are best developed on the basis of actualistic studies in modern ecosystems.

Biofilms develop in nearly every environment where water and available carbon and energy sources exist. Even at the upper temperature limit for life, hyperthermophilic biofilms develop on hydrothermal mineral precipi-

tates.[37] It has been estimated that the numbers of prokaryotic cells in subsurface microbial populations exceed by at least an order of magnitude the numbers of cells found within soils and the open ocean.[38] Subsurface biofilms in the deep subterranean biosphere have become the subject of intense study[39] since microorganisms display a propensity to form biofilms on all available surfaces in environments that favor their proliferation. The presence of microfossil-like objects in the mineral-filled fractures of the martian meteorite ALH84001,[40] a subsurface igneous rock, serves as a bellwether regarding the potential importance of subsurface paleobiological repositories.

The need to search for microbial biosignatures in any structural discontinuities in rocks from Mars through which mineralizing fluid has passed freely is exemplified by the discovery on Mars of recently young surficial effluents.[41] Sedimentary deposits associated with surficial effluents on Earth are extremely fine grained, having precipitated rapidly from solutions supersaturated most commonly by cooling, evaporation, or fluid mixing. Any particulate matter, including microorganisms, entrained in the subsurface fluids that escape from such effluents is often sequestered in fine-grained mineral deposits. Even if evidence of a subsurface biosphere was not discovered in the deposits that precipitated around the effluents discovered on Mars, their study would provide detailed information about the geochemistry of the planet's near-surface aquifers.

Effects of Solution Chemistry in Determining How Fossilization Occurs

In order to retain high cellular fidelity, permineralized microfossils must be preserved intra- and extracellularly within a fine-grained mineral matrix prior to extensive cellular degradation. Fine-grained mineral matrices form when numerous, aqueously precipitated crystal nuclei reach, nearly simultaneously, the critical dimensions needed for energetically favored crystal growth. Mineral nuclei of such critical dimensions can form by the random collisions of ions or atoms within a supersaturated solution (i.e., homogeneous nucleation) or on preexisting surfaces (i.e., heterogeneous nucleation). Since reactive sites that promote surface sorption and chemical bonding occur on microbial cell surfaces, heterogeneous precipitation of mineral nuclei at these sites can result in mineral encrustation of the cell.[42] Whether encrustation will lead to the formation of a permineralized cell depends upon whether, and when, minerals precipitate on the inside of the cell wall, obviously post mortem, but prior to complete degradation of the cell wall. A limited amount of microbial cellular decomposition, accompanied by early mineralization, appears to have enhanced the preservation of cellular remains in the geological record.[43] Post mortem fossilization of microorganisms favors the formation of fine-grained mineral matrices, since more reactive sites for mineral nucleation become available once organisms begin to decay.

Environments containing aqueous fluids likely to reach saturation for either heterogeneous or homogeneous mineral nucleation include (1) evaporated or rapidly cooled subaerial and subaqueous hydrothermal systems and the edges of playa lake basins, (2) locations where fluids of different composition mix (e.g., at the edges of all types of aquifers), and (3) sites where fluids are stratified across chemoclines and thermoclines (e.g., in stratified lake basins, in water-filled caves, and within biofilms and microbial mats). All of these environments should be considered in the search for paleobiological repositories on Mars.

Relative Timing of Fossilization, Cellular Degradation, and Post-fossilization Events

The amount of cellular fidelity displayed by permineralized and nonmineralized cells ultimately depends upon the amount of cellular degradation that occurs prior to fossilization. In their study of microbial preservation along outflow channels of mineralizing thermal spring ecosystems, Gerasimenko and Krylov demonstrated how the apparent paleobiodiversity preserved in the modern hot spring deposits resulted from the fossilization of morphologically similar taxa that were fossilized at various stages of cellular decomposition.[44] The degree to which nonmineralized cells in detrital sediments retain their cellular fidelity depends upon whether they were buried to depths where anaerobic conditions prevailed prior to extensive cellular degradation. Preservational biases resulting from variation in the amount of cellular degradation have been quantified by Bartley,[45] who established a rating system for analyzing statistically the effects of degradation on cell morphology. Post-fossilization events can either enhance or limit the potential for long-term preservation of permineralized microfossils or nonmineralized cellular remains. For example, preservation is enhanced in detrital sediments if they are cemented

by a fine-grained mineral assemblage prior to physical disruption, exposure to oxidants, or metamorphic alteration. On the other hand, preservation of cellular fidelity and long-term preservation are limited in mineralizing environments if intracellular and extracellular mineral growth continues unabated.[46,47]

Diagenesis and Preservation of Microbial Biosignatures

Once microorganisms are fossilized, the potential for long-term preservation of their cellular components depends upon the recalcitrance (resistance to biodegradation and major chemical transformations such as hydrolysis and oxidation) of the macromolecular biomolecules.[48] The preservation potential of various types of organic biomolecules has been reviewed by Logan et al. and by Allison and Briggs.[49,50] Factors that degrade organic matter and determine their recalcitrance have been studied in great detail.[51] The organic remains of microorganisms source much of the world's petroleum and gas reserves. Of the four principal classes of biomolecules, the various classes of lipids, especially glycolipids and lipopolysaccharides, are most resistant to degradation in all types of depositional environments. Lipids tend to resist chemical attack, are insoluble in water, and can be incorporated in kerogens, thereby increasing their preservation potential. Although the conversion of primary lipids to their diagenetic counterparts (i.e., geolipids) involves information loss through structural alteration (e.g., hydrogenation of double bonds, aromatization of rings, loss of functional groups), the original class of lipid can often be identified even after diagenetic changes.[52]

The diagenetic history of the minerals that entomb microfossils in paleobiological repositories ultimately determines the length of time microfossils can be preserved in the geological record. In general, diagenesis occurs when an increase in the temperature and/or pressure of a deposit rises to the point where it alters the structural configuration of the primary organic molecules and mineral phases. Microbial cells are usually preserved initially within thermodynamically metastable mineral assemblages, and their transformation to thermodynamically stable mineral assemblages occurs as a result of diagenetic processes that operate over time scales from tens to millions of years (e.g., burial and tectonic alteration). For example, mineral diagenesis alters chert deposits with time via the transformation of primary opaline silica phases to microcrystalline quartz varieties (microquartz, macroquartz, chalcedony, quartzine, "lutecite," "moganite") through intermediary cryptocrystalline opal phases.[53,54]

Importance of Chemical and Structural Discontinuities

Regardless of the mechanism by which a microbe is fossilized or the degree to which it is altered from its primary state, the microfossil must differ either in composition or in structural organization from the mineral matrix that surrounds it in order to be detected. For example, chemical and structural discontinuities occur between nonmineralized cells or permineralized microfossils and their mineral matrix. Compositional differences between a microfossil and its mineral matrix also develop when microorganisms concentrate—actively or passively, intra- or extracellularly—heavy metals or other ions from solution. Environmental perturbations can produce gradational compositional differences between microorganisms and their mineral matrix. The geochemical changes that accompany the evaporation of fluids can be preserved in the laminated crusts that develop around microbial cells. The geochemically predictable sequence of minerals around the microfossils preserves not only a cast of the microorganisms, but also paleoenvironmental and paleogeochemical information.

It is worth noting that if mineral-replaced microbial cells are preserved within a mineral matrix of the same composition, it will be extremely difficult to establish the biogenicity of the microfossil-like objects. Even when minerals display habits similar to the morphology of microbial cells, it will be necessary to demonstrate that such minerals could not have formed abiotically. As discussed by Buick,[55] a certain degree of cellular elaboration must be displayed by a purported microfossil, although the level of morphological complexity continues to be debated. It could also be possible that structural defects or structural differences between the matrix minerals and microfossil-like objects exist. If so, however, they must be interpreted with caution—mineral microstructural characteristics may reflect only differences in the relative amounts of diagenetic processing.

Conclusions

At the present time, only bona fide microfossils provide the direct evidence needed to establish extraterrestrial and earliest Earth life. Nonmineralized and permineralized cells contain multiple biosignatures indicative of their biogenicity, thereby distinguishing them from pseudofossils. Although cellular permineralization is likely to occur in nearly all types of mineralizing environments, the rarity of permineralized microfossils in the geological record suggests that much remains to be learned about how diagenetic processes affect long-term preservation. As improvements are made in the resolution limits of analytical instruments that can simultaneously "image" microfossil-like objects and detect and analyze the biochemical structure of their minute concentrations of carbonaceous compounds, we may find that permineralized remains are more common than heretofore realized.

Acknowledgments

The author gratefully acknowledges the NASA Exobiology Program (NASA Grant# NAG5-9579) and the National Science Foundation LExEn Program (NSF Grant #EAR-0096354) for support of basic research in areas described in this paper.

ELECTRON-BEAM TECHNIQUES FOR MICROFOSSIL CHARACTERIZATION

David McKay
NASA Johnson Space Center

Abstract

Electron-beam techniques are extremely valuable in searching for and characterizing microfossils of all kinds. Electron-beam techniques combined with optical microscopy, ion probe, and organic microanalysis techniques can usually provide definitive information on whether a candidate feature is truly a microfossil, even if it has been considerably altered by diagenesis. In our laboratory, we use scanning electron microscopy (SEM) and transmission electron microscopy (TEM) combined with detailed electron microprobe (EMP) analysis. Specialized techniques that may enhance textural data include ion etching of polished surfaces and small grains, acid etching of polished surfaces, and oxygen plasma etching of polished surfaces and small grains. Chemical mapping of polished surfaces with EMP can reveal subtle differences in chemistry and textures that may be associated with original biogenic features, even if the original biogenic features have been destroyed. Our general sequence of analysis is optical microscopy, followed by SEM petrography, EMP chemical mapping, quantitative EMP analysis of specific phases and areas, and TEM analysis. Examples for some of these techniques are illustrated. Some samples may then be analyzed in other laboratories by ion microprobe, time-of-flight (TOF) secondary ion mass spectroscopy (SIMS), or Raman spectroscopy.

Introduction

Indigenous microfossils in rocks are absolute proof that life was once active in the environment from which the rock came. Other than finding living organisms, finding true microfossils is perhaps the most reliable proof that life was present in the environment represented by the sample. However, some important pitfalls must be considered. The purported microfossil must be shown to be a true fossil of an organism or trace of an organism. This requires detailed textural information and often requires chemical information, sometimes including isotopic ratios. Next, the microfossil must be shown to be indigenous and not contamination. For some purposes, the microfossil must be shown to be contemporaneous with the enclosing rock, but for other purposes, finding microfossils added to the rock at a later time may be quite useful. Microfossils found in igneous rocks, for example, were clearly not present during the initial cooling and crystallization of the melt, but were added after the rock cooled, generally into cracks and pores of the original rock or by alteration of its phases.

Finding and characterizing microfossils in rocks can be greatly facilitated by using electron-beam techniques, particularly in combination with other techniques. Basic instruments used in our laboratories include SEM including attached energy-dispersive x-ray analyzer (EDXA), TEM, and EMP. Petrographic doubly polished thin sections of the rock samples are necessary for the preliminary examination by petrographic optical microscope. Images from this microscope serve as maps for the more detailed mapping and analysis by SEM or TEM. For samples that will be prepared for SEM or TEM, a polished thin section is not always necessary and small chips can be used. It is usually helpful to study the chips with a good binocular microscope.

The basic information desired is the texture of the rock and its included microfossils, the location of the microfossils, their morphology, and their dimensional measurements. Basic chemical information includes maps of major and minor elements and quantitative analyses of minerals, glasses, cements, and microfossils. Ideally, each suspected microfossil feature should have well-documented texture or fabric and feature morphology in two or three dimensions, quantitative chemistry, and chemical maps showing variation from place to place of each major and minor element. The chemistry of true microfossils is not likely to resemble the chemistry of the original organism; replacement or void filling may produce a composition such as iron oxide or SiO_2 totally unlike the original reduced carbon, water, and other components of the living organism. Consequently, it is necessary to document and understand the chemical effects of diagenesis and replacement that create the fossilized version of the original organism. This, in turn, requires considerable experience, familiarity with the literature, or even experimental laboratory studies of fossilization.

Sample Preparation

In our laboratory, we typically prepare samples using the following processes:

1. A representative rock chip (generally 1 to 10 mm) is used for making one or more polished petrographic thin sections, typically 30 μm thick. We make these sections in the Curatorial Thin Section Laboratory here at Johnson Space Center. Such thin sections are used for optical petrographic mapping and mineral identification, followed by SEM petrographic mapping and EDXA analysis, EMP compositional mapping, and quantitative EMP mapping of particular phases, traverses, or features.
2. A small chip (0.1 to 2 mm) containing interesting features identified in a binocular microscope is mounted on an SEM stub and coated with a conductive coating of Cr, C, or Pt for SEM examination and photography. For some purposes, the sample is left uncoated and examined at low voltage.
3. Additional small chips (0.05 to 0.1 mm) are embedded in epoxy for microtome thin-section making, and several thin sections of ~50 to 80 nm are produced for TEM examination. The objective in TEM work is to document the mineralogy with electron diffraction, high-resolution imaging, and EDXA chemistry, and to determine the ultrastructure with low- to high-magnification images. Many TEMs have electron energy-loss spectrometers (EELS) attached, which can help with chemical and mineral identification.
4. Similarly, some polished thin sections may be etched in an ion beam at low angle to bring out textures related to ion erosion resistance.[56,57] Ion etching may bring out extremely delicate textures not visible in optical, SEM secondary, or SEM backscattered images of polished samples. This technique is particularly useful for detecting amorphous material such as glass or polymerized gels that develop distinctive bumpy textures in the ion etching device.
5. For some applications, one of the polished thin sections is lightly etched in hydrofluoric acid fumes for a few minutes to bring out hidden textures.[58]
6. Another useful technique is to place the section or chip in an oxygen plasma-etching device for a few minutes. This technique removes many kinds of organic material from the exposed surface and may differentially etch away organics creating a multilevel surface. Before-and-after SEM images can then be used to locate etched organic material.

Analysis Objectives

Generally, we want to find features that might be the remains of microbes or might have been formed or influenced by microbial life. We wish to relate these features to their environment or surroundings. If the features are fossilized microbes, we try to determine the changes in texture, morphology, and chemistry that have taken place during diagenesis and fossilization. Next we want to relate the microbial remains or traces to the original environment and the original form and type of microbe. We try to determine whether nonbiologic processes that mimic the results of biologic processes could have made the feature. The value of a biomarker such as a microfossil is greatest for forms and features that cannot easily be made by inorganic processes. Finally, we try to determine whether features are indigenous or are contaminants added later. Every effort should be made to interpret the features as artifacts, inorganic products, and contamination. Only after such interpretations are eliminated can a microfossil origin be seriously considered.

Examination Steps

Petrographic optical microscopy of the polished thin section is generally the first step. This technique can rapidly locate areas of interest that may contain potential microfossils and can provide maps for subsequent, more detailed study with an electron microscope or electron microprobe. This technique can rapidly identify many minerals. Petrographic microscope examination can also show the relationship of the potential microfossils to the rest of the rock. We use optical images as maps to guide initial study by electron backscatter petrography in the SEM or EMP.

Next, SEM studies of polished thin sections are generally done with backscatter- and secondary-electron detectors. Backscatter-electron detectors can detect small differences in average atomic number and display them as brightness variations. If the polished surface has been etched by HF fumes, ion sputtering, or oxygen plasma, the secondary electrons will show the differences in topography caused by etching and will provide data complementary to the backscatter electrons.

SEM images and EDX analyses of features on small sample chips provide a simple way to search for possible biogenic morphologies. Features may include complete fossilized microbes or their colonies, fragments of microbes, products of microbes including fossilized biofilm, or traces of microbes including molds, casts, nucleation centers for mineral precipitation, and a number of other features.[59]

TEM imaging, chemical analysis, and mineral identification are usually done on separate small chips sliced by microtome. We sometimes use ion thinning rather than microtoming to prepare TEM thin sections. We have also developed a technique for coring a small interesting area from a polished thin section and making the core into a TEM thin section.

EMP analysis including chemical mapping and spot or traverse quantitative analysis of micrometer-size spots can provide much useful data on the minerals, the potential microfossils, and the matrix material. Quantitative EMP data using proper standards may provide very high quality data with a standard deviation of less than 1 percent for major elements. It can also detect and analyze trace elements down to a few tens of parts per million for many elements. Our microprobe is fully automated and computer driven, and can perform analyses and make chemical maps unattended over nights and weekends.

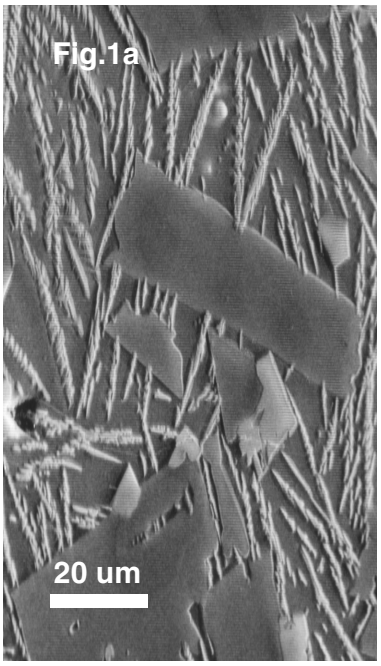
Occasionally we need other types of data that we cannot collect in our own laboratory, so we take the samples to another laboratory that has the necessary instruments such as an ion microprobe. For example, isotope ratios for some elements (e.g., S, C, O, H, N, and Fe) can sometimes be used to help discriminate between biogenic and nonbiogenic processes or to determine whether a feature formed on Mars or on Earth. Mass-dependent fractionation of S and C are among the most common indicators of biogenic fractionation for many kinds of terrestrial samples. Hydrogen fractionation providing deuterium-rich water appears to be a distinctive flag for indigenous martian water.

Organic species analysis of mapped features is another valuable technique that can be used on mapped thin sections or chips. We have used both double laser mass spectrometry and TOF-SIMS. Other useful techniques include gas chromatography-mass spectrometry (GC-MS); however, this is difficult to use on thin sectioned samples. Laser Raman spectroscopy provides some information on molecular species present. Three-dimensional relationships within the 30-μm polished thin sections can be determined using laser confocal microscope techniques. Staining with various organic stains and examination with a fluorescence microscope can be used to detect traces of DNA, cell walls, lipids, proteins, and other specific products of microorganisms.

Examples

Figures 1 through 5 provide examples of the use of some of the above-mentioned techniques.

The examples shown for the martian meteorites are not intended to be claims that the features are fossilized martian microbes. These examples simply illustrate features identified by electron-beam techniques, which—based on their morphologic similarity to known biogenic features in terrestrial samples—are candidates for additional investigation to collect detailed data on mineralogy, chemistry, isotopes, and ultrastructures. Only a full set of such data can provide convincing determination of either biogenetic origin or lack of it. The small size and the complexity of these features points out the need for additional types of microprobe instruments capable of isotopic and organic chemical analysis at the submicrometer scale.



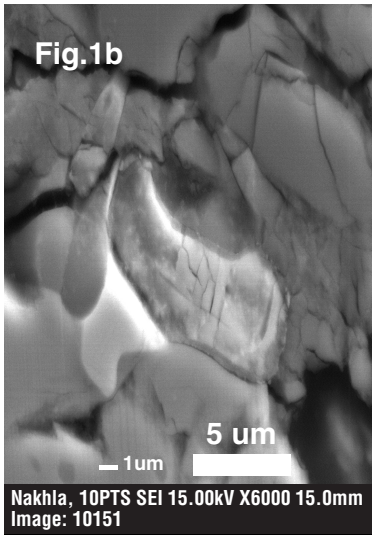


FIGURE 1. (a) SEM view of ion-etched polished section of lunar volcanic ash (sample 74002). Here, the delicate thin branching textures of the ilmenite are clearly shown in high relief against the glassy matrix. The medium-gray crystals are olivines, and the bright crystals are spinels. Ion etching was used to characterize the mineralogy, textures, and cooling history of these volcanic particles[60] and to reveal subtle matrix textures in impact-breccias.[61] This technique may reveal structures below the resolution limits of ordinary backscattered electron mapping, and enables much higher spatial resolution secondary electron imaging to be used on polished thin sections. For lunar regolith samples, the technique revealed details not detectable by any other technique. (b) SEM view of a lightly ion-etched polished section of the martian meteorite, Nakhla. The U-shaped grain is iron sulfide (pyrite). Note the difference in relief among the various phases. A thin rim, somewhat lower than the calcium phosphate grain, completely surrounds it. This rim is not obvious on the unetched sample, but it may contain critical information on the change in conditions leading to incipient alteration of the pyrite.

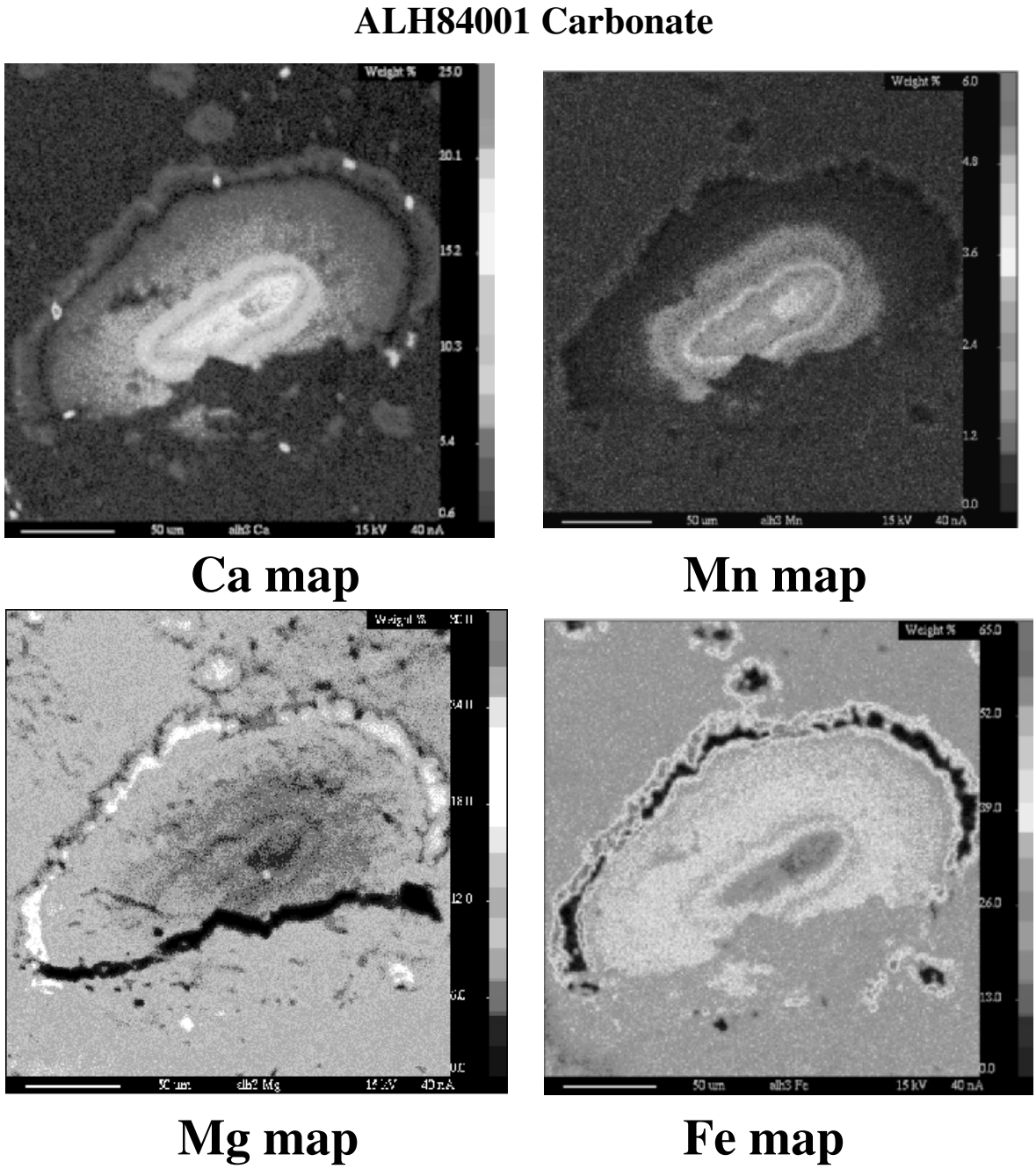


FIGURE 2. Chemical maps, made by the electron microprobe, of a single carbonate globule on a fracture surface of the martian meteorite ALH84001. The chemical zoning is apparent for all elements, but in particular for the two magnetite-rich zones. Most investigators now agree that the complex zoning is consistent with precipitation of the carbonates from liquid water or brine at relatively low temperatures. Of particular importance is the presence of small high-Ca regions within the magnetite-rich zone. These high-Ca regions correspond to high P regions (not shown). This presence of Ca phosphate in the magnetite-rich carbonate may be an important constraint on models for formation of both the carbonate and the magnetite.

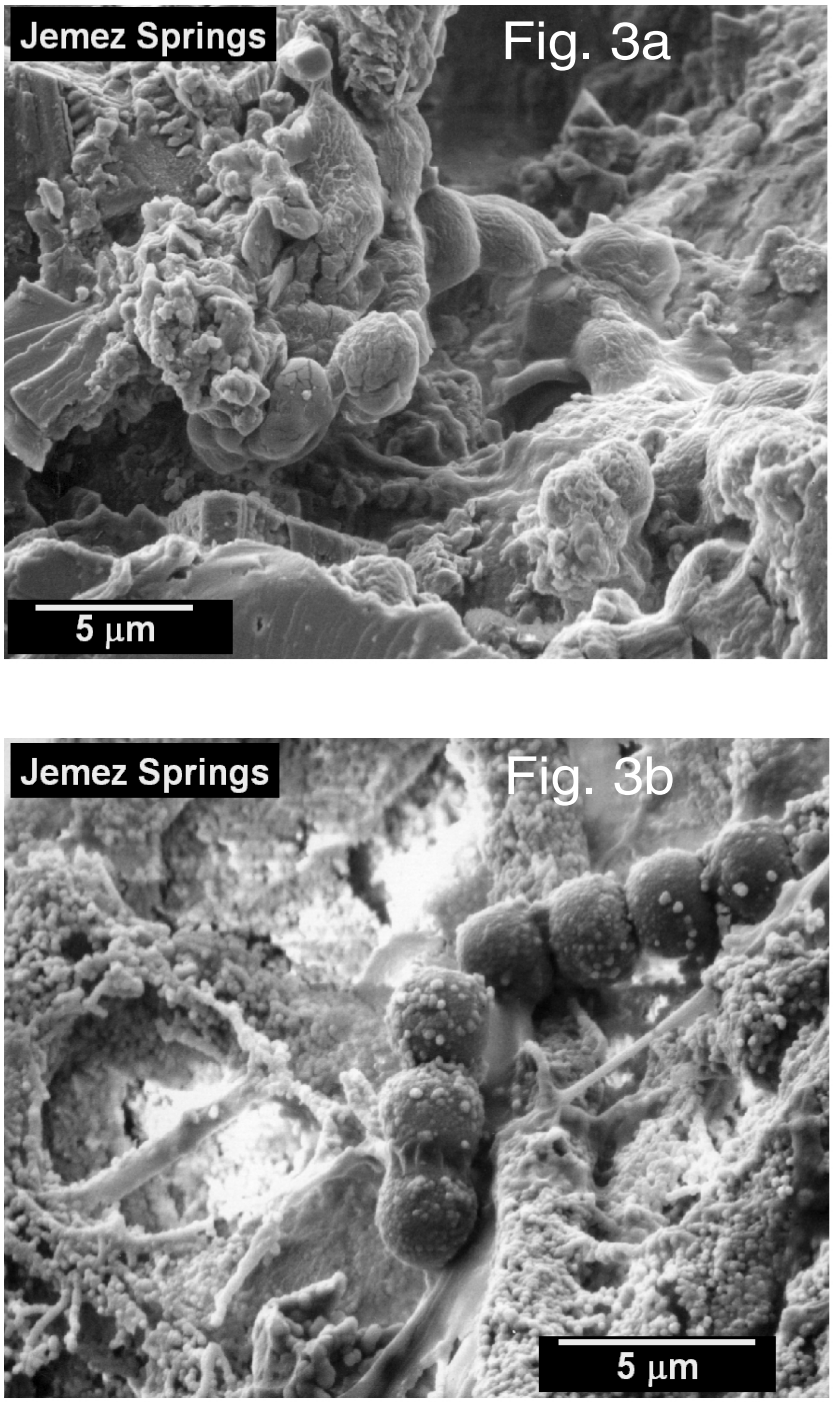
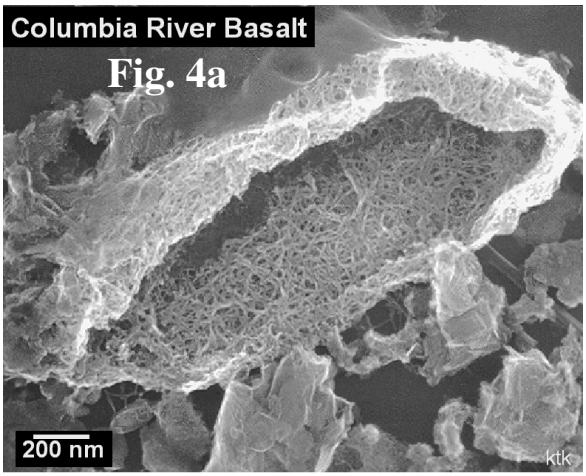


FIGURE 3. (a) SEM view of recent microbes in hot springs samples from Jemez Springs, New Mexico. These microbes are being replaced and fossilized by deposits of silica precipitating from the hot cooling water. The microbes are partially embedded in a biofilm, which is also being converted to silica. It has only recently been realized that biofilm may fossilize and be preserved, often better than microbial cells. (b) SEM view of coccoid bacteria from Jemez Springs, New Mexico. The bacteria were multiplying and forming chains. Both bacteria and surrounding biofilm are in the process of being replaced by silica. Images by Carlton Allen.



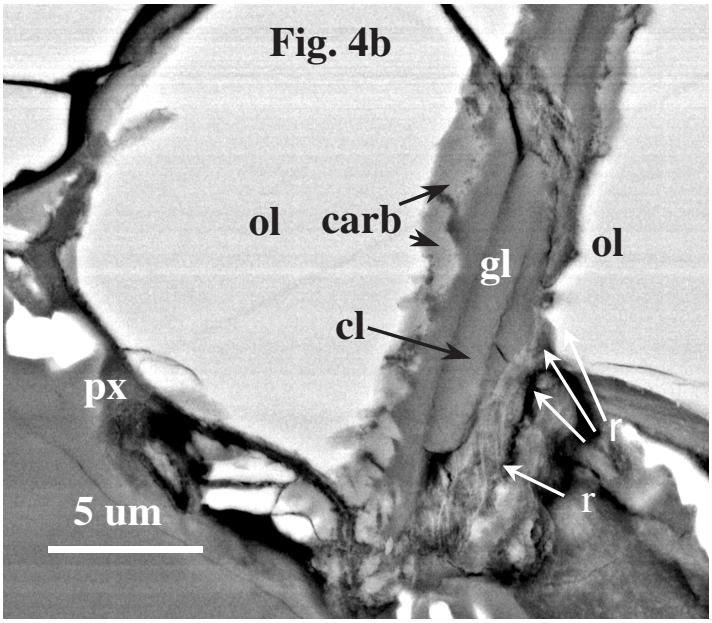


FIGURE 4. (a) SEM view of elongated bacteria form, which has been covered by a fine network of iron hydroxide fibers (ferrihydrite). The organism, which provided the form for the coating, has been lysed or dissolved away. Individual ferrihydrite fibers are only ~10 nm in diameter. This sample was from a laboratory experiment that grew and fossilized the microbes using basalt chips and groundwater from deep within the Columbia River basalt in Washington State.[62] (b) Backscatter electron (BSE) image of a polished thin section of the martian meteorite Nakhla. It illustrates cracks in the olivine (*ol*) and pyroxene (*px*) grains filled with smectite-like clay mineral (*cl*), carbonates (*carb*), and a glass (*gl*). This image shows the ability of BSE to display subtle differences in average atomic number at a fine scale and to illustrate fine-grained textures. The spatial BSE resolution here is less than 50 nm at 15 kV. Several ~2-μm-round features (*r*) are present which might be mineral concretions. However, these forms are candidates for possible fossil microbes. Additional analyses are indicated, including EMP carbon analysis and mapping, search for other possible biogenic traces such as P or N, search for organics using TOF-SIMs or fluorescent staining techniques, and possibly coring for TEM imaging and mineral identification. If carbon is present, isotopic analysis and mapping by ion microprobe may be indicated. Deuterium analysis of the clay matrix would likely determine whether the clay was formed on Mars (the current interpretation for these secondary phases in Nakhla) or on Earth.

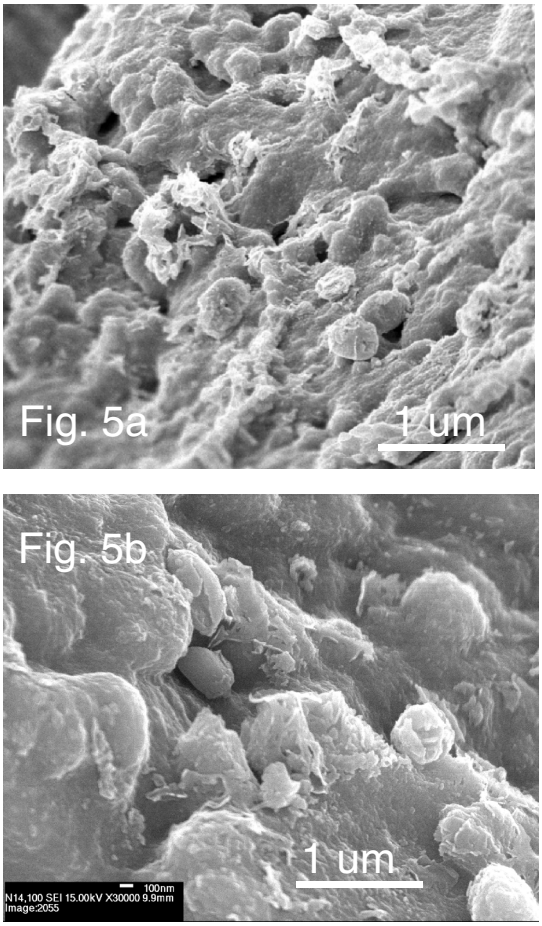


FIGURE 5. (a) SEM view of a complex fracture surface from a chip of the martian meteorite, Nakhla. The features resemble in some ways the fossilized biofilms and bacteria cells illustrated in Figure 3. Composition data on the larger rounded features reveal that they are enriched in Fe and O compared to the substrate. The bright, filmy material is enriched in Si and O compared to the substrate. These features require further analysis to determine whether they are biogenic and indigenous to the Mars meteorite. (b) SEM view of another fracture surface of a chip of Nakhla. The fracture surface is full of rounded and bumpy forms in a complex three-dimensional matrix. The meteorite appears to break apart preferentially along preexisting fracture surfaces, which are often filled with secondary mineral deposits as illustrated in Figure 2. These features require further analysis to determine whether they are biogenic and indigenous to the Mars meteorite.

Summary

Electron-beam techniques combined with optical microscope surveys form a powerful method of finding and characterizing microfossils in almost any kind of rock. We and others are developing criteria for determining what features are truly microfossils and what features are inorganic structures that may mimic microfossils. In addition, detailed chemistry and mineralogy of the microfossil and its surrounding matrix may help determine whether the feature is indigenous. As the spatial and chemical resolution of electron-beam instruments improves, these instruments will become more valuable in finding, documenting, and understanding fossil organisms or their traces. If identified, such fossils and their traces constitute robust biosignatures in early terrestrial rocks and possibly in nonterrestrial rocks. The application of these techniques to samples from beyond Earth is just beginning, but promises to be a major part of future astrobiology. The complexity of the required characterization requires that samples be returned to terrestrial laboratories for detailed, multitechnique analysis.

ORGANIC DETECTION

Luann Becker
Department of Geological Sciences
University of California, Santa Barbara

Abstract

The search for biogenic organic matter on Mars and other moons and planets in our solar system is rapidly emerging as a result of technological advancements and the study of early "life" on our own planet. As we have learned from our previous mission to Mars and the examination of martian meteorites, the criteria for establishing life require the appropriate strategy. Thus, a viable approach will require careful mapping of the surface from orbit for the selection of appropriate landing sites, robotic space missions equipped with several life detection in situ techniques for selection of samples, and sample return missions for additional verification of in situ results and laboratory measurements. The development of life detection techniques for future missions to Mars and beyond may require the appropriate Earth analogue to test the viability of these methods and further maximize our chances for scientific success.

Introduction

NASA is now entering a new phase of planetary exploration with the current scheduled missions to Mars and future missions to some of the moons of Jupiter and Saturn (e.g., Europa and Titan, respectively). Our interest in Mars is motivated by the recent studies of martian meteorites that suggest that the early history of the Red Planet was remarkably similar to the Earth's, where life apparently arose 4 billion years ago. If this is indeed the case, then Mars was presumably a much warmer, wetter, planet than it is today. This hypothesis is further supported by orbital imagary returned by the Viking, Mariner, and Mars Global Surveyor (MGS) spacecrafts that all show compelling evidence that copious liquid water existed on the surface of Mars in the past. In fact, new images provided by MGS suggest that there may be current sources of liquid water at or near the surface of the Red Planet. Other MGS data suggest that an ocean may have once existed at high northern latitudes, and valley networks apparently once carried water into the northern basin. All of these data suggest that life could have arisen on Mars in liquid water environments.

The search for *extinct organic matter* (i.e., organic matter generated by now-extinct organisms, in rocks, sediments, and ices from Mars and other planets and moons in our solar system) is critical to the determination of where life existed. Missions to Mars have the potential to address whether life arose there in a separate origin and may further provide information about our own prebiotic evolution, a record that has all but been erased from Earth's crust. Future missions to moons like Europa may enable us to search for clues of life in a liquid water environment. It seems clear that the potential for learning about life beyond our own planet is one of considerable interest to scientists and the general public alike. Yet, as we learned from the Viking missions, the search for life signs is problematic and requires an appropriate strategy that will maximize our opportunities to properly examine these compelling questions. This report discusses what is known about the organic matter in martian samples and further addresses techniques and strategies for future missions to Mars and beyond.

The Viking Missions

One of the major objectives of the 1976 Viking missions was to search for extinct organic compounds preserved in soils on the surface of Mars and to determine their structure and abundances.[63] The determination of the structure of organic compounds would provide the information needed to assess both "abiotic" (meteoritic) and "biotic" (intact martian organics) syntheses of organic compounds. Thus, a gas chromatograph coupled to a mass spectrometer (GC-MS) was the method of choice selected to combine both sensitivity and structural specificity.

Developing a GC-MS instrument that would be capable of flight and still achieve the goal of detecting "intact" organic compounds on Mars was a formidable one and required some modifications from the conventional

instrumentation. In particular, the chemical extraction of organics used to assess terrestrial samples was far too complex to miniaturize and operate reliably on the Viking landers. Therefore, thermal volatilization for the detection of organics and their degradation products was selected as the simplest and most flight-compatible approach. Another aspect of the original GC-MS design, which was later discarded to simplify the package, was a sample oven tied directly to the mass spectrometer for the detection of more complex, less volatile (kerogen-like) compounds.

The Viking GC-MS revealed that very little, if any, organic material is present in the soils sampled at the surface of Mars. The absence of organic carbon was attributed to the unusual oxidizing properties of the soil detected during measurements of the martian atmosphere. A recent assessment of this latter hypothesis demonstrated that nonvolatile salts such as benzenecarboxylic acids, oxalic acid, and acetic acid are metastable intermediates of meteoritic or martian organic debris under oxidizing conditions.[64] Organics converted to carboxylic derivatives would be difficult, if not impossible, to detect using GC-MS. Thus, in evaluating the overall Viking strategy, future instrumentation for the detection of extinct organic matter should include the following:

- The capability to volatilize the more complex kerogen-like component (e.g., a high-temperature oven)—such an improvement may also address the composition of the minerals found in the martian surface materials, a point that we have yet to address with either the Pathfinder or the MGS missions; and
- Instrumentation that is capable of detecting the alteration products of meteoritic and intact biologically mediated martian organics.

Organic Compounds in Martian Meteorites

Although the Viking landers were unable to address the question of life on Mars, the orbiters did provide important measurements of the martian atmosphere that led to the identification of a unique suite of meteorites on Earth as martian in origin. To date, all that is known about the organic matter on Mars comes from the examination of these meteorites. Perhaps the most famous of the group (16 total) is the Allan Hills (ALH84001) meteorite that was reported to have evidence of both putative martian fossil remains and organics in the form of polycyclic aromatic hydrocarbons (PAHs).[65] The announcement of the ALH84001 results has led to a reassessment of how to search for life on Mars and elsewhere. In fact, the techniques used to evaluate the origin of organic matter in ALH84001 and other martian meteorites are central to any discussion of the detection of extinct organic matter on Mars. Much of the controversy surrounding ALH84001 centers on stable carbon isotope studies ($\delta^{13}C$) of the carbonate and the associated organic matter. Thus, subsequent studies of ALH84001 have focused on determining the $\delta^{13}C$ values of specific organic compounds isolated from various mineral phases to assess the sources (biotic versus abiotic) of this material.

Stable Isotope Measurements of Martian Organic Matter

Two independent investigations of the stable carbon isotopic compositions of the organic matter in ALH84001 indicate that a small portion (~50 out of 250 parts per million [ppm]) of this material has a $\delta^{13}C$ value of –15‰.[66,67] A $\delta^{13}C$ value of –15‰ would be unusual for martian organics since $^{13}C/^{12}C$ measurements of trapped gases in some martian meteorites indicate that two distinct carbon reservoirs exist on Mars:

1. An isotopically heavy component (atmosphere) enriched in $\delta^{13}C$ (+36‰) and
2. A high-temperature igneous (i.e., mantle) component ($\delta^{13}C$ –20 to –30‰).

On the other hand, a $\delta^{13}C$ value of –15‰ is consistent with a "kerogen-like" component in carbonaceous chondrites. Thus, some portion of the organic matter in ALH84001 is extraterrestrial in origin and likely derived from meteoritic or cometary debris that is exogenously delivered to the surface of Mars. Recent measurements of the organic matter isolated from the Nakhla meteorite also indicate that a portion of this material is extraterrestrial in origin with a $\delta^{13}C$ value of –15‰.[68] These new data further support the notion that extraterrestrial meteoritic

or cometary debris has been accumulating on the martian surface and sequestered in the crust over geologic time (Nakhla has been dated at ~1.2 billion years old). The study of extinct organic matter in martian meteorites brings some new considerations to bear on the selection of analytical techniques for future life detection missions. These considerations include the following:

- Extraterrestrial organic matter is being incorporated into the soils and rocks on Mars;
- Measurements of the $\delta^{13}C$ compositions of organic compounds in martian samples could provide the information needed to distinguish abiotic and biotic sources of organic matter; and
- The detection of putative fossils in martian rocks (e.g., ALH84001) is not sufficient to determine that life evolved on Mars.

Future missions to Mars such as the European Mars Express will carry mass spectrometers capable of measuring stable carbon isotopic compositions in rocks and soils on Mars. The earliest of these measurements will come some three decades after the Viking landers produced null results regarding the existence of organic molecules at two places on the Martian surface.

Strategies in the Search for Extinct Organic Compounds on Mars and Beyond

The recognition of the martian meteorites has vastly expanded our knowledge of the chemistry, mineralogy, age, and isotopic composition of the martian crust. However, most of the meteorites we have are young, are igneous in nature, and appear to be from a similar location. In fact, isotopic dating of the Los Angeles meteorite, which was discovered in 1999, revealed an age of only 175 million years, contemporary in geologic terms. These new results suggest that Mars has been tectonically active in the recent past and may have a much younger surface then had previously been assumed (Meteoritical Society meeting, August 2000). As several studies have now indicated, however, none of these rock types appear to be conducive to the preservation and accumulation of organic matter.

In formulating a strategy to search for extinct organic matter on other moons and planets, we can draw some insight from our own geologic record. Experience gained from decades of searches for ancient evidence of past life on Earth suggests three major stages in the detection of extinct life on other planets. First comes the identification of specific sites of likely fossil preservation associated with the past presence of water; second is the selection of fossiliferous rocks for the studies of those locations; and third is the actual analyses of the rock themselves for structural, molecular, or isotopic evidence of past life. On other planets, site selection will depend upon geological and compositional observations made from orbit, rock selection based on chemical measurements made by landers or rovers, and either in situ analyses or sample return to Earth.

The most suitable lithologies for the preservation and accumulation of organic matter on Earth are sedimentary rocks that are typically fine grained and are characterized by well-defined aqueously derived mineral assemblages. We have yet to identify sediments on Mars or any other moon or planet; however, recent MGS images have provided new insight on locations that could offer the appropriate environment (aqueous) needed for the identification of these rock types. While it appears that evidence for fossil life cannot be resolved by orbital observations, future chemical investigations of the local environment may reveal the ecological imprint of biological activity. Ecological signatures, likely requiring broader spatial coverage to direct rovers and landers equipped with a network of instruments, may be reflected among local rocks in the chemical or mineralogical compositions or in gradients of these properties. On the other hand, depending on spatial resolution, orbital observations should be capable of detecting carbonate formations that are either directly or indirectly associated with biological activity on Earth, provided these rocks are not covered in dust that hinders their detection. Definitive evidence of fossil life, however, will require more direct and detailed observations of appropriately selected rocks. Rock selection serves two purposes:

1. To demonstrate that the sample formed in sedimentary environments, and
2. To show that it contains organic matter.

Although the absence of organic matter in a sedimentary rock would not preclude a biological imprint, a rock

containing organic matter would offer more lines of evidence to follow in establishing the presence of past life.

This strategy would, of course, require sample return to answer the more critical question of whether they contain biologically mediated martian organic matter. It may, however, be possible to obtain additional information about the associated organic matter present in these mineral assemblages in a single measurement that is both capable of flight and is nondestructive to the sample. Such an approach must address the fundamental question of the presence or absence of organic matter, its source (abiotic versus biogenic), and the associated mineralogy, thus fulfilling the requirement of establishing a martian biota without sample return.

A potential candidate compound to search for would be amino acids. Amino acids play an essential role in biochemistry as we know it and have properties such as chirality (handedness) that can be used to distinguish between abiotic and biotic origins.[69] These compounds occur as racemic mixtures (*D*- and *L*-enantiomers) in carbonaceous chondrites, and only the *L*-enantiomers are used in the proteins and enzymes in life on Earth. In fact, the detection of enantiomerically pure amino acids in a martian sample could be compelling evidence that life had occurred on Mars.

However, the identification of amino acids that seem to have all of the essential requirements needed to determine the origin of life on Mars, could be compromised if we do not sample properly. On Earth, temperature changes due to seasonal fluctuations and/or climate change result in an enhancement in racemization of amino acids. Recently, the effect of seasonal fluctuations in shallow lakes and Siberian permafrost on the rate of racemization was evaluated, and it was concluded that such changes were affecting the preservation of the amino acids.[70]

For sample selection sites on Mars where recent soil deposition is due to aeolian rather than fluvial or sedimentary processes, much of the soil, even at depth, would be considered well mixed with regard to exposure to high temperatures. Under these conditions, racemization of all amino acids would be complete even if life existed in the first billion years of martian history. However, in deep ancient (i.e., not gardened) rock and even in surface rocks at northern polar latitudes, the temperature maxima are currently low enough to allow incomplete racemization of most amino acids over martian history.

Thus, the search for viable organic matter depends, not only on the appropriate biomarker, but also on the proper location for sample selection and preservation of the organic signature. The detection of in situ organic matter may ultimately depend upon a variety of techniques with differing sensitivity, molecular structural information, sample preparation and degree of reliability in distinguishing biogenic and abiogenic production mechanisms.

Methods for the Detection of Extinct Organic Matter in Martian Samples

Raman Spectroscopy and Biomineralization

A major improvement in the study of complex biological systems has been the use of resonance Raman spectroscopy.[71] Due to the rapid advancements in laser technology, most wavelengths between 190 and 1064 nm are available and easily implemented in a flight-compatible package. Although fluorescence can interfere with Raman spectra, most bacterial taxonomic markers (pigments) absorb in the fluorescence-free ultraviolet region (e.g., detection limits of 1 ng/ml for a single bacterial cell). The application of Raman spectroscopy is being considered by several groups participating in future missions for the analysis of complex mineralogies such as meteorites and the detection of small quantities of biologically important chemical species (e.g., sulfur, carbon).

While the current wavelengths being studied (far red or near infrared) are not ideal for the detection of specific organic compounds (amino acids, lipids, etc.), they are suitable for material such as microbial pigments (biominerals) and their residues (e.g., phycocyanin in cyanobacteria at 530-560 excitation). For example, Raman spectra of deep-sea corals (Figure 1, left) closely resemble spectra for magnesium calcite with the exception of pink coral, which shows additional bands in the region 1010-3758 cm^{-1} that arise from a carotenoid pigment.[72]

More recently, Raman spectra (excited at 1064 nm) of epilithic Antarctic lichen encrustation embedded in Beacon sandstones (Figure 1, right) indicate vibrational bands for β-carotene (1136 cm^{-1}) and α-quartz (463 cm^{-1}).[73] The Antarctic geobiological systems are the closest analogues we have on Earth to potential martian ecosystems. Thus, Raman spectroscopy offers a nondestructive approach suitable for the assessment of biomolecules of exobiological importance.

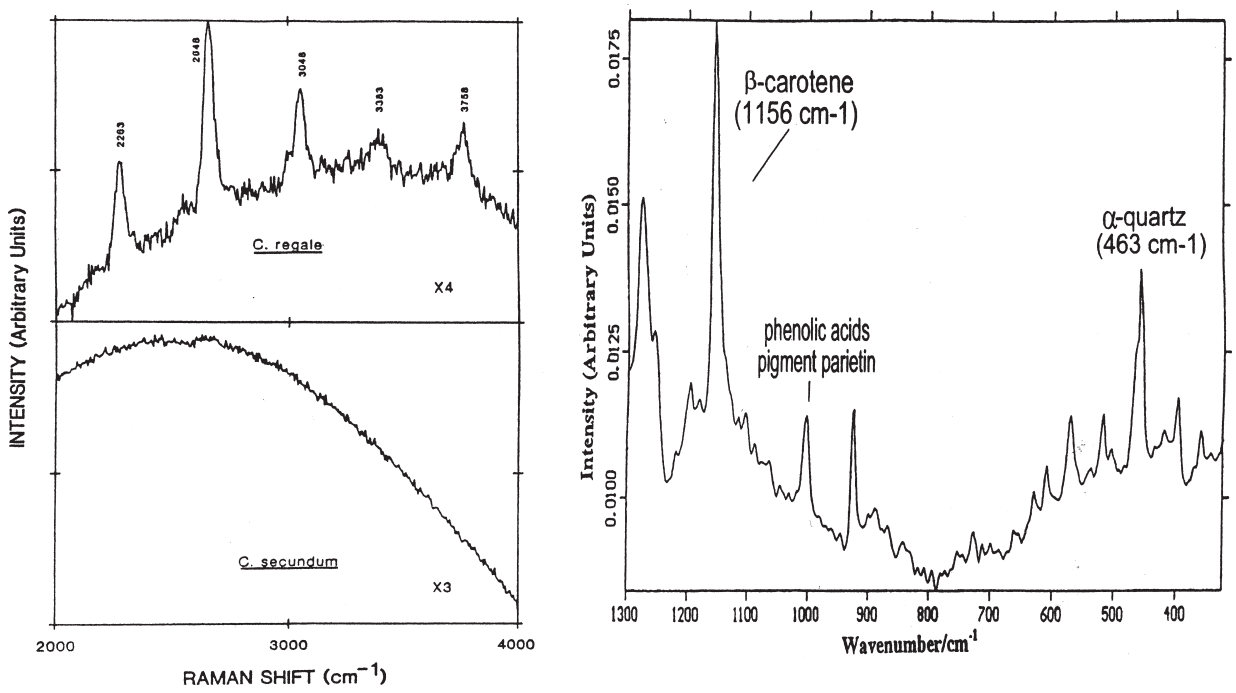


FIGURE 1. (left) Raman spectra (2000-4000 cm^{-1}) of pink and white corals (J. Urmos, S.K. Sharma, and F.T. Mackenzie, "Characterization of Some Biogenic Carbonates with Raman Spectroscopy," *American Mineralogist* 76:641, 1991); (right) Fourier transform Raman of *Xanthoria elegans* lichens from Crater Cirque, Victoria Land, Antarctica (H.G.M. Edwards, D.W. Farwell, M.M. Grady, D.D. Wynn-Williams, and I.P. Wright, "Comparative Raman Microscopy of a Martian Meteorite and Antarctic Lithic Anologues," *Planet. Space Sci.* 47:353, 1999).

Laser Desorption Mass Spectrometry

One of the methods used for the detection of extinct organic matter in martian meteorites has been laser desorption mass spectrometry (LDMS).[74,75] LDMS has the capability of examining organics on very small (~10 μm) to large (millimeter) sized grains in low amounts (nanomoles) "intact" without any further sample preparation. The very fine spot size of the laser allows for detailed analyses of an individual particle and a critical assessment of the distribution (heterogeneous versus homogeneous) and abundances (parts per billion [ppb] to ppm) of individual organic compounds. LDMS has also been used extensively for the detection of biomolecules and their fragments (DNA, proteins, peptides, etc.; see Session 3, "Detecting Extant Life").

The first organic compounds identified in ALH84001 were PAHs. These are ubiquitous in the universe, and on the Earth, they are the products of slow geochemical diagenetic reactions and the burning of biomass. The chemical architecture of PAHs, however, precludes any unique interpretation of their synthesis or source (i.e., biotic or abiotic). When coupled with the proper technique, however (e.g., carbon isotopes), LDMS is a useful approach for the detection and structural interpretation of intact organic compounds and their degradation products.

A recent examination of PAHs in the Nakhla meteorite (observed to fall in northern Egypt in 1911) revealed several peaks that were interpreted as the oxidative derivatives of PAHs.[76] These degradation products (Figure 2) may be the first indication of the potent oxidant present in the martian regolith that converts most or all of the organic compounds to carbon dioxide in a relatively short period of time if left unprotected.[77]

Unlike Nakhla (Figure 2, bottom), the ALH84001 PAHs (Figure 2, top) do not indicate significant oxidation suggesting that these compounds were quickly sequestered into minerals and sediments where they were protected from the effects of the strongly oxidizing surface environment. Thus, LDMS may be a good technique for evaluating the degree of oxidation and stability of organic compounds that are formed in situ (martian organics) or are delivered exogenously to the surface of Mars.

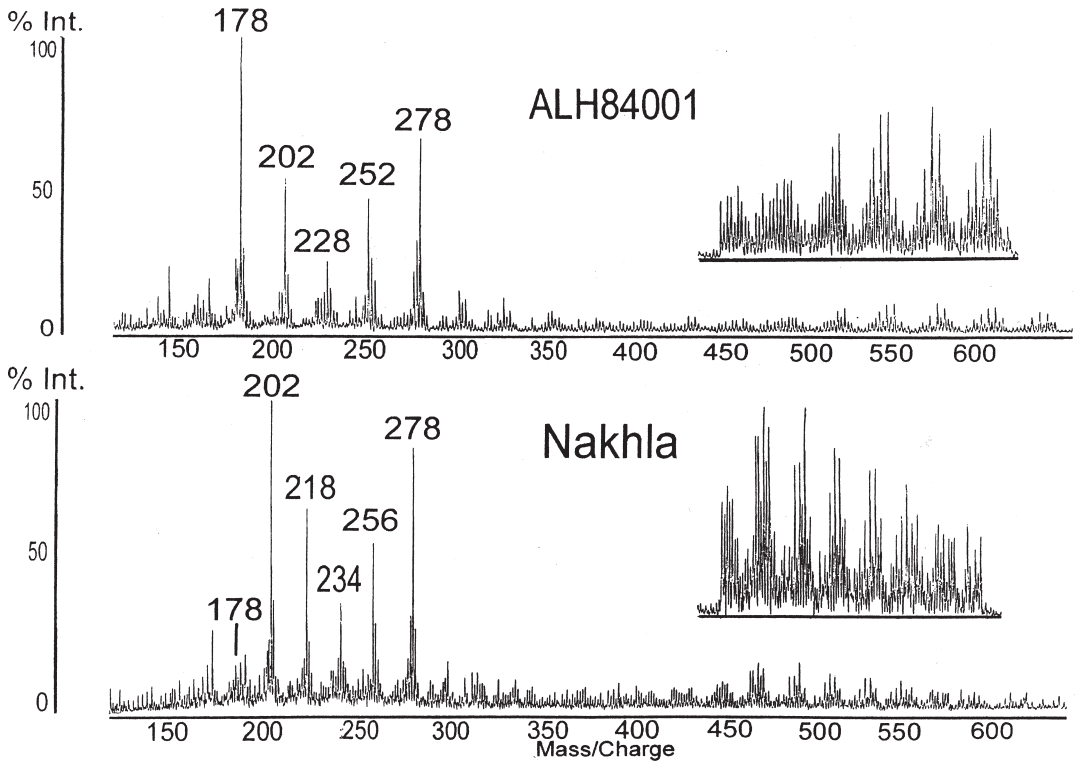


FIGURE 2. Laser desorption mass spectrometry of polycyclic aromatic hydrocarbons in carbonates examined in the ALH84001 (top) and the Nakhla (bottom) martian meteorites. The PAHs detected in ALH84001 are identical to those reported by McKay et al. in 1996. The Nakhla PAHs also display some of the same PAHs as ALH84001; however, evidence for oxidation of pyrene (mass = 202) is evident with the detection of mass = 218 (202 + 1 oxygen) and mass = 234 (202 + 2 oxygens). This may be evidence for the exposure of organic compounds to the "superoxide" atmosphere detected by Viking in 1976. The recognition of these oxygenated PAHs (or oxy-PAHs) may have been impossible to detect using the pyrolysis GC-MS available on the Viking lander.

Iron Isotopes as Biomarkers

As we have learned from examining the martian meteorites, isotopic fractionation studies of the lighter elements (i.e., carbon, sulfur, etc.) are extremely useful in determining the origin of extinct organic compounds (biogenic versus abiogenic). Recently, advances in mass spectrometric techniques have led to research into the fractionation of Fe and other transition metals (e.g., Cu, Zn, Ti, Mo). Since none of these elements are radioactive, nor are they the products of long-lived radioactive decay, the isotopic variations observed must result from mass-dependent fractionation. Some of these metals have been studied in the laboratory to ascertain their biological and nonbiological fractionation processes. Isotopes of iron (Fe) are of particular interest since Fe-bearing phases, including biominerals, are widespread, are resistant to alteration, and on Earth, have been linked to biologically mediated processes.

Like carbon, Fe can serve as an electron donor, providing metabolic energy to some microbes under both aerobic and anerobic conditions. Fe is also intimately associated with the oldest rocks on Earth. Coupled with its availability for carbon fixation (Fe_2^+), its association with banded iron formations (BIFs) and some of the deepest organisms on the phylogenetic tree, Fe usage appears to be an early invention of life. Thus far, most of the research on Fe isotopes has involved establishing the degree of fractionation in biological and nonbiological processes. For example, it has been shown that $\delta^{56}Fe$ in marine sediments is shifted by –1.5‰ compared to igneous rocks. However, some complications have been recognized such as the similarity in fractionations of iron

meteorites, loesses, and paleosols (only 0.3‰) to igneous rocks.[78] Thus, as is the case for any new tracer, more measurements are needed to establish the various fractionation patterns and to further determine the usefulness of Fe isotopes as a biomarker of life. Nevertheless, the research to date clearly shows the potential for the use of Fe isotopes in biogeochemical life studies and should be closely watched as, perhaps, a unique and novel approach in the search for life signs on Mars and beyond.

Extinct Fossil Life (Electron-beam Techniques)

Evidence of extinct fossil structures can be sought in detail at various detection levels ranging from macroscopic stromatolitic structures, to microfossils, to the intermolecular distribution of carbon isotopes and organic compounds of the associated organic matter. Depending on their depositional environment, fossilization mechanism and diagenetic history, both macro- and microscale biogenic structures, including biofilms, can be preserved with varying amounts of their original organic contents. In all cases, especially the absence of organic matter, the field "context" of the sample site and the texture and fabric of the structures are critical in determining biogenicity. Since the latter information also undergoes degradation over time, laboratory and field studies of both the fossilization and the subsequent diagenesis processes are needed to determine the time scales over which biogenic features are lost or preserved under various environmental conditions.

Electron-beam techniques (scanning and transmission electron microscopy) combined with optical microscopy provide powerful tools for the characterization of putative fossil structures, often in three dimensions, with respect to their location within the mineral matrix, their morphology, texture, and size. Energy-dispersive x-ray analyzer (EXDA) and electron energy-loss (EEL) attachments, as well as electron microprobes (EM), provide essential chemical and mineralogical measurements that support biogenic origin. Similarly, ion beam techniques like time-of-flight secondary ion mass spectrometry (TOF-SIMS) afford high spatial resolution imaging of organic matter and even stable isotopic measurements on specific structures or locations within structures.

None of these techniques alone, however, can adequately address the question of biogenicity. Nowhere is this more evident then in the assessment of ALH84001 where this array of methods was used to evaluate potential putative martian fossils, yet agreement amongst the scientific community that such evidence was biological could not be achieved. The absence of organic remains within potential fossil structures detected using this approach makes the problem even more difficult. In this context, studies aimed at determining what biological morphologies, fabric, and features are not produced by inorganic processes take on high priority. These considerations suggest that fossil life detection by microscopy alone remains a hope for the future rather than a promise for the present.

In Situ Techniques for Life Detection

The Grand Challenge

As part of NASA's strategy in the search for life signs on Mars and elsewhere, the Jet Propulsion Laboratory (JPL) in Pasadena, California, has initiated the Grand Challenge Program (GCP) to develop in situ measurement techniques that are capable of detecting chemical signatures of life. The GCP will invoke interdisciplinary research goals combining science and technologies that will go beyond the current state of the art and will be implemented in the next decade for future missions in space science exploration. Funding for the program will also remain independent from the NASA R&D funding commitments, allowing participants to continue to develop technologies unimpeded by the normal fluctuations in the agency's budget.

Among the topics of interest for Phase 1 of the GCP program are the following:

1. Identifying chemical signatures for life that are "non-Earth centric" and independent of specific molecules or unusual life properties on Earth;
2. Developing techniques and statistical strategies for identifying signatures in a background of "non-life" and testing them on Earth; and

3. Devising concepts for how measurements can be implemented in situ with emphasis on the development of "miniaturized" in situ instruments.

The GCP concept so far shows great promise in the development of new approaches and instrumentation that will emphasize these topics to explore for life on Mars and elsewhere in the solar system.

Sensor Web

A promising new technique being developed at JPL is a *sensor web system* that consists of several spatially distributed sensor pods that can be deployed to monitor and explore a variety of environments.[79] Of particular interest is the use of the sensor web to conduct long-term monitoring of biogenic gases (e.g., H_2S, FeS, NH_3, or CH_4 oxidation) in extreme environments on Earth, as well as on Mars and bodies in the outer solar system.

The small size and minimal energy and weight requirement of the sensor web (essential for any flight-compatible life detection technique) are demonstrated by considering an analogy to Sojourner rover, which weighed about 15 kg. At a minimum communication distance of 50 m between pods, a monitoring area of 500×500 m^2 area is possible. This would be a true advantage over a rover like Sojouner that only moved a radial distance of 7 m on the surface of Mars. On the other hand, a sensor web deployed with a rover could provide information needed to track down appropriate samples to evaluate. Thus, sensor web represents the type of new technology that can be used for future life detection missions.

Earth Analogues and the Search for Extinct Organic Matter Beyond Mars

Europa

If liquid water exists beneath the surface ice layer on Europa, then one of the environmental requirements for life as we define it will have been met. Additional observations of a source of energy for metabolism and the recognition of the availability of key biogenic elements would be a further indication that life may have originated and could still be present on Europa. The detection of energy sources and possible biogenic elements, however, is not a trivial one. The major goal for exploration of Europa is to establish the presence of an ocean beneath the ice cap. If this is indeed the case, then the emphasis will shift to exploration at depth within the ice to address the question of europan life.

Like Mars, the ices of Europa require analytical approaches that will allow one to differentiate between abiotic and biotic sources of organic material. However, much of what may be delivered exogenously or deposited in situ (europan organic matter) in the ice layers could be destroyed by the intense radiation at the surface of Europa. On the other hand, as has been suggested by the Galileo images, layers of dark material (organics or hydrated minerals) incorporated just a few millimeters beneath the ice may remain unaffected by irradiation. Nevertheless, the severe conditions existing at the surface of Europa present a significant challenge that will require appropriate tests to enhance our opportunity to successfully detect extinct organic compounds. The development of specific instrumentation that is directly related to life present in the ice and ocean environment of Europa will require the following:

1. In situ chemical approaches capable of probing for specific cations, anions, and organic compounds essential to metabolism and life;
2. Impact penetrator-type devices for burrowing through the ice;
3. A cryobot for exploring the ice environment; and
4. A hydrobot for exploring the aqueous environment underneath the ice cap.

Such approaches are truly in their infancy and, thus, require further testing with respect to their development and reliability.

Lake Vostok

Since the exploration of Europa will necessitate an advanced technological approach, testing the equipment using an appropriate Earth analogue, such as Lake Vostok near the South Pole in Antarctica, would greatly facilitate the development of in situ instrumentation. Exploration of the ice cap and lake at Vostok will provide a unique environment for the deployment of a variety of instruments. In addition, several advanced drilling approaches have been developed to recover ice from Lake Vostok.[80] Ice core studies have already revealed significant information pertaining to the nature of climate change over the past ~400,000 years.[81] Moreover, recent analyses of portions of Vostok ice cores revealed between 2×10^2 and 3×10^2 bacterial cells per milliliter and low concentrations of potential growth nutrients, suggesting that Lake Vostok may contain viable microorganisms (Figure 3).[82,83] The detection of microorganisms under a thick ice cap (4000 m) in Lake Vostok supports the

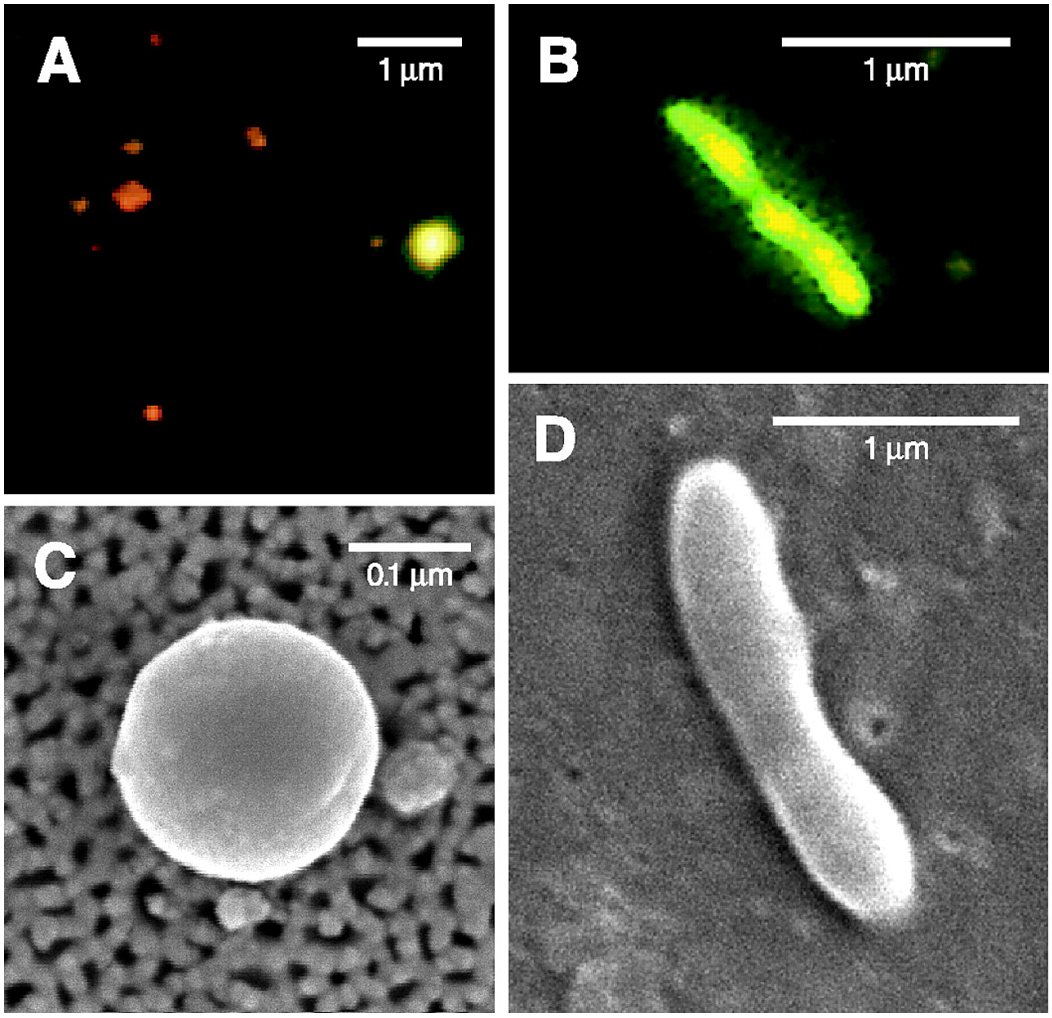


FIGURE 3. The examination of accreted ice from the Lake Vostok ice core drilling project revealed between 200 and 300 bacterial cells per milliliter of meltwater and low concentrations of potential growth nutrients suggestive of viable organics living in the lake underneath 4000 m of ice. Studies of Earth analogues such as Lake Vostok may be necessary to prepare for future missions to moons like Europa, which may have a liquid ocean beneath a thick ice cap. SOURCE: D.M. Karl, D.F. Bird, K. Bjorkman, T. Houlihan, R. Shackelford, and L. Tupas, "Microorganisms in the Accreted Ice of Lake Vostok, Antarctica," *Science* 286:2144-2147, 1999.

notion that the lake could serve as a terrestrial analogue to guide the design of samplers and experiments to be used in life probe missions to Europa and beyond (e.g., Titan, Callisto, Ganymede).

Hydrothermal Vents

Another possible terrestrial analogue for testing advanced technological approaches would be extreme environments such as those around hydrothermal vents. The potential for assessing life at high temperatures can be tested by identifying the sources of energy, the quantity of energy, and the extent to which the energy can be focused. These kinds of studies would greatly facilitate our effort to establish the minimum energy requirements needed to support life beyond Earth. Current studies to quantify energy sources have focused on organisms (chemoautotrophs, organisms that gain energy from chemical reactions) that are capable of capturing energy from environments to generate organic compounds from inorganic forms (e.g., carbon, nitrogen, sulfur, phosphorus). As a complement to these empirical and laboratory efforts, in situ measurements in the hydrothermal environment would allow us to further constrain the energy requirements needed to support organisms. Efforts are under way to use submersible technology to assess the energy sources available and to further test the reliability of the instrumentation in these environments.

Conclusions

The search for life on planets like Mars and perhaps the moons of Jupiter is a testable hypothesis that is of immense interest to the public and researchers worldwide. Our future missions to Mars should begin with the examination of the latest MGS images that will aid in the assessment of the proper landing sites and will maximize the probability of detecting any record of a past martian biosphere. The instrumentation selected for the detection of extinct organic compounds must be capable of differentiating between mechanisms of formation (biogenic versus abiogenic) and the sources (martian or meteoritic) of the material. Beyond these in situ efforts, samples should be "cached" for sample-return missions.

Future exploration efforts beyond Mars should begin with observations carried out in orbit and possibly the deployment of rovers and long-term sensor systems that will provide preliminary measurements of the environment. In addition, testing of instrumentation in the appropriate environment on Earth is essential to the development and implementation of these analytical approaches in a martian or europan environment. The information gained from such approaches can then be used in the development of a series of missions to explore for life signs.

BIOMARKERS AND BIOSIGNATURES OF FOSSIL ORGANIC COMPOUNDS

J. Michael Moldowan
Department of Geological and Environmental Sciences
Stanford University

Abstract

Biosignatures in organic lipids include specific biomarker compounds and other features (stereochemistry, nonrandom distributions) of the lipid fingerprint that cannot be explained by abiogenetic synthesis. In a general screening for organic biosignatures both lipid distributions and the occurrence of complex molecular structures requiring biosynthesis (biomarkers) would seem important. In screening for biomarkers it may be possible to recognize whether any of the three extant domains, Bacteria, Archaea, and Eukarya, were present by including hopanes, head-to-head isoprenoids, and steranes, respectively, in the search protocol. Chirality, and specifically the enatiomeric ratio, is a potentially important biosignature of some biomarkers related to their origins of enzyme assisted synthesis. Enantiomeric excess is preserved through diagenesis in lipids, such as steranes and hopanes, which have quaternary asymmetric carbon atoms in their structures. Relatively mild diagenetic conditions can result in racemization of molecules with only tertiary asymmetric carbon atoms, such as acyclic isoprenoids and amino acids. Distributions of non-biomarker compounds can amount to a biosignature. An example would be a normal alkane distribution that shows predominance of the even (or odd) carbon numbered compounds, reflecting biosynthetic pathways that homologate by two carbon atom increments. Measurements of stable carbon isotope ratios on individual compounds could suggest enzymatic intervention by offering the possibility to recognize big carbon isotopic fractionation effects in individual molecules, thus improving the legibility of biosignatures.

Introduction

In the search for what evidence could be indicative for extraterrestrial life, the analysis of lipids should be very important. Most organic lipids on Earth are derived from organic cellular components. The more complex ones found in sediments or the environment that can be unambiguously related to enzymatic synthesis have been called biological markers or biomarkers since the 1960s.[84,85] Recently, the search for evidence for extraterrestrial life has catalyzed a popular extension of the use of the term "biomarker" to cover all manner of evidence for life. However, at a recent workshop (Biosignatures Workshop, 2000) it was agreed by consensus that "biomarker" would be used in the classical sense to refer to molecular fossils and that any type of evidence for life, including the occurrence of the so-called molecular fossil biomarkers, would be referred to as a "biosignature."[86]

Discussion

Definitions

Biomarkers or biological markers have been defined as "complex organic compounds composed of carbon, hydrogen and other elements which are found in oil, bitumen, rocks and sediments and show little or no change in structure from their parent organic molecules in living organisms. Most, but not all, biomarkers are isopentenoids, composed of isoprene sub-units. Biomarkers include pristane, phytane, steranes, triterpanes, porphyrins, and other compounds."[87] This definition references some of the compounds that are most commonly identified as biomarkers. They typically show complex structures that are not particularly stable compared to other possible organic molecules of the same size. Yet, kinetic energy barriers to their destruction are significant and they survive for billions of years if kept at low temperatures in the absence of oxygen.[88] They show the result of enzymatic syntheses that assemble molecular structures frequently having a shape and size necessary to perform a specific function in the cell.[89] Often this is accomplished at some energy expense for the organism, compared to assembling other less complex lipids of greater thermal stability.

In general, lipids are found as complex mixtures. We can recognize biosignatures in these mixtures when their composition is out of balance with what could be formed from abiotic synthesis alone. Some characteristics of a given biosignature may include the following:

1. Excesses of some structural isomers or homologues over others outside the realm of possible random (abiotic) synthesis;
2. Repeating structural subunits in a molecule, for example, the C_5 isopentene subunits in isoprenoids; and
3. Enantiomeric or other stereochemical excesses that do not reflect relative thermal stability.

Structures and Their Significance

The most abundant individual hydrocarbons produced either by abiogenetic processes or by enzymatic synthesis are the normal alkanes. A large component of these are biosynthetically produced as functionalized compounds, such as fatty acids. Diagenetic processes convert them to *n*-alkanes. Fischer-Tropsch synthesis, an abiotic process, also produces these straight hydrocarbon chains, again mostly with functionality.[90] Figure 1

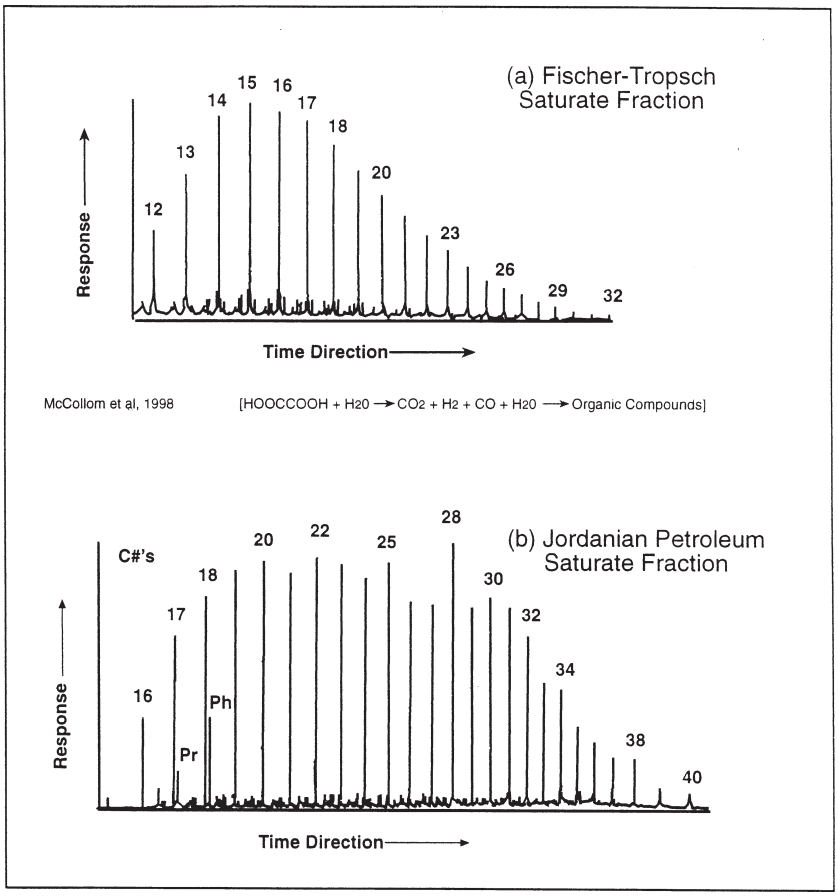


FIGURE 1. Mass chromatographs at m/z 85 monitoring for acyclic alkanes from a saturate fraction (a) produced by a Fischer-Tropsch-type synthesis (from T.M. McCollom, G. Ritter, and B.R.T. Simoneit, "Lipid Synthesis Under Hydrothermal Conditions by Fischer-Tropsch-type Reactions," *Orig. Life Evol. Biosph.* 28:1-14, 1998) and (b) of a petroleum sample. The Fischer-Tropsch synthesis, which serves as a model for abiogenic synthesis, gives a smooth distribution of *n*-alkanes. Petroleum and rock extracts generally show uneven *n*-alkane distributions, and the biomarkers pristane and phytane are usually present. These features can be recognized as a biosignature.

contrasts the distribution of saturate hydrocarbons obtained by Fischer-Tropsch synthesis with that of a petroleum sample. The smooth distribution of *n*-alkanes in the Fischer-Tropsch product typifies what would be expected from abiotic synthesis. There is no outstanding excess or lack of any particular homologue. The envelope of distribution of the homologues may be controlled by conditions of the synthesis, such as temperature, pressure, and catalyses. The oil sample shows multiple expressions of biological synthesis and, therefore, a clear biosignature. Relative abundance of several *n*-alkanes (C_{25}, C_{28}, C_{30}, C_{34}, C_{38}) above the envelope of *n*-alkane peaks indicates preferential synthesis for their precursors in one or a group of organisms. The predominant even carbon-numbered alkanes in this sample could be from sedimentary reduction of fatty acids that are almost entirely even carbon numbered. The isoprenoids pristane and phytane, classical biomarkers having structures with regularly repeating isopentenyl subunits, show prominently in the mass chromatogram. Branched hydrocarbons that occur between the *n*-alkanes are in greater abundance than those formed in the Fischer-Tropsch synthesis, and this may be another biological attribute. In fact, simple methyl-branched alkanes where the methyl group shows a positional preference along the carbon chain may be a key biosignature, because of the link between these lipids and primitive organisms.[91]

Many biomarkers carry taxon specificity in which they identify input from various kingdoms, families, and even individual species. Figure 2 gives examples showing taxon-specific biomarkers from the three domains, Bacteria, Archaea, and Eukarya. Squalene is a widely used isoprenoid intermediate in the biosynthesis of many polycyclic biomarkers, but the cyclization varies in different domains. Bacteria cyclize squalene to make hopanes, while in eukaryotes, squalene lies on the pathway that eventually leads to sterols. Archaea have a unique way of joining isoprenoids to form a "head-to-head" linkage.

What is known about the chemistry of bacterial lipids suggests there are relatively few themes used by these organisms. As mentioned, many prokaryotes synthesize squalene and cyclize it to hopene. Hopene can be further

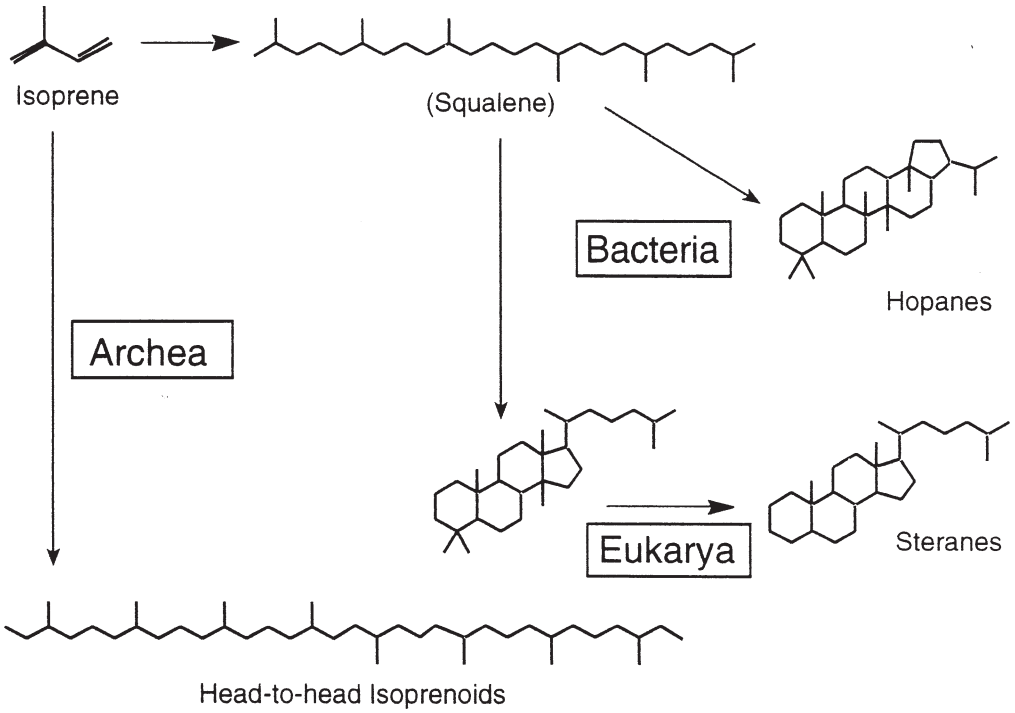


FIGURE 2. Syntheses of many lipids that are recognized as biomarkers incorporate isoprene as a repeating subunit. Three domains of extant life, Archea, Bacteria, and Eukarya, carry signature membrane lipids—head-to-head linked isoprenoids, hopanes, and sterols—respectively. Squalene (shown as squalane without double bonds) acts as intermediate for hopane and sterol biosynthesis. Sterols are eventually preserved as steranes in sediments.

extended by adding a five-carbon chain derived from a sugar (ribose) to give bacteriohopane.[92] Thus, the hopane molecules, which have been said to comprise the most abundant large-molecule repository of carbon on Earth,[93] derive almost entirely from two biomolecular types: hopene (C_{30}) and bacteriohopane (C_{35}). These basic compounds, degraded and rearranged by catalytic and thermal effects of diagenesis and catagenesis, form hundreds of hopanoid products that can be found in sediments.[94] Postdepositional degradation alters the biochemistry, but often does not erase it. Many of the products can still be recognized as biomarkers, albeit partially altered ones. Figure 3 shows some of the diagenetically altered hopane structures that can be found among the saturate hydro-

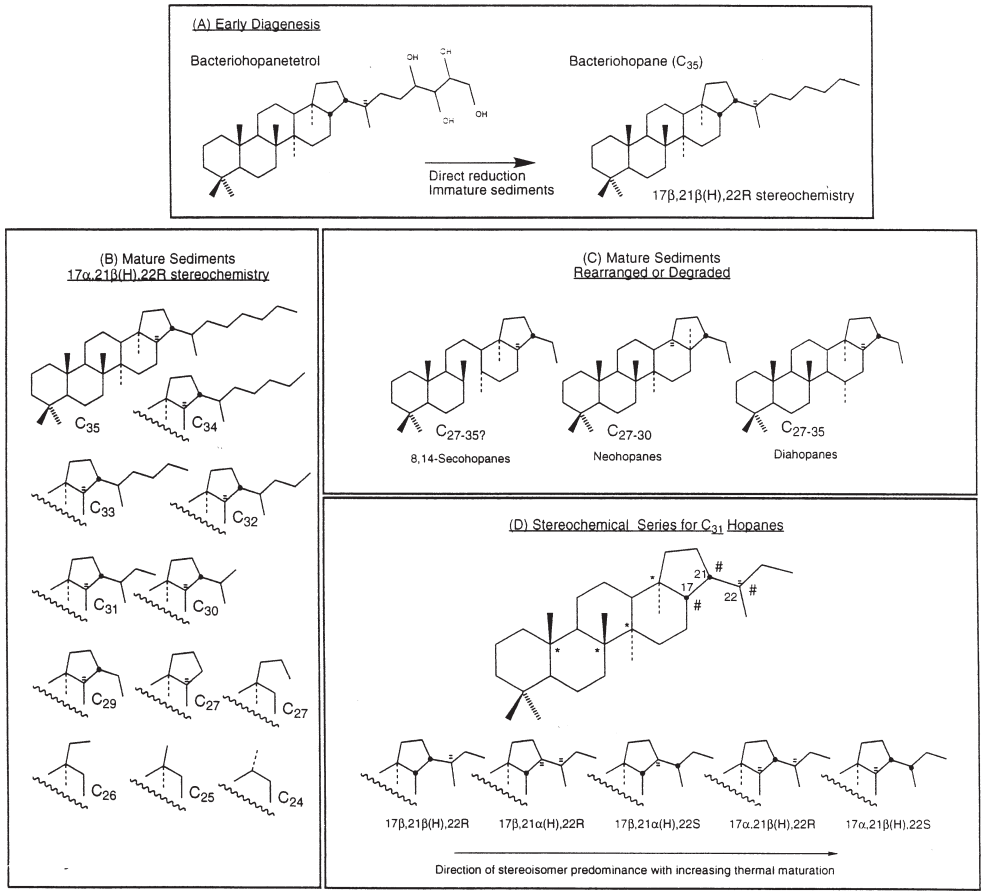


FIGURE 3. Examples of saturate biomarkers formed from bacteriohopanetetrol during diagenesis. Bacteriohopanetetrol (C_{35}) and diplopterol (C_{30}, not shown) and closely related compounds may account for most biomarkers of the hopane series. In addition to those shown and other known saturate hopanoids, numerous aromatic analogues are known. Altogether, more that 200 sedimentary hopanoids are known. (A) Reduction of bacteriohopanetetrol under mild diagenetic conditions results in complete preservation of the carbon structure in immature sediments. (B) The commonly encountered pseudohomologous series of saturate hopanoids likely formed by degradation of the side chain and E-ring. (C) Degraded and rearranged hopanes showing the known carbon number ranges. (D) Epimerization in hydrocarbons occurs only at ternary asymmetric carbon atoms. Stereochemical configuration is frozen at quaternary carbon atoms. For C_{31} hopane, stereochemically frozen quaternary carbon atoms and those tertiary carbon atoms known to epimerize are indicated by * and #, respectively. The known diastereomeric series of C_{31} hopanes is shown. Hopanes with 17β,21β(H), 22*R* stereochemistry occur in sediments with a very mild thermal history and are absent in thermally mature sediments. Hopanes with 17α,21β(H) stereochemistry predominate over 17β,21α(H)-hopanes and 17α,21β(H),22S predominates over 17α,21β(H),22R in thermally mature sediments (e.g., see Figure 4).

carbons of ancient sediments and oil. Figure 4 shows a typical m/z 191 chromatogram that monitors for hopanes and related compounds and some other terpanes.

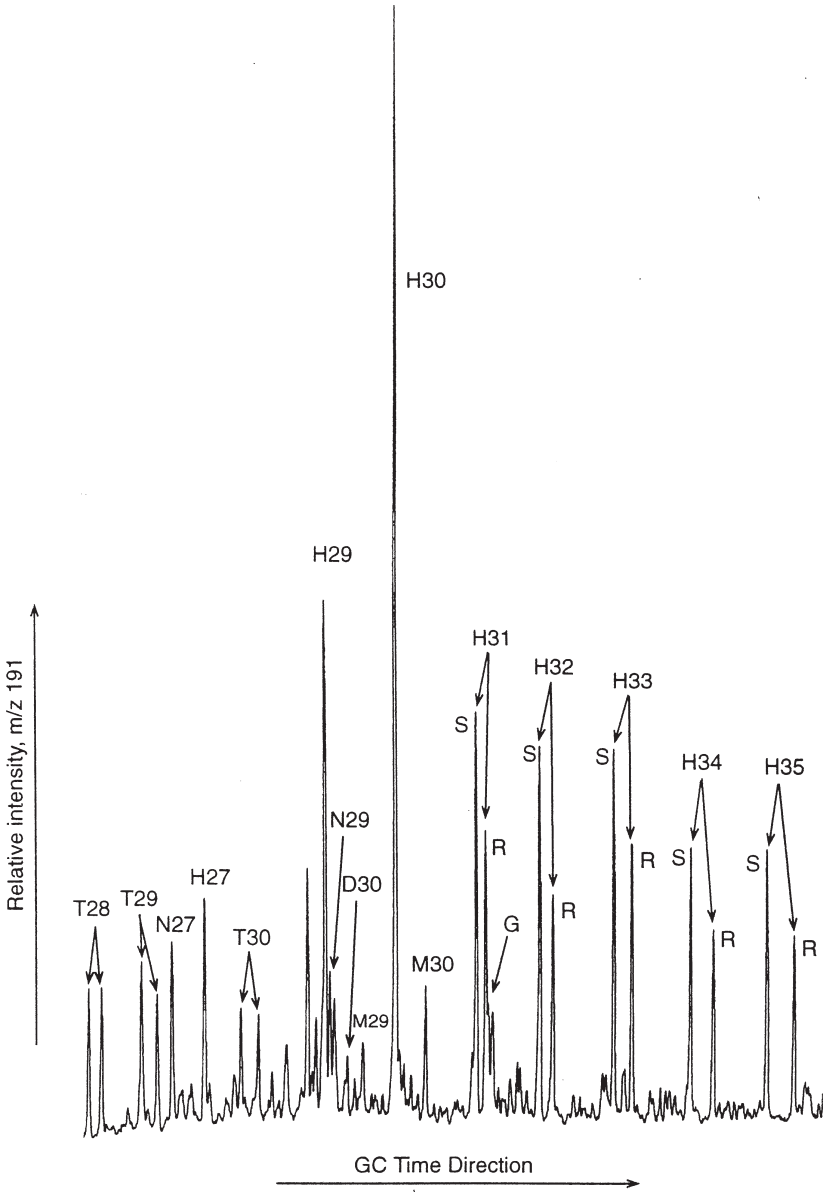


FIGURE 4. Partial mass chromatogram of the m/z 191 response for the saturate fraction of an oil from the North Caspian Basin, Kasakhstan, shows peaks indicating biomarkers of microbial and algal origin. Labeled peaks are as follows: Numbers refer to number of carbon atoms in the chemical structures. Ts are tricyclic terpanes (cheilanthanes) possibly related to tasmanites, a single-celled alga; G is gammacerane, attributed to bactivorous ciliates; Ns are neohopanes, hopanes with rearranged structures; D is diahopane, a rearranged hopane; Ms are moretanes; Hs are 17α-hopanes, diastereomers of biosynthetic hopanes (17β), with various side chains (see Figure 3) accounting for the range in carbon number 27 to 35. Hopane homologues ≥ H31 show stereochemical antipodes at C-22 indicated by R or S. SOURCE: J.M. Moldowan, "Biomarkers," in *McGraw-Hill Yearbook of Science and Technology,* McGraw-Hill, Boston, Mass., 2000, pp. 36-39.

In most cases, lipids that have asymmetric carbon atoms (carbon atoms with four different groups attached) are enzymatically biosynthesized as one or the other antipode. For example, amino acids are always biosynthesized as the *L*-enantiomer rather than the *D*-enantiomer. Some biomarkers show multiple asymmetric centers. However, diagenesis has a tendency to invert certain asymmetric centers. Thus, ancient organic matter that still contains amino acids may tend to show a racemic mixture (50:50 mixture) of *L*- and *D*-amino acids. In the analysis of lipid biomarkers we are seldom concerned with simple enantiomers, because they are difficult to resolve by the available analytical technology. However, compounds with multiple asymmetric centers can be resolved as diastereomers, in cases where some but not all of the asymmetric centers have been inverted. Asymmetric centers carrying a hydrogen atom (tertiary carbon centers) tend to invert, while asymmetric quaternary carbon centers cannot. Inversion at asymmetric centers is called epimerization and does not generally result in a 50:50 mixture of the two configurations (e.g., see Figure 4) when multiple asymmetric centers are involved unless all centers are free to invert. Inversion at quaternary centers during diagenesis is not possible, and disruption of these centers results in molecular rearrangements and loss of the base structure.

Archaea may be even more primitive than bacteria, but less has been published about the structures of their lipids. The head-to-head isoprenoids appear to be Archaea specific.[95] These compounds include some with rings and possibly others with shorter carbon chains. There may also be simple branched hydrocarbons that are peculiar to Archaea. Both Archaea and Bacteria are known to produce smaller isoprenoids, like phytane. Thus, acyclic isoprenoids could be useful to indicate even very primitive life.

Recent articles on the analysis of early Earth sediment extracts, revealed that a 2700-million-year old rock from Australia contained a variety of complex biomarkers.[96,97] These workers showed the occurrence of phytosynthesis even at those early times, by the presence of 2-methylhopanes, biomarkers common to photosynthetic cyanobacteria. Even more striking was the discovery that a homologous series of steranes, comparable to that found in Proterozoic through Phanerozoic marine sediments, was also present. This strongly suggests the presence of Eukarya (Figure 2), and indeed, a quicker evolution of Eukarya by as much as a billion years, than had been previously suggested based on fossil evidence.[98,99] The quick advancement into cellular organelles on Earth indicated by these studies suggests that analysis for Eukarya-specific biomolecules should not be excluded in the search for extraterrestrial life.

Isotope Ratio Gas Chromatography-Mass Spectrometry

Another research tool that could add important information about biosynthetic origin of compounds would be isotope ratio gas chromatography-mass spectrometry (IRGC-MS). It provides information about carbon isotope fractionation on individual hydrocarbons. In mixtures of highly degraded or thermally altered hydrocarbons it may still be possible to detect excess carbon isotopic fractionation, compared to that which might be available from abiotic synthesis, by using IRGC-MS. Certainly, this would be an added piece of information to consider should sufficient lipid extract be available for the analysis.

ORGANIC CHEMISTRY OF METEORITES: CRITERIA FOR ABIOTIC ORIGINS

Sherwood Chang
SETI Institute

Abstract

The Murchison meteorite contains a rich inventory of organic matter. The distribution of molecular structures and C, H, and N isotopic compositions among the organic components exhibit characteristic abiotic patterns distinctly different from those of terrestrial biochemistry. Together, these patterns provide criteria that can be used with cautious confidence to distinguish between organic matter of biological and nonbiological origins in extraterrestrial samples.

Introduction

Although telescopes may eventually take the search for extraterrestrial life beyond the confines of our solar system, a capability for detecting signs of life on extrasolar planets remains outside our current reach.[100] Within our grasp lie Mars and Europa, and for these bodies, sample return missions and robotic experiments loom in the foreseeable future as opportunities for seeking life beyond Earth.

Whether data are obtained at remote sites or extracted from returned samples, the credibility of any conclusion drawn concerning the presence or absence of alien life will depend critically on the ability to establish and apply criteria that distinguish unambiguously the products of biological from nonbiological processes.

A decades-long search for the earliest evidence of life on Earth has produced molecular, isotopic, and stereochemical properties that are useful for assessing the biogenic origins of extraterrestrial organic matter. The value of these traits for discerning alien life hinges on how common they are to all biochemistries;[101] the worth of criteria that characterize abiogenic organic matter depends on their absence in all biochemistries.

Why the Organic Matter in Carbonaceous Meteorites?

Carbonaceous meteorites are some of the most studied objects in the solar system, principally because of the presence of condensates from our solar nebula and other stars, primitive bulk elemental compositions, mineral phases formed in liquid water (in CI and CM types), and diverse organic components. The critical connection between liquid water and life on Earth lends special significance to the earliest known occurrence of liquid water and abundant organic compounds in the solar system.[102] Apparently, prebiotic evolution occurred early on some parent bodies of carbonaceous meteorites, but fell short of the origin of life.

Samples from carbonaceous meteorites offer a rich and accessible source of information for identifying model characteristics for organic matter that may have formed during prebiotic evolution on Mars, Europa, and other extraterrestrial environments. They also exhibit spectral properties that resemble those of the abundant C-type main belt asteroids.[103] The proximity of the asteroid belt to Mars ensures that the planet's surface contains carbonaceous meteoritic debris and interplanetary dust particles.[104] Organic matter, whether prebiotic or biological and asteroidal or martian, would have been best preserved in sedimentary rocks. Such samples are the highest-priority targets for remote analyses or return to Earth.

Molecular Structural Characteristics of Meteoritic Organic Matter

Before the fall of the Murchison meteorite (CM type) in 1969, terrestrial contamination obscured the organic chemistry of carbonaceous meteorites. In preparing to study lunar samples returned that same year, state-of-the-art analytical methods designed to minimize terrestrial contamination were developed that ushered in a new generation of meteorite analyses focusing on Murchison samples. Ensuing investigations uncovered an extensive inventory of compounds whose structures, stable isotopic compositions, and stereochemistry reveal a rich organic chemistry.[105]

The 2 percent total carbon in the Murchison meteorite takes on a variety of forms: The mole fraction of carbon occurring as water- and solvent-soluble organic compounds varies between 0.2 and 0.3 from sample to sample. The bulk of the carbon, ranging in mole fraction from 0.6 to 0.8, resides in a high molecular weight, solvent- and acid-insoluble component whose detailed properties remain poorly understood. This material appears to be a mixture of submicron carbonaceous grains resembling the CHON particles detected in the coma of comet Halley.[106] The insoluble fraction also contains minor amounts of extrasolar condensates in the form of nanometer-sized diamonds, silicon carbide, and graphite grains.[107] Carbonates formed during the epoch of liquid water make up the remaining 0.02 to 0.1 mole fraction of carbon.

The classes of meteoritic organic compounds that have familiar biochemical counterparts include amino acids, fatty acids, purines, pyrimidines, and sugars (G. Cooper, personal communication). In addition there are alcohols, aldehydes, amides, amines, mono- and dicarboxylic acids, aliphatic and aromatic nonpolar hydrocarbons, polar hydrocarbons as heterocyclic aromatics, hydroxy acids, ketones, phosphonic and sulfonic acids, sulfides[108] and ethers.[109] Concentrations of compound classes vary widely from less than 10 (amines) to tens (amino acids) to hundreds of parts per million (carboxylic acids).[110] Chromatographic analyses of virtually all classes of acyclic compounds reveal complex molecular assemblages containing homologous series of compounds up to C_{12} in some cases (carboxylic acids).

Three distinctive patterns of structural variation with molecular weight emerge from studies of these mixtures, no one of which is exhibited by the classes of compounds used in living systems. Data for amino acids and hydroxy acids are chosen to illustrate these patterns, the former for their centrality to terrestrial biochemistry, and both for their wide range of possible structural variations and the availability of relevant meteoritic data. Undoubtedly, future analysts of extraterrestrial organic matter will target amino acids because an alien biochemistry is likely to evolve catalytic polymers consisting of similar multifunctional building blocks.

1. *Molecular abundances decrease with increasing carbon number.* As illustrated by the α-methyl branched- and straight-chain series of α-amino acids, plots of their concentrations (log nanomoles per gram) versus carbon numbers yield linear correlations with declining slopes of about –7.[111] These trends suggest growth of amino acid carbon skeletons by single carbon additions.

2. *Abundances of branched-chain isomers exceed those of the straight chain.* For example, for compounds containing the same number of carbon atoms, the concentration of the α-methyl- and the β-methyl-branched isomers each surpasses that of the straight-chain isomer.

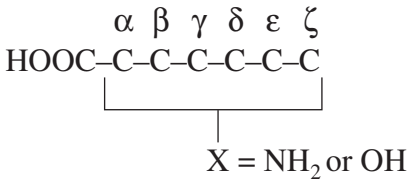
3. *Complete structural diversity prevails at the lower carbon number.* This last pattern is illustrated by Table 1, which compares the numbers of known isomers of the acyclic monoamino monocarboxylic acids and the acyclic monohydroxy monocarboxylic acids with the numbers of theoretically possible structures. Overall, 57 amino acids occur among the 159 possible C_2 to C_7 isomers. Analytical sensitivity and diminishing abundances at higher molecular weights limit further identifications. Remarkably, almost all structural isomers from C_2 to C_7 occur in the α-amino and α-hydroxy acids; from C_2 to C_5 nearly all isomers of β- and γ-substituted acids appear. Note that Murchison also contains assemblages of structurally diverse acyclic unsubstituted, and monoamino- and monohydroxy-substituted *di*carboxylic acids.[112]

In contrast, life on Earth employs only 20 common protein α-amino acids and all of these have an α-H. Biochemical counterparts account for less than one-third of the 33 listed in the second column of Table 1. Life on Earth uses a ubiquitous but restricted number of isomers taken predominantly from a few classes of organic compounds to fulfill its requirements for structure and function. Biosynthetic pathways manifest themselves in patterns of structural variation in these classes,[113] just as abiotic pathways are reflected in the patterns exhibited by meteoritic compounds (see below).

Structural diversity also occurs among cyclic meteoritic compounds. A plethora of polycyclic aromatic hydrocarbons up to 750 daltons have been found in Murchison samples, along with a multitude of C_{15} to C_{30} branched alkyl-substituted mono-, di-, and tricyclic alkanes.[114] Becker et al. also found an extensive suite of high molecular weight C_{72} to C_{270} fullerenes.[115] Polycyclic compounds together with the alkyl phosphonic and sulfonic acids comprise the most thermally stable species in the Murchison inventory. They and the acid-insoluble material are the likeliest meteoritic components to survive delivery to and diagenesis on Mars.

TABLE 1. Comparison of Numbers of Acyclic Monoamino Monocarboxylic Acids and Monohydroxy (shown in bold) Monocarboxylic Acids of the Murchison Meteorite with the Number of Possible Theoretical Isomers (shown in bold italic) (compounds with other substituent groups are omitted). (After J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.)

C Atoms	α	β	γ	δ	ε	ζ	Unknown
2	***1,*** 1, **1**	—	—	—	—	—	—
3	***1,*** 1, **1**	***1,*** 1, **1**	—	—	—	—	—
4	***2,*** 2, **2**	***2,*** 2, **2**	***1,*** 1, **1**	—	—	—	—
5	***3,*** 3, **3**	***6,*** 6, **3**	***3,*** 3, **3**	***1,*** 1, **0**	—	—	**1**
6	***8,*** 8, **8**	***12,*** 3, **1**	***11,*** 4, **0**	***4,*** 2 ,**0**	***1,*** 1, **0**	—	**2**
7	***18,*** 18, **12**	***29,*** 0, **0**	***29,*** 0, **0**	***20,*** 0, **0**	***5,*** 0, **0**	***1,*** 0, **0**	**2**



The observed patterns of variation in molecular structure and abundance with increasing carbon number suggest synthesis routes entailing small free-radical initiators and intermediates.[116] Such pathways tend to produce all possible structural isomers at lower carbon numbers by more or less random synthesis. Primary reactions in interstellar clouds produced mixtures of nitriles and other compounds. When exposed to liquid water on the parent body, the nitriles were converted to various substituted and unsubstituted carboxylic acids, including the amino acids mentioned earlier. Other classes of compounds were likely to have formed in secondary reactions. For example, the Strecker synthesis of cyanohydrins and aminonitriles from HCN, aldehydes or ketones, and ammonia or amines, followed by hydrolysis, could account for synthesis of some of the α-amino- and α-hydroxy acids. Amino acids substituted at more distant positions from the carboxyl group require other synthetic pathways.

Chirality of Amino Acids in the Murchison Meteorite

Syntheses of organic compounds do not produce chiral products in the absence of a chiral agent, whether it be an energy source (e.g., circularly polarized light), a surface substrate (e.g., quartz crystals), or a reactant. A recent evaluation of abiotic mechanisms proposed for the origin of chiral molecules on the primitive Earth concluded that they are "inapplicable and implausible."[117] The absence of chirality in products of prebiotic evolution experiments strengthened the presumption that natural abiotic synthesis invariably produces racemic compounds. Indeed early analysts of Murchison amino acids declared them racemic.[118] Under the looming specter of contamination, later reports of *L*-enantiomeric excesses in several meteoritic counterparts of biological amino acids generated a swirl of controversy.[119–121] In 1997, Cronin and Pizzarello resolved the issue in favor of modest *L*-enantiomeric excesses of 2 to 9 percent.[122] They avoided the pitfalls of contamination by making measurements on 2-amino-2,3-dimethylpentanoic acid, α-methylnorvaline, and isovaline. All three compounds are α-methyl substituted; the first two have no known biological counterparts, and the third has a restricted distribution in fungal antibiotics. Engel and Macko have found evidence for nonracemic Murchison amino acids using an isotopic approach.[123]

Bailey et al. suggest that the observed enantiomeric excesses could have been induced by circularly polarized light arising from dust scattering in regions of high-mass star formation.[124] These sources occur more widely than do the supernova remnants or pulsars that were first proposed by Rubenstein et al. as sources of circularly polarized synchrotron radiation.[125] Regardless of production mechanism, the nonracemic amino acids testify to the reality of a naturally occurring abiotic chiral process. Future researchers will need to be cautious in using chirality to assign biotic origins to extraterrestrial compounds.

Isotopic Compositions of Carbon, Hydrogen, and Nitrogen in Murchison Organic Matter

Although bulk analyses of the isotopic compositions of C, H, and N of the Murchison meteorite hold no surprises, detailed examination of organic components reveals anomalies that call for unusual production mechanisms and environments of origin. The organic matter generally contains deuterium enrichments that start above the upper limits of the terrestrial range (δD +100‰) and extend to +2500‰, approaching those of interstellar molecules.[126] Values of $\delta^{13}C$ range from –18 to +40. Results are displayed in Figure 1 for organic components on which simultaneous measurements of $\delta^{13}C$ and δD are available.

The heavy isotope enrichments in C and H vary widely from component to component and extend well beyond the limits of terrestrial experience. This is also true for $\delta^{13}N$ values, which range from +18 to more than +98‰.[127] Note that the strongest heavy-isotope enrichments occur in polar organic compounds. Variations are large even within compound classes. Such variability suggests different reaction pathways or different formation

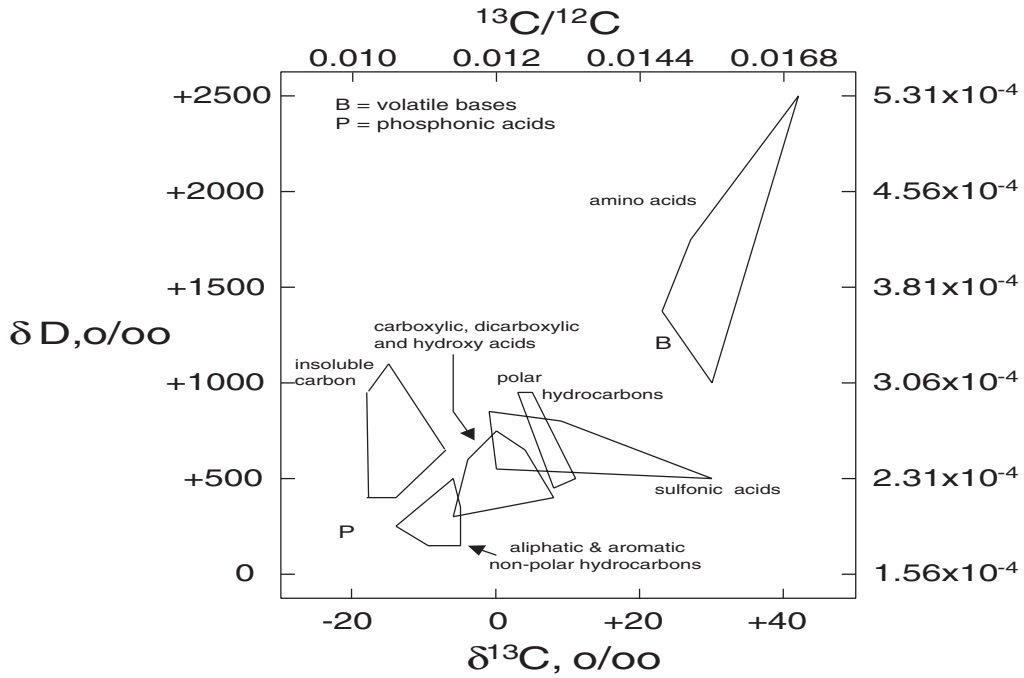


FIGURE 1. C and H isotopic composition of organic components in Murchison meteorite. SOURCE: J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258, and G.W. Cooper, M.H. Thiemens, T.L. Jackson, and S. Chang, "Sulfur and Hydrogen Isotope Anomalies in Meteorite Sulfonic Acids," *Science* 277:1072-1074, 1997.

temperatures, sampling of isotopically dissimilar reactant reservoirs, or some combination of these and other factors. Temperature effects accord with the view that the deuterium enrichments arise from gas phase (ion-molecule) and grain surface (atomic or free radical) reactions occurring at low temperatures in interstellar clouds.[128] Sampling of isotopic reservoirs are consistent with the presence in Murchison of originally interstellar molecules and stellar condensates containing distinctive nucleosynthetic isotopic compositions.[129]

In sharp contrast, biochemical processes typically do not yield such wide isotopic variability within or among compound classes, nor do the products of laboratory prebiotic syntheses.[130,131] The isotopic record of fossil life preserved in sediments of increasing age generally shows a relatively narrow range of carbon isotopic compositions for both extractable[132] and insoluble organic fractions.[133,134] The 20-35‰ difference ($\Delta\delta$) between the organic carbon and the isotopically heavier coeval carbonate (representing the source reservoir) is generally taken as an isotopic biomarker. Murchison organics preserved in ancient sediments on Mars, however, might well yield a similarly narrow range of compositions, as diagenesis destroys the minor polar components leaving the thermally stable insoluble carbon and hydrocarbons as relics. The $\delta^{13}C$ of carbonate in Murchison is about +42‰; therefore, the $\Delta\delta$ could range from +20 to +60‰, overlapping the terrestrial biomarker values. As with chirality, isotopic criteria must be used cautiously for distinguishing biological from nonbiological materials, particularly in an Earth-like environment with a single dominant isotopic reservoir for each of C, H, and N.

Compounds extracted from returned martian samples can be subjected to intermolecular and intramolecular isotopic analyses. Measurements on individual Murchison compounds show each carboxylic acid containing ~16‰ more ^{13}C than the light hydrocarbon of corresponding carbon number. In both homologous series, $\delta^{13}C$ values decline in parallel fashion as carbon number increases.[135] Intramolecular measurements on acetic acid indicate that the carboxyl group is relatively enriched in ^{13}C by 15 to 30‰. These results suggest synthesis of carboxylic acids by stepwise addition of the elements of CO_2 to hydrocarbons. Cooper et al. also carried out intramolecular carbon, hydrogen, and sulfur isotopic measurements on individual Murchison sulfonic acids.[136] Measurements at similar levels of molecular detail may prove invaluable in establishing sources and production mechanisms; they should certainly be included in the isotopic arsenal for future studies of extraterrestrial organic matter.

Organic Survey Analysis of the Murchison Meteorite

Survey analyses for volatile and organic components will be among the key preliminary measurements carried out on rocks returned from Mars. One such technique was used successfully in critical experiments on the 1976 Viking lander. Martian soil samples were progressively heated under vacuum to ~500˚C followed by product analysis with a combined gas chromatograph-mass spectrometer (GC-MS).[137] The measured ion abundances set a 4-part-per-billion upper limit on organic matter in martian soil, which argued persuasively for the absence of detectable signs of life at the Viking landing sites.

In a similar experiment, the data shown in Figure 2 were produced from mass spectra recorded every 17 seconds as a sample of a Murchison-like meteorite was heated under vacuum from 25 to 1400˚C. The mass pyrograms show gas evolution in the temperature ranges over which water-bearing clays, carbonates, and sulfates release their H_2O (250 to 600˚C), CO_2 (500-550˚C), and SO_2 (400-800˚C). Dehydration, decarboxylation, and oxidative desulfurization of organic compounds also yield these same species over the range <200 to 600˚C. Hydrocarbons and N- and O-containing organic compounds evolve from 100 to ~700˚C due to pyrolysis of parent organic matter of differing thermal stabilities. The CO_2, CO, and N_2 released above ~600˚C represent refractory carbonaceous material that has already lost its alkyl and functional groups and is undergoing oxidation by inorganic silicates and oxides. While this hardy material would have been the most likely organic component to have survived on the martian surface, it would not have been detected by the Viking pyrolysis GC-MS with its 500˚C temperature limitation.

Survey analyses of this sort, while incapable alone of addressing the origin of organic compounds, would provide means for detecting organic matter and identifying sedimentary rock samples either in terrestrial laboratories or on planetary surfaces. Such screening would be invaluable for identifying samples for return to Earth or for determining the relative abundances of rock types at landing sites. The analytical methodology is well established,

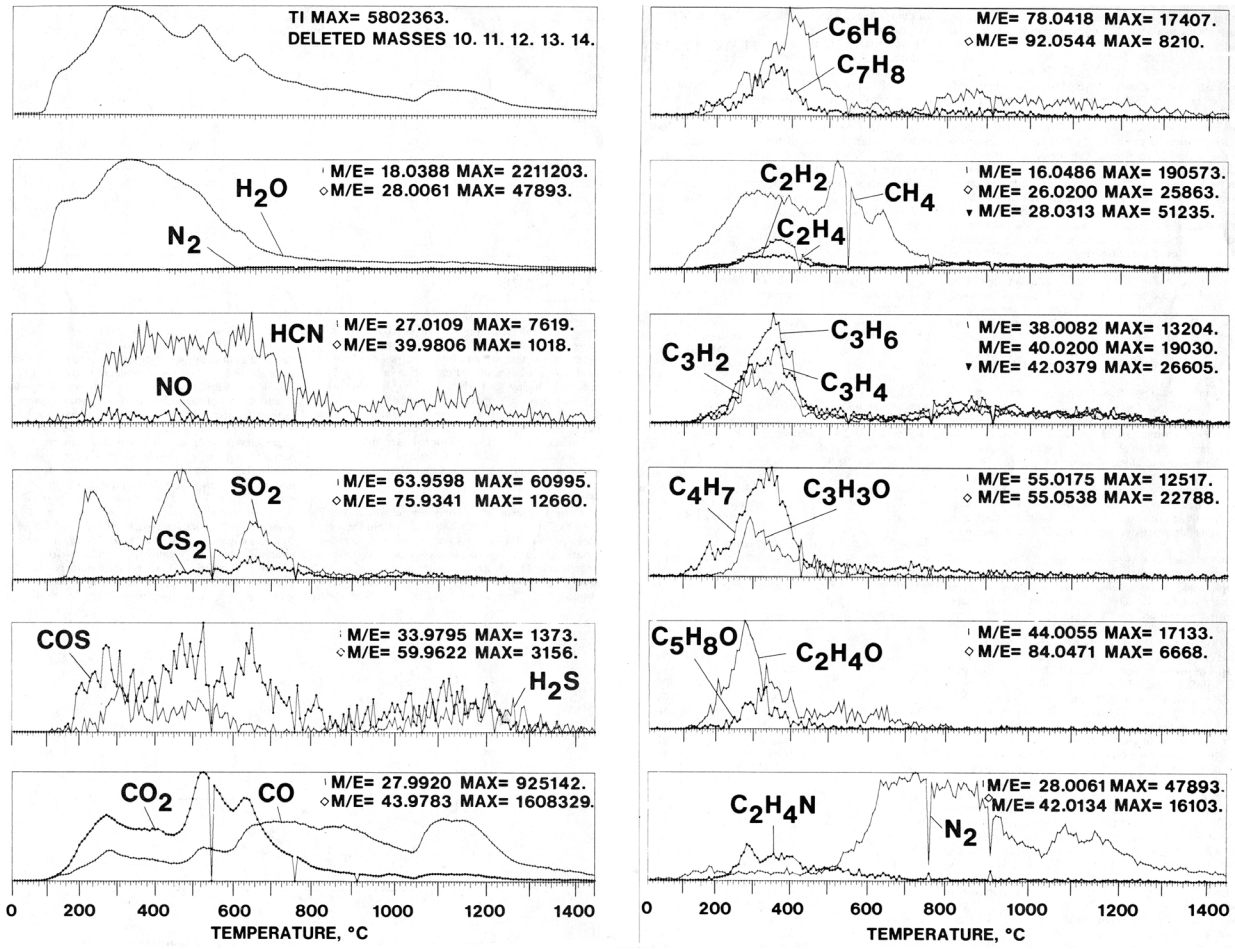


FIGURE 2. High-resolution mass pyrograms for the Jodzie (CM) inclusion. SOURCE: T.E. Bunch, S. Chang, U. Frick, J.M. Neil, and G. Moreland, "Carbonaceous Chondrites: I—Characterization and Significance of Carbonaceous Chondrite (CM) Xenoliths in the Jodzie Howardite," *Geochim. Cosmochim. Acta* 43:1727-1742, 1979, Figure 6.

and it could be readily adapted to modern instrumentation for robotic exploration. A pyrolysis-GC-*isotope ratio*-MS, however, could have potential for life detection.

Conclusions

Analyses of Murchison organic matter reveal characteristic patterns of variation in molecular composition. These include the following:

1. Complete structural diversity within compound classes among isomers of the lower carbon homologues;
2. Molecular abundances in a class declining with increasing carbon number;
3. Branched-chain isomers predominating at a given carbon number; and
4. C, H, and N isotopic compositions varying widely among and within compound classes.

Since these attributes may not be held in common by all abiotic reservoirs of extraterrestrial organic matter, it is unwarranted to view the observed traits as universal abiotic indicators. Molecular assemblages of biological origin, however, will rarely if ever exhibit these patterns. Therefore, these criteria may be used with cautious confidence to distinguish nonbiological from biological organic matter. The discovery of *L*-enantiomeric excesses of abiotic origin in some Murchison amino acids means that chirality is not a foolproof molecular indicator of biology.

ISOTOPES OF IRON: BIOMARKER PROSPECTS

Ariel D. Anbar
Department of Earth and Environmental Sciences
University of Rochester

Abstract

The isotopes of iron are fractionated during microbially mediated reduction of ferrihydrite. The isotopic composition of iron in modern and ancient ferromanganese sediments displays a range of values similar to those produced biologically, which could reflect biological production of iron in nature. Because of the ubiquity of iron-bearing phases in nature and the diverse uses of iron in microbial metabolism, these observations demonstrate the potential of iron isotope research for the detection of ancient life. Nonbiological chemical processes can similarly fractionate Fe isotopes, but the importance of nonbiological fractionation in nature has yet to be determined.

Introduction

Isotopic indicators are extremely useful in the detection of life in the geologic record. Compared to both molecular fossils and microfossils, isotopic indicators can be more robust against alteration processes. A notable example is the observation of ^{12}C-enriched organic residues in strongly metamorphosed sediments deposited >3.85 billion years ago.[138] This constitutes the earliest evidence of life on Earth.

Study of isotope fractionation of elements lighter than S has been ongoing for nearly 50 years. In contrast, until recently there was virtually no research into the fractionation of Fe and other transition metal isotopes—biogenic or otherwise. Such research was hampered by difficulties in making precise measurements of the isotopic compositions of these elements.

Recent advances in mass spectrometric tools and techniques have greatly simplified such measurements, leading to rapid progress. Natural variations in the isotopic compositions of Fe (masses 54, 56, 57, 58), Cu (63, 65), Zn (64, 66, 67, 68, 70), Tl (203, 205), and Mo (92, 94, 95, 96, 97, 98, 100) have been reported.[139–145] Because these isotopes are neither radioactive nor the products of long-lived radioactive decay, these variations must result from mass-dependent fractionation of metal isotopes. For some of these metals, biological and nonbiological fractionation processes have been studied in the laboratory.[146–150]

Isotopes of Fe are of particular interest because Fe-bearing phases, including well-known biominerals, are widespread in nature and are more robust against alteration than many C-bearing materials, and because biological fractionation of Fe isotopes has been reported by Beard et al.[151] Hence, biogenic Fe isotope signatures could prove useful for detection of life in the geological records of Earth or Mars.

Research on the Fe isotope system is advancing rapidly. This paper reviews the basis for interest in Fe isotopes and the status of Fe isotopes as biomarkers.

Why Iron Isotopes?

When considering the utility of Fe (or any other metal) for isotopic biomarker studies, four key questions must be answered:

1. Is the isotopic mass difference large enough to potentially produce a detectable fractionation?
2. Does the biological use of Fe justify attention?
3. Do biogenic fractionations exist?
4. Are biogenic fractionations larger than those resulting from natural nonbiological processes?

The first two questions, which should be addressed prior to isotopic study, are discussed below. The latter two, the subjects of ongoing research, are addressed in the following section.

Fractionation Potential

To answer the first question, it is useful to understand why mass-dependent fractionation occurs at all. Briefly, isotope fractionation results from differences in the zero-point energies (ZPEs) between chemical bonds that are identical except for isotopic substitution. In the simple case of a diatomic molecule, A-B, ZPE = $1/2h\nu$, where ν is the vibrational frequency of the bond and h is Planck's constant. Approximating the bond as a harmonic oscillator, $\nu = 1/2\pi(k/\mu)$, where k is the force constant and μ is the reduced mass of the bond ($\mu = m_A m_B/(m_A + m_B)$). Hence, bond strengths are mass dependent. Because bond strength is inversely related to ZPE, bond strength increases upon substitution of heavier isotopes. Thus, if we consider two isotopes of A, A_1 and A_2, where $m_{A_1} > m_{A_2}$, bond A_1-B is stronger than A_2-B.

This mass dependence leads to differences in reaction rate constants: A_1-B will react more slowly than A_2-B. This is the origin of "kinetic" isotope effects in the case of unidirectional or incomplete reactions. An example is the fractionation of ^{12}C from ^{13}C during photosynthesis, where $^{12}CO_2$ is processed faster than $^{13}CO_2$. Kinetic isotope effects favor reaction of the lighter isotope.

Mass dependence also leads to differences in equilibrium constants and, hence, "equilibrium" isotope effects. Such effects favor partitioning of the heavier isotope into the stronger bonding environment. Although complete equilibrium is often not achieved, especially in biological systems, such free-energy differences can drive isotope fractionation in nature. An example is the fractionation of C isotopes between CO_2 gas and the carbonate ion (CO_3^{2-}).

A rough guide in assessing the *relative* potential for fractionation of different elements can be made by considering the simplified case in which $m_B >> m_A$. Here, $ZPE_{A_1\text{-}B}/ZPE_{A_2\text{-}B} \sim (m_{A_2}/m_{A_1})$. The relative values of (m_{A_2}/m_{A_1}) for a variety of biologically important elements are shown in Table 1. It is immediately apparent that while the values for transition metals are generally smaller, they are of comparable magnitude to values for other elements. Hence, it is reasonable to expect that transition metals, including Fe, could show natural variations in isotopic composition similar to those of lighter isotopes of order 1 to 10 parts per thousand (1 to 10‰).

Table 1
Some Elements of Biological Interest

	Stable Isotopes[a]	$\nu(m_{A_2}/m_{A_1})$
C	^{12}C, ^{13}C	1.041
N	^{14}N, ^{15}N	1.035
S	^{32}S, ^{34}S	1.031
Ca	^{40}Ca, ^{44}Ca	1.049
Se	^{76}Se - ^{78}Se, ^{80}Se, ^{82}Se	1.039
Fe	^{54}Fe, ^{56}Fe, ^{57}Fe	1.027
Ni	^{58}Ni, ^{60}Ni, ^{61}Ni, ^{62}Ni	1.034
Cu	^{63}Cu, ^{65}Cu	1.016
Zn	^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn	1.031
Mo	^{92}Mo, ^{94}Mo - ^{98}Mo, ^{100}Mo	1.043

[a]Isotopes with average abundance >1 %.
A_2 is the heaviest and A_1 the lightest isotope.

However, it must be emphasized that for each element, the extent of isotope fractionation depends on the chemical reactions in which the element is involved. For example, while C and Ca compare favorably in Table 1, the natural variability of $^{40}Ca/^{44}Ca$ is about one-tenth that of $^{12}C/^{13}C$. This reflects differences in the environmental biogeochemistry of these elements. Therefore, to assess the relative utility of Fe for biomarker studies, its use in biology must be considered.

Iron in Biology: Present and Past

From a strictly biological perspective, Fe is of interest because it has the most diverse usage of any metal in biochemistry. Briefly, Fe is found in cofactors and enzymes such as porphyrins, which are ubiquitous in electron-transfer cytochromes; in enzymes that bind CO, O_2, and NO; and in various oxidases; Fe-S proteins, such as ferredoxin, a component in the electron transfer chain of Photosystem I and the Fe_4-S_4 cofactors in nitrogenase; methylreductase; and Fe-based superoxide dismutase, present in prokaryotes, chloroplasts, and mitochondria.

Most interesting is the fact that Fe, like Mn, can be used as an oxygen substitute in respiration.[152] Like C, it can also serve as an electron donor, providing metabolic energy to Fe-oxidizing microbes under both aerobic and anaerobic conditions. Because there is only one stable isotope of Mn, *Fe is the only metal involved in this manner in microbial respiration that also has the potential for detectable fractionation effects.* Hence, as with C, there is potential for kinetic fractionation of Fe isotopes during enzyme-catalyzed unidirectional reactions in Fe metabolism. The signature of such processes could be found in the isotopic composition of Fe minerals formed from biologically processed iron.

In addition, the uptake of Fe by microorganisms involves conversion from inorganic complexes to extremely strong organic complexes with Fe-specific biogenic ligands ("siderophores"). Hence, there is potential for both kinetic and equilibrium fractionation of Fe isotopes during Fe uptake. Such uptake can be extremely efficient and, because of mass balance considerations, leads to the possibility that biogenic Fe isotope signatures might also be found in residual Fe not taken up by organisms.

Fe also stands out compared to other metals because, from an evolutionary perspective, it was probably particularly important for early life. For example, in the anoxic environment of the Archaean Earth, the availability of Fe may have led to the widespread use of Fe^{2+} as an energy source for carbon fixation. Both anaerobic Fe oxidation and Fe reduction may have been important in the formation of banded iron formations (BIF).[153] The deepest organisms on the phylogenetic tree contain various Fe-bearing proteins. Hence, it is all but certain that the uptake and use of Fe was an early invention of life on Earth. It is likely that biological Fe utilization would also have been an early invention of life on Mars, if life existed there.

Therefore, it is reasonable to expect that microbial processing of Fe generates isotopic signatures that will prove useful for detection of ancient life. This expectation has motivated Fe isotope research.

Recent Research Findings

To test this expectation, three complementary research approaches have been pursued by several research groups:

1. Determination of the extent of Fe isotope variability in nature;
2. Study of Fe isotope fractionation processes in laboratory model systems; and
3. Theoretical modeling of the magnitude of Fe isotope fractionation.

The results of this research are summarized below and in Figure 1. In this discussion, variations in the isotopic composition of Fe are presented using the "δ" notation:

$$\delta^{56}Fe_{sample} = [(^{56}Fe/^{54}Fe)_{sample}/(^{56}Fe/^{54}Fe)_{standard} - 1] \times 1000‰.$$

Natural Variations

Variations in the isotopic composition of Fe have been determined in a range of terrestrial and lunar igneous rocks, iron meteorites, and loesses, as well as in ancient and recent sediments.[154,155] *These measurements provide unequivocal evidence that the isotopic composition of Fe varies in nature.*

Beard and Johnson first observed that $\delta^{56}Fe$ in marine sediments is more variable than in igneous rocks.[156] Fe isolated from Pacific and Atlantic ferromanganese nodules is shifted by ~ –1.5‰ compared to the range for

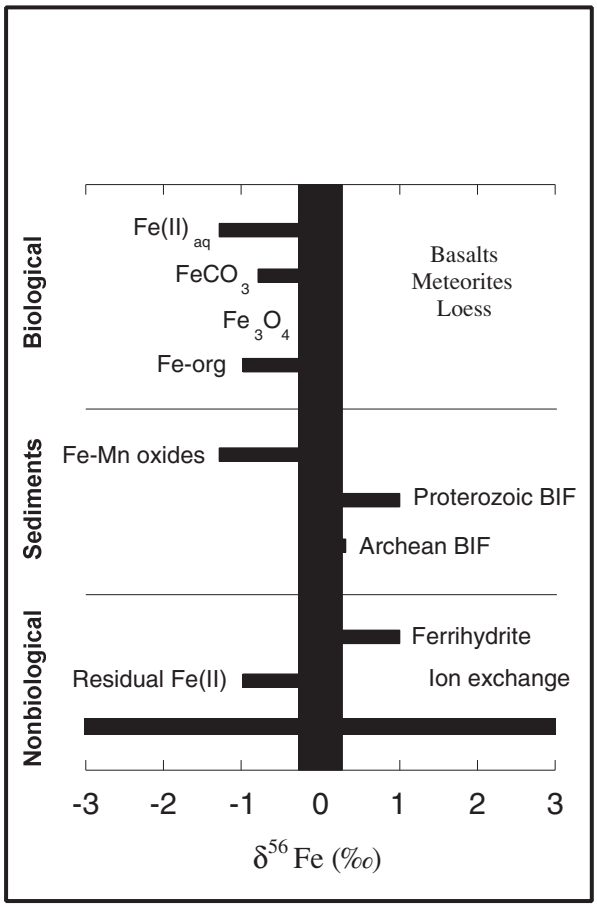


FIGURE 1. Summary of all δ^{56}Fe data in refereed and unrefereed literature as of April 2000. See text for details and sources.

igneous rocks, while Fe isolated from the light-colored, Fe-poor layer of a Proterozoic BIF is shifted by ~ +1‰ compared to the darker, Fe-rich layers. By comparison, Fe in igneous rocks varies over a range of $< \pm 0.3$‰. This contrast strongly suggests that Fe isotopes are fractionated during (bio)geochemical processing of Fe in the environment. Because microbial activity is believed to play an important role in such processing, this observation is at least consistent with the existence of biogenic fractionation effects.

Zhu et al. carried out a detailed examination of the isotopic composition of Fe in a well-characterized Atlantic ferromanganese crust.[157] They found that δ^{56}Fe varies over a range of ~1‰, with an average value of ~ –1‰ compared to a laboratory Fe standard, iron meteorites, and loess (note that Zhu et al. report their data as $\varepsilon^{57}Fe_{sample} = [(^{57}Fe/^{54}Fe)_{sample}/(^{57}Fe/^{54}Fe)_{standard} - 1] \times 10^4$. These values have been translated to δ^{56}Fe for comparison here. Assuming mass-dependent fractionation of Fe isotopes, $\delta^{56}Fe \sim 0.067 \times \varepsilon^{57}Fe$).

Direct comparison of these two studies is slightly problematic because different standard materials were used, and there has been, as yet, no interlaboratory calibration. However, the total range of values reported by Zhu et al. for iron meteorites, loesses, and paleosols is only ~ 0.3‰.[158] This is similar to the range for igneous rocks reported by Beard and Johnson.[159] Hence, both data sets reveal that the greatest variability of δ^{56}Fe is in ferromanganese sediments and that δ^{56}Fe in recent marine sediments is shifted by ~ –1‰ compared to all other Fe (with the exception of Proterozoic BIF).

The isotopic composition of Pb ($^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$) in the ferromanganese sediments is also reported by Zhu et al.[160] The Pb isotopic system differs from the Fe system in that the ^{206}Pb and ^{208}Pb are the products of

U radioactive decay. Nevertheless, correlated, secular trends are observed in $\delta^{56}Fe$ and the Pb ratios. Because mass-dependent fractionation of Pb isotopes is expected to be minuscule, this correlation argues against in situ Fe isotope fractionation in the sediments. Instead, the secular variations in $\delta^{56}Fe$ apparently reflect correlated changes in the sources of Fe and Pb to the sediments. However, it must be stressed that this conclusion in no way invalidates the observation that Fe in these sediments is isotopically fractionated from other Fe and provides no direct information about fractionation processes.

Laboratory Studies—Biological

Substantial laboratory effort has been devoted to biological fractionation processes. The most extensive published biological studies, performed by Beard et al.,[161] focused on Fe isotope fractionation during microbially mediated reduction of ferrihydrite (an Fe(III) oxyhydroxide precipitate) by *Shewanella algae. S. algae* is a mesophilic, dissimilatory Fe-reducing organism that uses Fe(III) as an electron acceptor during respiration. Hence, this organism is a good candidate for Fe isotope fractionation research.

In a series of experiments conducted under carefully controlled conditions, it was clearly demonstrated that the initial $\delta^{56}Fe$ of the dissolved Fe(II) produced by *S. algae* is shifted ~ –1.3‰ compared to the initial isotopic composition of the ferrihydrite substrate. *These data provide evidence that Fe isotopes are fractionated during microbially mediated reduction of ferrihydrite.*

The fractionation mechanism is not yet clear. However, the observation that the Fe(II) product is isotopically lighter than the ferrihydrite reactant strongly suggests preferential uptake and/or reduction of ^{54}Fe versus ^{56}Fe, consistent with a kinetic isotope effect. Such a kinetic effect could occur during extraction of Fe(III) from the ferrihydrite or during enzyme-catalyzed reduction.

Consistent results have been reported for other Fe-reducing bacteria grown on ferrihydrite substrate.[162] Brantley et al. have presented evidence that Fe leached from the mineral hornblende in the presence of organic ligands (including siderophores) is fractionated by ~ –1‰ compared to the mineral-bound Fe.[163] This may provide insight into the mechanism of biogenic fractionation, suggesting that fractionation occurs during uptake of Fe rather than during reduction.

In contrast to these studies, Mandernack et al. found no evidence of fractionation of similar magnitude in intracellular magnetite produced by magnetotactic bacteria.[164] It is not yet known whether this reflects basic differences in Fe metabolism or the sensitivity of fractionation to growth conditions. Regardless, this finding reveals that biogenic Fe is not *necessarily* fractionated with respect to Fe sources.

Laboratory Studies—Nonbiological

Fractionation of Fe isotopes by chemical processes in the absence of biology was demonstrated by Anbar et al.[165] during elution of Fe in HCl media from small (~4 cm) columns packed with anion-exchange resin. The range of values observed in the elution fractions (~6‰) was larger than seen in biological experiments to date. Integrated over all fractions (which accounted for 100 percent of the loaded Fe), $\delta^{56}Fe = 0$, as expected from mass balance. *This study provides unequivocal evidence that Fe isotopes can be fractionated by nonbiological chemical processes.*

It was proposed that this fractionation results from equilibrium isotope exchange between dissolved $FeCl_4^-$, which binds to the resin, and cationic and neutral Fe-Cl complexes ($FeCl_3$ and $FeCl_2^+$). The former is a tetrahedral complex, while the latter are octahedral, providing a difference in bonding environment that could drive such fractionation. This is not a unique interpretation; it is possible that kinetic effects could also explain the data.

Because this particular ion exchange system is not representative of chemical processes in the environment, experiments with other nonbiological systems are urgently needed. As yet, there are no such studies in the peer-reviewed literature.

Bullen et al. have presented data from controlled experiments in which dissolved Fe(II) is slowly oxidized by addition of O_2 at constant pH and precipitated as ferrihydrite.[166] In these experiments, $\delta^{56}Fe$ of the initial ferrihydrite was fractionated ~1‰ compared to the dissolved Fe. As expected from mass balance, as the fraction

of Fe precipitated approaches unity, δ^{56}Fe of the accumulated precipitate approaches 0‰, while the residual dissolved Fe approaches ~ –1‰.

Theoretical Studies

The magnitude of equilibrium fractionation between simple complexes can be predicted from experimentally determined vibrational frequencies. Such data are not always available for transition-metal compounds of interest in biomarker applications (e.g., common inorganic and organic complexes), and application to minerals is less straightforward. However, Schauble et al.[167] have predicted equilibrium fractionation of 1 to 10‰ between dissolved Fe complexes. Using a novel approach based on Mossbauer data, Polyakov and Mineev predict equilibrium fractionation of 1 to 10‰ between common Fe-bearing minerals.[168] *However, these predictions have not yet been tested against measurements.*

As yet there have been no theoretical studies of kinetic effects applied to Fe isotopes.

Synthesis

The evidence accumulated to date (Figure 1) demonstrates that the isotopic composition of Fe in ancient and modern Fe-rich sediments varies over a range of ~2.5‰. This variation is nearly 10 times larger than that of all other natural materials analyzed. Laboratory studies indicate that fractionations of per-mil magnitude can be produced by dissimilatory Fe-reducing bacteria and by leaching of Fe from minerals by biogenic ligands.

These observations demonstrate the potential of Fe isotope measurements for the detection of ancient biological activity. As an example, Beard et al. suggest that the value δ^{56}Fe ~ +1‰ in the light-colored, Fe-poor layer of a Proterozoic BIF reflects biological conversion of insoluble Fe(III) oxyhydroxides to soluble Fe(II) by Fe-reducing bacteria.[169] Such a process would be expected to preferentially remove ^{54}Fe, producing a residue with δ^{56}Fe > 0.

While such applications of the Fe isotope system are credible, they should be approached with some caution. First, as pointed out by Zhu et al.,[170] fractionation in sediments does not necessarily reflect in situ processes. This is an important caveat that may invalidate some interpretations of sedimentary δ^{56}Fe variations. However, it must be recognized that this point has no direct bearing on whether natural δ^{56}Fe variations are of biological or nonbiological origin. For example, given the results of Brantley et al.,[171] it is easy to envision biogenic Fe isotope fractionation during chemical weathering or during dissolution of aeolian material in seawater, which would deliver already-fractionated Fe to the sediments.

Second, and more importantly, it has not yet been demonstrated that nonbiological processes are incapable of producing comparable fractionations in nature. Clearly, chemical fractionation of Fe isotopes in the absence of biology is possible; the entire range of observed natural variations has been readily produced in a contrived laboratory system.[172] The preliminary data of Bullen et al. suggest that nonbiological processes could be particularly important in ferromanganese sediments.[173] However, many more such studies, covering a wider range of phases and reactions, are obviously needed.

The ultimate impact of such studies on biomarker applications is unclear. However, regardless of the outcome, it is very likely that the Fe isotope system will prove to be useful in biogeochemical research. Similar debates animated the development of both the C and the S isotope systems. In the case of C isotopes, it is now widely accepted that nonbiological processes typically occurring at Earth's surface cannot easily reproduce the magnitude of fractionation produced by photosynthesis. Hence, C isotopes are readily employed for detection of ancient life. On the other hand, in the case of S isotopes, such a simple distinction between biological and nonbiological fractionation magnitudes is not possible. Nevertheless, S isotope studies have been profoundly useful in biogeochemical research, in both modern and ancient environments. Research into Fe and other metal isotope systems is likely to be at least as useful as S isotope research in the study of ancient environments and life, and may yet prove as useful as C.

Acknowledgments

The author thanks S. Brantley and T. Bullen for providing prepublication data, C. Johnson for stimulating discussions, and the National Science Foundation-LExEn (CHE 9714282) and the NASA Astrobiology Institute for financial support.

DEVELOPMENT OF IN SITU MEASUREMENT TECHNIQUES FOR DETECTING THE CHEMICAL SIGNATURES OF LIFE

Wesley T. Huntress, Jr.
Geophysical Laboratory
Carnegie Institution of Washington

Abstract

A "Grand Challenge" Research Program has been instituted at Caltech's Jet Propulsion Laboratory (JPL) to develop in situ measurement techniques that are capable of detecting the chemical signatures of life. The JPL-Caltech concept of a Grand Challenge, the events leading to adoption of the current theme, and the participating investigators and their research goals are described.

A Short History of JPL Grand Challenges

A Grand Challenge is a Caltech-JPL institutional investment in a long-term research and development program to enable exciting new missions in space science and exploration. They are generally intended to develop the science and technologies that will enable accomplishments in a new realm of exploration well beyond the current state of the art. Grand Challenges set long-term interdisciplinary research goals, combining science and technology objectives, to try and accomplish something never done before. They should be doable in 10 to 15 years, progress must be measurable, and it must be clear when the program is finished. Grand Challenges are long-term Caltech investments in major technological innovations that will lead to the establishment of a new NASA program with a major impact on the future of space exploration. These new NASA programs would replace or extend current investments in the JPL mission in space.

The Grand Challenge concept is also a means to provide stable funding at the investigator level independent of the annual and often large fluctuations in NASA R&D funding commitments. NASA R&D funding in any one field and at any one center is subject to fierce competition among a large number of constituencies including other NASA centers, universities, NASA Headquarters, the Administration, and Congress—which leads to a long history of large fluctuations that seriously hinder progress toward any particular long-term goal.

There have been two previous JPL Grand Challenges. The first was "Detection of Planets Around Stars" (1984 to 1996). This program was triggered by an image of the dust disk around β Pictoris taken in 1981. The program tackled this difficult problem by investing in the development of near-infrared optical interferometery. A 100-meter interferometer using 1-meter optics was first built at Palomar to develop the optical component technology, followed by investment to scale the technology up to the Keck twin 10-meter telescopes. These investments have enabled the future Space Interferometer Mission (SIM), which has been funded by NASA, and a larger Terrestrial Planet Finder mission scheduled after SIM. The research conducted in this Grand Challenge was key to enabling the NASA Origins Program, and the JPL program was terminated with NASA establishment of the Origins Program.

The second JPL Grand Challenge to be established was "In-Situ Planetary Exploration and Robotics" (1990). This was triggered after every planet in the solar system (except Pluto) had been visited by at least one spacecraft. The event was the Voyager flyby of Neptune at the edge of the solar system in 1989. At that time, there also were three orbiters in development—Magellan to Venus, Galileo to Jupiter, and Cassini to Saturn—so that by 2005, orbiters would have explored the planets from Venus to Saturn. The next stage after flybys and orbiters was exploration of planetary surfaces, so this Grand Challenge was instituted to develop the technologies and science instruments for surface exploration. This Grand Challenge enabled the Sojourner rover for Mars Pathfinder and is still under way in JPL's Center for In-Situ Instrumentation.

Selection and Management of the New JPL Grand Challenge

Three potential topics for the third Grand Challenge emerged from an extensive consensus-building process at JPL and the Caltech campus:

1. *In situ detection of extraterrestrial life.* This topic was triggered by discoveries in biology, planetary exploration, and astronomy that have led to the impression that life might be more commonplace in the universe than previously expected. The topic is also perfectly complementary to the ongoing Grand Challenge in in situ planetary exploration and robotics.
2. *Interstellar travel and exploration.* This topic was triggered by discoveries of extrasolar planets and the prospect for learning more about them. It suffered a real disadvantage because of the immense technological challenge posed to propulsion and spacecraft reliability.
3. *Robotic colonies in space*. This topic is also synergistic with the current new phase of planetary surface exploration. It envisioned a new paradigm for robotic planetary exploration, carried out by a semi-intelligent, self-tasking, and self-servicing robotic infrastructure on a planetary surface operating only on high-order goals from humans on Earth. These robotic colonies were seen as a preface to eventual human arrival and the establishment of a joint human-robotic outpost.

After selection of topic 1 by the JPL director in January 1999, an external expert review was called to assess the prospects for this challenge, and recommendations were forwarded to JPL's director in April of 1999. Caltech and JPL established an institutional funding line at about $1 million per year, with a funding schedule in two phases. The first 18-month phase was for concept development, followed by a second phase of about 4 years for development of a consensus measurement strategy and instruments. Phase I investigations were solicited in May from the Caltech-JPL community and reviewed by an External Review Panel composed of the first four members of what became the External Review Board:

Wesley Huntress (chair)	Carnegie Institution of Washington
James Ferris	Rensselaer Polytechnic Institute
Harold Morowitz	George Mason University
Steven Squyres	Cornell University
Kathy Olsen	NASA Headquarters, Chief Scientist
William Schopf	University of California, Los Angeles
Norman Pace	University of Colorado

Recommendations for selection and funding were made to JPL's chief scientist and director in September. It is expected that after results are examined from the first phase, the second phase will call for new proposals and could result in program restructuring. Funding was received by the investigators in November 1999.

The Phase I solicitation required that the principal investigator be from JPL, but also required teaming with outside investigators at other academic institutions and strongly encouraged Caltech campus participation. The solicitation also asked that each team have the capability to carry out research on three objectives:

1. *Identifying the chemical signatures of life.* Define the most likely non-Earth-centric signatures; universal properties for living systems, not dependent on specific molecules or peculiar properties of life on Earth. Define a strategy for employing them in a systematic search for extraterrestrial life.
2. *Developing measurement techniques to detect these signatures.* Develop measurement techniques and statistical strategies for identifying these signatures in the background of nonlife. Test these methods on Earth samples with prepared controls.
3. *Conceptualizing the possibilities for miniaturizing in situ instrumentation.* Devise concepts for how the identified measurement techniques can be implemented in-situ on a remote planetary surface. This task should lead to proposals for the development of miniaturized in situ instruments.

The response, however, did not meet this objective. The proposals received generally fell into four categories:

- *Category I*—addressed all three goals: proposed to define chemical signatures, identify required measurements, and conceptualize potential miniaturization schemes;
- *Category II*—adopted a particular chemical property or process as a signature of life and proposed a series of predefined measurements to identify the property or process;
- *Category III*—submitted by instrument scientists offering their technique as a de facto signature and proposing to develop their instrument for measuring in situ samples; and
- *Category IV*—unresponsive.

There was only one proposal in Category I; there were three in Category II, twelve in Category III, and four in Category IV. This distribution should have been expected from a science community at JPL heavy in instrument scientists. The selected proposals are the following:

- *Category I: Signature Definition Studies*
  - Conrad — Biological Signature Definition
- *Category II: Specific Signatures with Defined Multiple Measurements*
  - Beauchamp — Chirality—mass spectroscopy/chromatography/electrophoresis/chirooptical
  - Mojarradi — Metabolism—e-transport/porphyrins/heat production/DNA
  - Grunthaner — Diagenesis—XPS/mass spectroscopy/microfluidics
- *Category III: Instruments Proposed as Individual Signature Detectors*
  - Webster — Laser spectroscopy (and mass spectroscopy) isotope ratios
  - Kanik — Ion mobility spectrometer
  - Bearman — Chemical imaging
  - Sinha — Fe isotope mass spectroscopy
  - George — X-ray fluorescence.

JPL has uncharacteristically chosen a nonresident scientist to manage this Grand Challenge Program, Wesley Huntress of the Geophysical Laboratory, Carnegie Institution of Washington; the local project scientist is Kenneth Nealson of the Jet Propulsion Laboratory. The role of the project manager is to bring the Grand Challenge Research Program to a successful conclusion, where success is defined as a credible, consensus in situ measurement strategy for the detection of life. He will also conduct external scientific and technical reviews during Phase I on 6-month centers. The role of the project scientist is to provide on-site scientific guidance, to ensure progress toward the common goal for in situ detection of life, and to instill a sense of common scientific purpose among the investigators.

This new research program has only just begun and is not yet 6 months old. There are no firm results yet to report. The work done so far does show that even should the ultimate goal prove elusive, there will be a great deal of excellent science accomplished. Some confidence that the program will reach its goal is found in the strategy this program has adopted for life detection. The approach is to look for structures in samples that appear to be nonrepresentative of the common—particularly structures that seem out of equilibrium with the remainder of the sample. One methodology is to look for these structures through elemental analysis, which has no preconceived notion of the particular structure or composition used by life other than that the residue of life must have a distinguishable structure and a chemistry out of equilibrium with the environment. This methodology, applied to terrestrial samples, would seek out structures in rock samples with enrichment of carbon, nitrogen, sulfur, and phosphorus; depletion of aluminum, silicon, iron, and other metals; and association of C, N, O, S, and P both amongst themselves and with Fe, Ni, Co, and other enzymatic metals.

MINIATURIZED IN SITU INSTRUMENTS FOR AMINO ACID DETECTION ON SOLAR SYSTEM BODIES

Jeffrey L. Bada
Scripps Institution of Oceanography
University of California, San Diego

Abstract

The Mars Organic Detector (MOD) is a simple, compact instrument designed for the sensitive in situ detection of organic compounds derived from either prebiotic organic chemistry or extinct/extant organisms. MOD, which was recently selected for the NASA HEDS (Human Exploration and Development of Space) 2005 Mars Explorer Program mission, uses sublimation-based extraction coupled with fluorescence detection to carry out highly sensitive analyses for amino acids or amines and polycyclic aromatic hydrocarbons (PAHs) directly on the martian surface. MOD also has a tunable diode-laser spectrometer that measures the amount of evolved water and carbon dioxide in order to better understand the mineralogy associated with any detected organic compounds, as well as provide a better evaluation of the inventory of water and carbonates in the martian regolith. The next generation of MOD will include a microfabricated capillary electrophoresis system capable of determining the enantiomeric composition (*D/L* ratios) of any detected amino acids in order to ascertain whether they were of biotic or abiotic origin. MOD-type instruments could also be used to carry out similar in situ analyses on other solar system bodies.

Introduction

As we begin this new millennium, one of the major scientific questions that confronts humanity is whether life exists beyond Earth. There are reasons for optimism. Many of the organic compounds thought to be necessary for the origin of life appear to be widespread throughout the universe. Extrasolar planets seem to be omnipresent companions of main sequence stars. If the conditions that resulted in the origin of life on Earth are common on these extrasolar worlds, then it is reasonable to assume that life could be prevalent in the universe. Within our own solar system, whether life originated on Mars, and perhaps even still exists there today, is an area of intense debate. Although we have sent spacecraft to Mars to search for life and analyzed martian meteorites for possible evidence of life, these efforts have left us with no definitive answers. With increasing evidence of a large subsurface water ocean on the jovian moon Europa, there is the possibility that this body could have a primordial soup similar to that which gave rise to the origin of life on Earth or could potentially even harbor living organisms.

Because of technological limitations, the direct search for extraterrestrial life is presently (and will be for a long time) confined to bodies in our solar system, mainly Mars and Europa. During the next decade the search for evidence of extinct or extant life on Mars will be a central focus of both NASA and the European Space Agency (ESA) as a flotilla of spacecraft explore the martian surface and return martian samples back to Earth for comprehensive state-of-the-art analyses. The challenges are daunting. Missions to Mars are costly and risky, as evidenced by the recent losses of the Mars Climate Orbiter and Mars Polar Lander spacecraft. Collecting and returning samples to Earth, while appealing because of the direct hands-on analytical advantages they provide, are constrained by the amount of material than can be returned and containment issues related to potential biological hazards associated with possible extant martian organisms being transported back to Earth. Spacecraft-based robotic instruments designed to carry out direct analyses for evidence of prebiotic chemistry or life are constrained by mass and power limitations, as well as challenges associated with obtaining samples suitable for analysis.

A major potential impediment to the exploration of Mars for traces of life is the forward contamination of the planet with either terrestrial organisms or biomolecules. This problem makes it essential that organic analyses be carried out as early as possible in the Mars exploration program in order to provide a useful baseline data set for comparison with future analyses.

Searching for Organic Compounds on Mars

In searching for evidence of extinct or extant life on Mars and elsewhere, the detection of organic compounds is considered to be of fundamental importance. Carbon-based polymers such as nucleic acids and proteins make up the core molecules required to carry out the central biological functions of replication and catalysis. Without these functions, life as we know it could not exist.

One of the primary objectives of the 1976 Viking missions was to determine whether organic compounds were present in martian surface soils. Using a pyrolysis procedure, in combination with a highly sensitive gas chromatograph-mass spectrometer (GC-MS), Viking did not detect any organic compounds above a level of a few parts per billion in surface samples at two different landing sites.[174] Despite the negative results, it is nevertheless possible that organic compounds, or even living organisms,[175] are present on the martian surface at levels below the detection limit of the Viking GC-MS. For example, it is now apparent that the Viking pyrolysis GC-MS instruments would not have detected the presence of nearly a million bacterial cells in 1 gram of soil.[176] In addition, oxidation reactions involving organic compounds, whether they are biotic or abiotic in origin, on the martian surface would likely produce nonvolatile products that would also not have been detected by the Viking GC-MS.[177]

Meteorites from Mars have also been investigated to assess whether they contain organic compounds possibly derived from life. Unfortunately, contamination of martian meteorites by terrestrial-derived organic compounds greatly compromise these investigations.[178] This contamination problem underscores the importance of doing in situ organic compound analyses on Mars before samples are returned to Earth, where even under the best of circumstances they will be exposed to some level of terrestrial contamination.

Because amino acids are the building blocks of proteins in terrestrial organisms, they are excellent target compounds in the search for life elsewhere. In addition, over 70 different amino acids have been identified in carbonaceous meteorites,[179] which have almost certainly been delivered to Mars throughout its history.[180] While it is not certain that martian biology would use the exact same set of amino acids as life on Earth, their ubiquity as constituents of organic material in the solar system suggests that amino acids would have been available for incorporation into living entities on Mars just as they were on Earth. Amino acids derived from either extinct or extant life, and from the infall of meteorites and cosmic dust, could be present on the surface of Mars.

Another class of organic compounds of interest are polycyclic aromatic hydrocarbons. Although PAHs have no known role in biochemistry on Earth, they can be produced from the long-term (tens of millions of years or more) decomposition of biologically derived organic compounds.[181] PAHs have been identified in the interstellar medium[182] and in carbonaceous meteorites.[183] They may be the most abundant single class of organic compounds in the universe, perhaps making up as much as 10 to 20 percent of the total carbon.[184] Given the infall of meteorites and cosmic dust throughout the history of Mars, PAHs would be expected to be one of the organic components of the martian surface, especially if samples contained fragments of carbonaceous chondrites.

The MOD Concept

To evaluate whether organic compounds are present on Mars, in-situ-based analytical techniques with sensitivities several orders of magnitude greater than those of the Viking GC-MS are required. In addition, given the limitations of available resources (especially cost), instruments must be compact and able to detect the targeted compounds without requiring extensive sample processing.

MOD is an in situ instrument that has been developed to search for traces of the key organic compounds, amino acids or amines and PAHs, directly on the martian surface. MOD is based on the following concepts:

- Amino acids and PAHs can be directly sublimed from natural samples by heating to 450°C under partial vacuum, thus eliminating the need for the aqueous and organic solvents used in laboratory analyses; and
- The sublimed amino acids are condensed on a cold finger coated with a reagent specific for amino acids and detected at very high sensitivities using ultraviolet fluorescence. Sublimed PAHs can be directly detected on the cold finger because they are naturally fluorescent when exposed to ultraviolet light.

Laboratory experiments have demonstrated the feasibility of these concepts. A mixture of dry amino acids, or a crushed sample of a fossil mollusk shell, was placed into a sublimation apparatus that was then evacuated to 5 to 6 torr to approximate martian atmospheric pressure.[185] A cold finger was cooled to –195°C with liquid nitrogen, and the apparatus was heated to 450°C for various time periods ranging from 30 seconds to several minutes. The material that sublimed onto the cold finger was then analyzed. No decomposition into amines, which are produced by amino acid decarboxylation, was observed with the pure amino acid mixtures. The behavior of amino acids in a fossil mollusk shell during sublimation was found to be more complex than pure amino acid mixtures. Virtually all of the amino acids were decomposed into amines. However, the amines can be readily detected with the same methodologies used for amino acid detection. Even when amino acid decomposition to amines is significant, the presence of amino acids in the original sample can still be inferred.

Primary amines were found to react with the reagent fluorescamine coated on a cold-finger surface to yield intensely fluorescent derivatives. Experiments indicate that the fluorescamine reaction proceeds in the dry state; no solvent is necessary. Amino acid or amine detection limits with this method are in the 10^{-13}–10^{-14} mole range. Thus, even if amino acids were present in a martian sample at a level of a few parts per trillion, they would be detectable by the fluorescamine-based method.

PAHs also readily sublime under the same conditions used for amino acids. The detection of sublimed PAHs can be carried out directly on the cold finger without the need for derivatization reagents because these compounds are extremely fluorescent when irradiated with near-ultraviolet light. Detection limits with this approach are in the subfemtomole (10^{-15} mole) range.

The 2005 MOD Instrument

Because of its advanced development and ability to address key scientific issues, MOD was recently selected to be part of the HEDS lander instrument package for the 2005 Mars Explorer Program mission. The 2005 version of MOD carries out two simultaneous complementary experiments: (1) simple sublimation-based extraction, fluorescence detection, and quantitation of amino acids, amines, and PAHs at subpicogram ($<10^{-12}$ gram) levels (i.e., ~1000 times more sensitive than Viking) and (2) the detection and quantitation of evolved water and carbon dioxide using a tunable diode laser (TDL) in order to assess the water and carbonate inventory of the martian surface.

The MOD instrument consists of the following components (Figure 1):

- A rock crusher that pulverizes samples;
- The organic detector, consisting of a sublimation cell, a chemical detector, and a fluorescence analyzer; and
- The TDL spectrometer (not shown in Figure 1), which consists of a Herriott cell, a dual laser system, and a miniature capacitance manometer and Pirani gauge to measure absolute pressure.

MOD operates by the stepwise heating of a crushed sample at martian ambient pressure to a temperature of 950°C. During the heating process, the target organic compounds will sublime from the sample. At the same time, bound water, along with carbon dioxide from the decomposition of various carbonate minerals, will be released. The sublimed material is collected on a cold finger for analysis. The detector plate on the cold finger is divided into two zones: one zone is coated with fluorescamine for amino acid detection; the other zone is uncoated and is used to directly detect PAHs, which do not require a reagent in order to produce an intense ultraviolet fluorescent signal. The quantities of water and carbon dioxide, and their isotopic compositions, evolved during the step-heating cycle are determined using the TDL spectrometer. Even if no organic compounds are detected, the TDL spectrometer provides information on the amounts of bound water and carbonate minerals contained in the Mars regolith. With minor modifications, the TDL spectrometer can also be used by measure the water and carbon dioxide content, and isotopic composition, of the martian atmosphere.

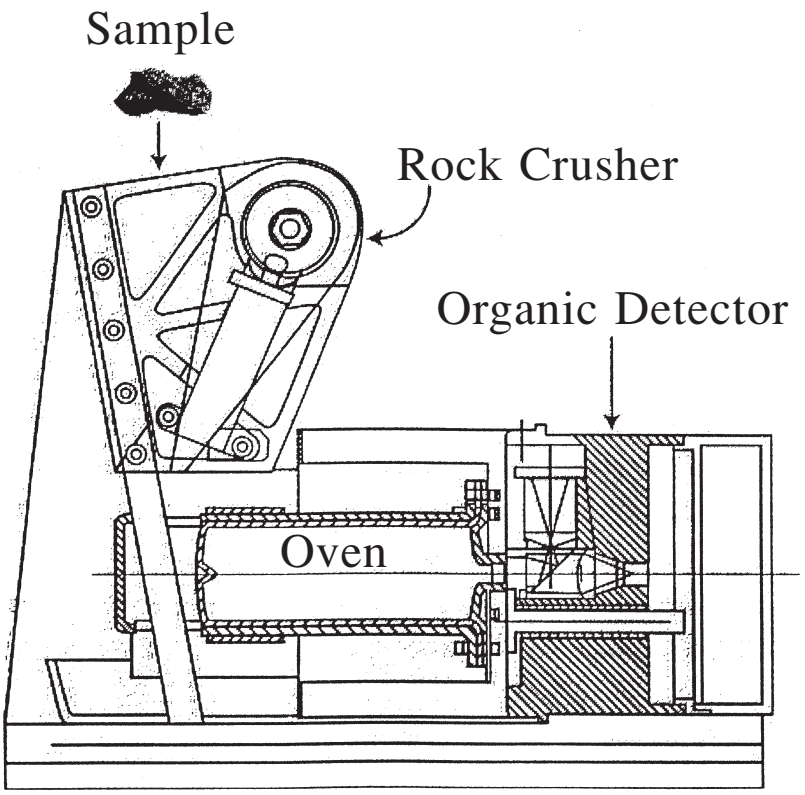


FIGURE 1. The MOD 2005 instrument. A sample collected by a drill or a scoop is dropped into the rock crusher, which pulverizes the sample and then drops it into the oven. After closing the oven at Mars ambient pressure, the crushed sample will be heated stepwise to 950°C. Amino acids and PAHs in the sample will be sublimed and collected on the sample wheel (right end of the oven), which is cooled to Mars nighttime temperatures (around –100°C). The condensed compounds are detected by fluorescence using laser-based sensors. A TDL spectrometer (not shown), which is on the far side of this view, measures the released amounts and isotopic composition of water as well as carbon dioxide produced from the decomposition of various carbonate minerals. The entire instrument weighs ~2 kg and fits in the palm of your hand.

MOD—The Next Generation

A central problem in amino acid analyses of martian samples is not only identifying and quantifying which compounds are present, but also distinguishing those produced abiotically from those synthesized by extinct or extant life.[186] Amino acid homochirality (enantiopurity) provides an unambiguous way of distinguishing between abiotic and biotic origins. Proteins made up of both *D*- and *L*-amino acids would not likely have been efficient catalysts in early organisms because they could not fold into bioactive configurations such as the α-helix. However, enzymes made up of all *D*-amino acids function just as well as those made up of only *L*-amino acids, but the two enzymes use the opposite stereoisomeric substrates. There are no biochemical reasons why *L*-amino acids would be favored over *D*-amino acids. On Earth, the use of only *L*-amino acids by life is probably simply a matter of chance. We assume that if proteins and enzymes were a component of extinct or extant life on Mars, then amino acid homochirality would have been a requirement. However, the possibility that martian life was (or is) based on *D*-amino acids would be equal to that based on *L*-amino acids.

The detection of a nonracemic mixture of amino acids in a martian sample would be strong evidence for the presence of an extinct or extant biota on Mars. The finding of an excess of *D*-amino acids would provide irrefutable evidence of unique martian life that could not have been derived from seeding the planet with terrestrial life (or the seeding of Earth with martian life). In contrast, the presence of racemic amino acids, along with abiotic amino acids such as α-aminoisobutyric acid, could be indicative of an abiotic origin or, alternatively, the racemization of biotically produced amino acids.[187]

A potential impediment to the search for life on Mars is the forward contamination of the planet with either terrestrial organisms or, more likely, terrestrial biomolecules. This problem would be of great importance in assessments of whether there are any amino acids indigenous to Mars. Because of the distinctive *L*-enantiomer signature of amino acids associated with terrestrial life, chiral amino acid analyses can be used to monitor the level of forward contamination of Mars that occurs during the course of planetary exploration. This requires that amino acid analysis data be acquired as early as possible in the Mars exploration program in order to provide a useful baseline data set for comparison with future analyses. A long-range monitoring program would be critical in assessing forward contamination during the eventual human exploration of Mars.

A relatively new technology that shows promise for spacecraft-based amino acid enantiomeric analysis is microchip-based capillary electrophoresis (μCE). With μCE, both the identity and the enantiomeric composition of amino acids can be determined at sub-part-per-billion levels. The μCE-based analyses are about an order of magnitude faster than analytical methods such as conventional capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Such short analysis times are an inherent advantage for robotic in situ measurements carried out from a spacecraft. In addition, μCE has a detection limit more than three orders of magnitude better than conventional HPLC. Thus, proportionally smaller samples (~100 pl or 10^{-10} l) can be analyzed, another important advantage for in situ spacecraft-based instruments.

A μCE chip system has been used to explore the feasibility of using such devices to analyze for amino acid enantiomers in extraterrestrial samples.[188] The test system consisted of a folded electrophoresis channel (19.0 cm long × 150 μm wide × 20 μm deep) that was photolithographically fabricated in a 10-cm-diameter glass wafer sandwich, coupled to a laser-excited confocal fluorescence detection apparatus providing subattomole ($<10^{-18}$ mole) sensitivity. The μCE analysis system consists of a stack of wafer-scale components that individually provide the liquid flow channels, the capillary separation zones, the electrophoretic controls, the fluid logic, and the detection system. This μCE system is more than an order of magnitude smaller in size than conventional laboratory bench top amino acid analytical instruments.

A critical aspect is that enantiomeric ratios can be rapidly and accurately determined using the microfabricated μCE chip instrument. Using a sodium dodecyl sulfate/γ-cyclodextrin pH 10.0 carbonate electrophoresis buffer and a separation voltage of 550 V/cm at 10°C, baseline resolution is observed for the enantiomers of valine, alanine, glutamic acid, and aspartic acid in only 4 minutes (Figure 2). Enantiomeric ratios of amino acids extracted from the Murchison meteorite using this μCE chip system closely matched values determined by HPLC.

The reduced time, resources, and sample requirements for microfabricated μCE translate into a significant reduction in mass, power, and volume. With an estimated mass of ~1 kg, a volume of ~1000 cm^3, and a power requirement of ~2 W, the μCE chip system provides a compact, low-mass instrument suitable for a wide variety of in situ exobiology applications. For spacecraft-based analyses, a microfluidics-based sample processing system will be needed in order to deliver an amino acid extract suitable for analysis by a μCE system. The design of such a system is presently under way.

Conclusions

This discussion has focused on amino acid detection systems tailored for missions to Mars. However, other solar system bodies, such as Europa, Saturn's moon Titan, asteroids, and comets, likely hold information about natural abiotic synthetic processes and the conditions necessary for synthesis of the organic compounds needed for the origin of life. In the case of Europa, compounds derived from living entities could possibly be present. In situ analyses carried out on these solar system bodies could thus potentially provide information about the suite of organic compounds that may have been present on prebiotic Earth and how the organic compounds used by

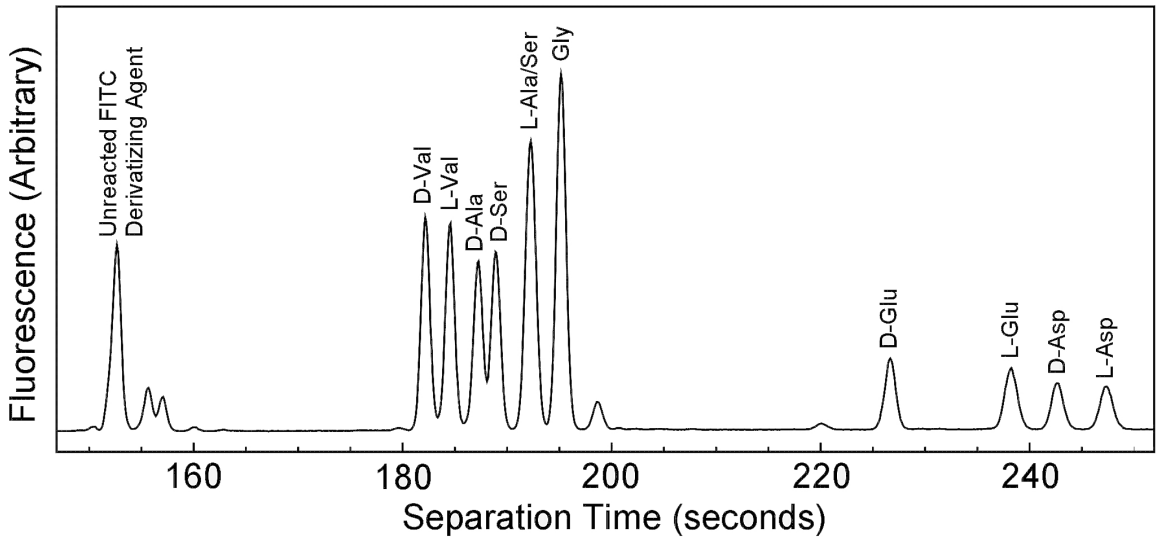


FIGURE 2. Baseline resolution of several amino acid enantiomers using the μCE chip system. SOURCE: L.D. Hutt, D.P. Glavin, J.L. Bada, and R.A. Mathies, "Microfabricated Capillary Electrophoresis Amino Acid Chirality Analyzer for Extraterrestrial Exploration," *Anal. Chem.* 71:4000-4006, 1999.

extraterrestrial life compare with those used by terrestrial organisms. The MOD instrument concept described here for investigations on Mars could easily be tailored for use on other bodies of interest in the solar system.

Acknowledgments

The development of MOD has been funded by the Planetary Instrument Definition and Development Program (PIDDP) and the Mars Instrument Development Program (MIDP) of NASA and the NASA Specialized Center of Research and Training in Exobiology at the Scripps Institution of Oceanography. The following members of the "MOD squad" contributed to this work: C. LaBaw, C. Mahoney, G. McDonald, O. Serviss, C.R. Webster, and F. Grunthaner (NASA-JPL); R. Mathies and L. Hutt (UC Berkeley); and G. Kminek, O. Botta, and D. Glavin (UCSD).

REFERENCES FOR PAPERS IN SESSION 4

1. J.W. Schopf, "Fossils and Pseudofossils: Lessons from the Hunt for Early Life on Earth," in *Size Limits of Very Small Microorganisms: Proceedings of a Workshop,* National Academy Press, Washington, D.C., 1999, pp. 88-93.

2. J.M. Schopf, "Modes of Fossil Preservation," *Review of Palaeobotany and Palynology* 20:27-53, 1975.

3. J.D. Farmer and D.J. Des Marais, "Exploring for a Record of Ancient Martian Life," *Journal of Geophysical Research* 104(E11):26977-26995, 1999.

4. Space Studies Board, National Research Council, *Size Limits of Very Small Microorganisms: Proceedings of a Workshop,* National Academy Press, Washington, D.C., 1999, p. 85.

5. See, for example, R.E. Summons and M.R. Walter, "Molecular Fossils and Microfossils of Prokaryotes and Protists from Proterozoic Sediments," *American Journal of Science* 290-A:212-244, 1990.

6. See, for example, M. Schidlowski, J.M. Hayes, and I.R. Kaplan, "Isotopic Inferences of Ancient Biochemistries: Carbon, Sulfur, Hydrogen, and Nitrogen," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983, pp. 147-186.

7. See, for example, T.J. Beveridge, M.N. Hughes, H. Lee, K.T. Leung, R.K. Poole, I. Savvaidis, S. Silver, and J.T. Trevors, "Metal-Microbe Interactions: Contemporary Approaches," *Advances in Microbial Physiology* 38:178-243, 1997.

8. See, for example, J.W. Schopf and M.R. Walter, "Archean Microfossils: New Evidence of Ancient Microbes," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983, pp. 214-239.

9. See, for example, R. Buick, "Microfossil Recognition in Archean Rocks: An Appraisal of Spheroids and Filaments from a 3500 M.Y. Old Chert-Barite Unit at North Pole, Western Australia," *Palaios* 5:441-459, 1990.

10. See, for example, J.W. Schopf, "Fossils and Pseudofossils: Lessons from the Hunt for Early Life on Earth," in *Size Limits of Very Small Microorganisms: Proceedings of a Workshop,* National Academy Press, Washington, D.C., 1999, pp. 88-93.

11. See, for example, J.W. Schopf and M.R. Walter, "Archean Microfossils: New Evidence of Ancient Microbes," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983, pp. 214-239.

12. See, for example, H.J. Hofmann and J.W. Schopf, "Early Proterozoic Microfossils," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983, pp. 321-360.

13. R. Buick, "Microfossil Recognition in Archean Rocks: An Appraisal of Spheroids and Filaments from a 3500 M.Y. Old Chert-barite Unit at North Pole, Western Australia," *Palaios* 5:441-459, 1990.

14. A.H. Knoll, "Exceptional Preservation of Photosynthetic Organisms in Silicified Carbonates and Silicified Peats," *Philosophical Transactions of the Royal Society of London* B 311:111-122, 1985.

15. See, for example, L.P. Knauth, "Petrogenesis of Chert," in *Silica: Physical Behavior, Geochemistry, and Materials Applications,* P.J. Heaney, C.T. Prewitt, and G.V. Gibbs (eds.), *Reviews in Mineralogy,* Volume 29, Mineralogical Society of America, Washington, D.C., 1994, pp. 233-258.

16. M.R. Walter and D.J. Des Marais, "Preservation of Biological Information in Thermal Spring Deposits: Developing a Strategy for the Search for a Fossil Record on Mars," *Icarus* 101:129-143, 1993.

17. S.L. Cady and J.D. Farmer, "Fossilization Processes in Siliceous Thermal Springs: Trends in Preservation Along Thermal Gradients," in *Evolution of Hydrothermal Ecosystems on Earth (and Mars?),* Ciba Foundation Symposium No. 202, G.R. Bock and J.A. Goode (eds.), John Wiley and Sons, Chichester, U.K., 1996, pp. 150-173.

18. J.D. Farmer, "Thermophiles, Early Biosphere Evolution, and the Origin of Life on Earth: Implications for the Exobiological Exploration of Mars," *Journal of Geophysical Research* 103:28457-28461, 1998.

19. See, for example, K.O. Stetter, "Hyperthermophiles in the History of Life," in *Evolution of Hydrothermal Ecosystems on Earth (and Mars?),* Ciba Foundation Symposium No. 202, G.R. Bock and J.A. Goode (eds.), John Wiley and Sons, Chichester, U.K., 1996, pp. 1-18.

20. See, for example, D.M. Ward, R. Weller, J. Shiea, R.W. Castenholtz, and Y. Cohen, "Hot Spring Microbial Mats: Anoxygenic and Oxygenic Mats of Possible Evolutionary Significance," in *Microbial Mats, Physiological Ecology of Benthic Microbial Communities,* Y. Cohen and E. Rosenberg (eds.), American Society for Microbiology, Washington, D.C., 1989, pp. 3-15.

21. See, for example, J.A. Baross and S.E. Hoffman, "Submarine Hydrothermal Vents and Associated Gradient Environments as Sites for the Origin and Evolution of Life," *Origins of Life* 15:327-345, 1985.

22. S.L. Cady and J.D. Farmer, "Fossilization Processes in Siliceous Thermal Springs: Trends in Preservation Along Thermal Gradients," in *Evolution of Hydrothermal Ecosystems on Earth (and Mars?),* Ciba Foundation Symposium No. 202, G.R. Bock and J.A. Goode (eds.), John Wiley and Sons, Chichester, U.K., 1996, pp. 150-173.

23. M.R. Walter and D.J. Des Marais, "Preservation of Biological Information in Thermal Spring Deposits: Developing a Strategy for the Search for a Fossil Record on Mars," *Icarus* 101:129-143, 1993.

24. S.L. Cady, M.R. Walter, D.J. DesMarais, and C.E. Blank, "Exopaleontological Search Strategy for Mars Exploration: A Case for Siliceous Epithermal Deposits," in *Lunar and Planetary Science Conference XXIII,* Lunar and Planetary Institute, Houston, Texas, 1997.

25. See, for example, R. Hesse, "Silica Diagenesis: Origin of Inorganic and Replacement Cherts," *Earth-Science Reviews* 26:253-284, 1989.

26. J.D. Farmer and D.J. Des Marais, "Exploring for a Record of Ancient Martian Life," *Journal of Geophysical Research* 104(E11):26977-26995, 1999.

27. D.S. McKay, E.K. Gibson, K.L. Thomas-Keprta, H. Vali, C. Romanek, S.J. Clemett, X.D.F. Chiller, C.R. Maechling, and R.N. Zare, "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001," *Science* 273:924-930, 1996.

28. F. Westall, "The Nature of Fossil Bacteria: A Guide to the Search for Extraterrestrial Life," *Journal of Geophysical Research* 104:16437-16451, 1999.

29. F. Westall, "The Influence of Cell Wall Composition on the Fossilization of Bacteria and the Implications for the Search for Early Life Forms," in *Astronomical and Biochemical Origins and the Search for Life in the Universe,* C. Cosmovici, S. Bowyer, and D. Werthimer (eds.), Editori Compositrici, Bologna, 1997, pp. 491-504.

30. See, for example, T.J. Beveridge, M.N. Hughes, H. Lee, K.T. Leung, R.K. Poole, I. Savvaidis, S. Silver, and J.T. Trevors, "Metal-Microbe Interactions: Contemporary Approaches," *Advances in Microbial Physiology* 38:178-243, 1997.

31. See, for example, F.G. Ferris, "Formation of Authigenic Minerals by Bacteria." in *Biological-Mineralogical Interactions,* J.M. McIntosh and L.A. Groat (eds.), Mineralogical Association of Canada, Ottawa, 1997, pp. 187-208.

32. D. Fortin, F.G. Ferris, and T.J. Beveridge, "Surface-mediated Mineral Development by Bacteria," in *Geomicrobiology: Interactions Between Microbes and Minerals,* J. Banfield and K.H. Nealson (eds.), *Reviews in Mineralogy,* Volume 35, Mineralogical Society of America, Washington, D.C., 1997, pp. 161-180.

33. See, for example, K.H. Nealson, "The Limits of Life on Earth and Searching for Life on Mars," *Journal of Geophysical Research* 102:23675-23686, 1997.

34. See, for example, J.D. Farmer and D.J. Des Marais, "Exploring for a Record of Ancient Martian Life," *Journal of Geophysical Research* 104(E11):26977-26995, 1999.

35. See, for example, J. Wingender, T.R. Neu, and H.-C. Flemming, *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function,* Springer-Verlag, Berlin, 1999.

36. See, for example, P. Stoodley, D. deBeer, J.D. Boyle, and H.M. Lappin-Scott, "Evolving Perspectives of Biofilm Structure," *Biofouling* 14:75-94, 1999.

37. See, for example, S.L. Cady and J.D. Farmer, "Fossilization Processes in Siliceous Thermal Springs: Trends in Preservation Along Thermal Gradients, " in *Evolution of Hydrothermal Ecosystems on Earth (and Mars?),* Ciba Foundation Symposium No. 202, G.R. Bock and J.A. Goode (eds.), John Wiley and Sons, Chichester, UK, 1996, pp. 150-173.

38. W.B. Whitman, "Prokaryotes: The Unseen Majority," *Proc. Natl. Acad. Sci. USA* 95:6578-6583, 1998.

39. See, for example, K. Pedersen, "Exploration of Deep Intraterrestrial Microbial Life: Current Perspectives," *FEMS Microbiology Letters* 185:9-16, 2000.

40. D.S. McKay, E.K. Gibson, K.L. Thomas-Keprta, H. Vali, C. Romanek, S.J. Clemett, X.D.F. Chiller, C.R. Maechling, and R.N. Zare, "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001," *Science* 273:924-930, 1996.

41. M.C. Malin and K.S. Edgett, "Evidence for Recent Groundwater Seepage and Surface Runoff on Mars," *Science* 288:2330-2335, 2000.

42. See, for example, F.G. Ferris, "Formation of Authigenic Minerals by Bacteria," in *Biological-Mineralogical Interactions,* J.M. McIntosh and L.A. Groat (eds.), Mineralogical Association of Canada, Ottawa, 1997, pp. 187-208.

43. See, for example, A.H. Knoll, "Exceptional Preservation of Photosynthetic Organisms in Silicified Carbonates and Silicified Peats," *Philosophical Transactions of the Royal Society of London* B 311:111-122, 1985.

44. L.M. Gerasimenko and I.N. Krylov, "Postmortem Alterations of Cyanobacteria in the Algal-bacterial Films in the Hot Springs of Kamchatka," *Dokl. Akad. Nauk* 272:215-218, 1983.

45. J.K. Bartley, "Actualistic Taphonomy of Cyanobacteria: Implications for the Precambrian Fossil Record," *Palaios* 11:571-586, 1996.

46. See, for example, S. Schultz-Lam, F.G. Ferris, K.O. Konhauser, and R.G. Wiese, "In Situ Silicification of an Icelandic Hot Spring Microbial Mat: Implications for Microfossil Formation," *Canadian Journal of Earth Sciences* 32:2021-2026, 1995.

47. See, for example, K.O. Konhauser and F.G. Ferris, "Diversity of Iron and Silica Precipitation by Microbial Mats in Hydrothermal Waters, Iceland: Implications for Precambrian Iron Formations," *Geology* 24:323-326, 1996.

48. E.W. Tegelaar, J.W. De Leeuw, S. Derenne, and C. Largeau, "A Reappraisal of Kerogen Formation," *Geochimica et Cosmochimica Acta* 53:3103-3106, 1989.

49. G.A. Logan, M.J. Collins, and G. Eglinton, "Preservation of Organic Biomolecules," in *Taphonomy; Releasing the Data Locked in the Fossil Record,* P.A. Allison and D.E.G. Briggs (eds.), Plenum Press, New York, 1991, pp. 1-24.

50. P.A. Allison and D.E.G. Briggs, "Taphonomy of Non-Mineralized Tissues," in *Taphonomy: Releasing the Data Locked in the Fossil Record,* P.A. Allison and D.E.G. Briggs (eds.), Plenum Press, New York, 1991, pp. 25-70.

51. See, for example, J.W. De Leeuw and C. Largeau, "A Review of Macromolecular Organic Compounds That Comprise Living Organisms and Their Role in Kerogen, Coal, and Petroleum Formation," in *Organic Geochemistry, Principles and Applications,* M.H. Engel and S.A. Macko (eds.), Plenum Press, New York, 1993, pp. 23-72.

52. R.E. Summons, "Biogeochemical Cycles: A Review of Fundamental Aspects of Organic Matter Formation, Preservation, and Composition," in *Organic Geochemistry, Principles and Applications,* M.H. Engel and S.A. Macko (eds.), Plenum Press, New York, 1993, pp. 3-21.

53. See, for example, S.L. Cady, K. Downing, and H.-R. Wenk, "HRTEM of Microcrystalline Opal in Chert and Porcelanite from the Monterey Formation, California," *American Mineralogist* 81:1380-1395, 1996.

54. See, for example, S.L. Cady, H.R. Wenk, and M. Sintuban, "Microfibrous Quartz Varieties: Characterization by Quantitative X-Ray Texture Analysis and Transmission Electron Microscopy," *Contributions to Mineralogy and Petrology* 130:320-335, 1998.

55. R. Buick, "Microfossil Recognition in Archean Rocks: An Appraisal of Spheroids and Filaments from a 3500 M.Y. Old Chert-Barite Unit at North Pole, Western Australia," *Palaios* 5:441-459, 1990.

56. G. Heiken and D. McKay, "Petrology of a Sequence of Pyroclastic Rocks from the Valley of Taurus-Littrow," in *Ninth Lunar and Planetary Science Conference, Houston, Texas, March 13-17, 1978, Proceedings,* Volume 2, Pergamon Press, New York, 1978, pp. 1933-1943.

57. W.C. Phinney, D.S. McKay, C.H. Simonds, and J.L. Warner, "Lithification of Vitric- and Clastic-matrix Breccias: SEM Petrography," in *Seventh Lunar Science Conference, Houston, Texas, March 15-19, 1976, Proceedings,* Volume 2, Pergamon Press, New York, 1976, pp. 2469-2492.

58. F. Westall, "The Nature of Fossil Bacteria: A Guide to the Search for Extraterrestrial Life," *Journal of Geophysical Research* 104:16437-16452, 1999.

59. F. Westall, "The Nature of Fossil Bacteria: A Guide to the Search for Extraterrestrial Life," *Journal of Geophysical Research* 104:16437-16452, 1999.

60. G. Heiken and D. McKay, "Petrology of a Sequence of Pyroclastic Rocks from the Valley of Taurus-Littrow," in *Ninth Lunar and Planetary Science Conference, Houston, Texas, March 13-17, 1978, Proceedings,* Volume 2, Pergamon Press, New York, 1978, pp. 1933-1943.

61. W.C. Phinney, D.S. McKay, C.H. Simonds, and J.L. Warner, "Lithification of Vitric- and Clastic-matrix Breccias: SEM Petrography," in *Seventh Lunar Science Conference, Houston, Texas, March 15-19, 1976, Proceedings,* Volume 2, Pergamon Press, New York, 1976, pp. 2469-2492.

62. K. Thomas-Keprta, D.S. McKay, and S.J. Wentworth, "Bacterial Mineralization Patterns in Basaltic Aquifers: Implications for Possible Life in Martian Meteorite ALH84001," *Geology* 26:1031-1034, 1998.

63. K. Biemann, J. Oro, P. Toulmin III, L.E. Orgel, A.O. Nier, D.M. Anderson, P.G. Simmonds, D. Flory, A.V. Diaz, D.R. Rushneck, J.E. Biller, and A.L. Lafleur, "The Search for Organic Substances and Inorganic Volatile Compounds in the Surface of Mars," *Journal of Geophysical Research* 82:4641-4658, 1977.

64. S.A. Benner, K.G. Devine, L.N. Matveeva, and D.H. Powell, "The Missing Organic Molecules on Mars," *Proc. Natl. Acad. Sci. USA* 97:2425-2430, 2000.

65. D.S. McKay, E.K. Gibson, K.L. Thomas-Krepta, H. Vali, C.S. Romanek, S.J. Clemett, X.D.F. Chillier, C.R. Maechling, and R.N. Zare, "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001," *Science* 273:924-930, 1996.

66. L. Becker, B. Popp, T. Rust, and J.L. Bada, "The Origin of Organic Matter in the Martian Meteorite ALH84001," *Earth Planet. Sci. Lett.* 167:71, 1999.

67. A.J.T. Jull, C. Courtney, D.A. Jeffrey, and J.W. Beck, "Isotopic Evidence for a Terrestrial Source of Organic Compounds Found in Martian Meteorites Allan Hill 84001 and Elephant Moraine 79001, *Science* 279:366, 1999.

68. A.J.T. Jull, J.W. Beck, and G.S. Burr, "Isotopic Evidence for Extraterrestrial Organic Material in the Martian Meteorite Nakhla, *Geochim. Cosmochim. Acta* 64:3763-3772, 2000, in press.

69. J.L. Bada, "Extraterrestrial Handedness," *Science* 275:942, 1997.

70. K. Brinton and G.D. McDonald, "Amino Acid Racimezation Studies in Siberian Permafrost," Abstract, Astrobiology Workshop, NASA Ames Research Center, April 2000.

71. W.H. Nelson, R. Manoharan, and J.F. Sperry, "UV Resonance Raman Studies of Bacteria," *Applied Spectroscopy Reviews* 27(1):67-124, 1992.

72. J. Urmos, S.K. Sharma, and F.T. Mackenzie, "Characterization of Some Biogenic Carbonates with Raman Spectroscopy," *American Mineralogist* 76:641, 1991.

73. H.G.M. Edwards, D.W. Farwell, M.M. Grady, D.D. Wynn-Williams, and I.P. Wright, "Comparative Raman Microscopy of a Martian Meteorite and Antarctic Lithic Anologues," *Planet. Space Sci.* 47:353, 1999.

74. D.S. McKay, E.K. Gibson, K.L. Thomas-Krepta, H. Vali, C.S. Romanek, S.J. Clemett, X.D.F. Chillier, C.R. Maechling, and R.N. Zare, "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001," *Science* 273:924-930, 1996.

75. L. Becker, B. Popp, T. Rust, and J.L. Bada, "The Origin of Organic Matter in the Martian Meteorite ALH84001," *Earth Planet. Sci. Lett.* 167:71, 1999.

76. See paper by L. Becker, Session 4, this appendix.

77. K. Biemann, J. Oro, P. Toulmin III, L.E. Orgel, A.O. Nier, D.M. Anderson, P.G. Simmonds, D. Flory, A.V. Diaz, D.R. Rushneck, J.E. Biller, and A.L. Lafleur, "The Search for Organic Substances and Inorganic Volatile Compounds in the Surface of Mars," *Journal of Geophysical Research* 82:4641-4658, 1977.

78. See paper by A. Anbar, Session 4, this appendix.

79. K.A. Delin and S.P. Jackson, "Sensor Web for In Situ Exploration of Gaseous Biosignatures," *IEEE Aerospace Conf. Proceedings,* Big Sky, Montana, 2000.

80. R. Bell and D.M. Karl (conveners.), *Lake Vostok Workshop Final Report,* National Science Foundation, Arlington, Va., November 7-8, 1998.

81. J.C. Petit, J. Jouzel, D. Raynaud, N.I. Barkov, J.M. Barnola, I. Basile, M. Bencer, J. Chappellaz, D. Davis, G. Delaygue, M. Delmotte, V.M. Kotlyyakov, M. Legrand, V.Y. Lipenkov, C. Lorius, L. Pepin, C. Ritz, R.E. Saltzman, and M. Stievenard, "Climate and Atmospheric History of the Past 420,000 Years from the Vostok, Antarctica," *Nature* 399:429, 1999.

82. D.M. Karl, D.F. Bird, K. Bjorkman, T. Houlihan, R. Shackelford, and L. Tupas, "Microorganisms in the Accreted Ice of Lake Vostok, Antarctica," *Science* 286:2144-2147, 1999.

83. J.C. Priscu, E. Adams, W.B. Lyons, M.A. Voytek, D.W. Mogk, R.L. Brown, C.P. McKay, C.D. Takacs, K.A. Welch, C.F. Wolf, J.D. Kirshtein, and R. Avci, "Geomicrobiology of Subglacial Ice Above Lake Vostok, Antarctica," *Science* 286:2141-2144, 1999.

84. G. Eglinton, P.M. Scott, T. Besky, A.L. Burlingame, and M. Calvin, "Hydrocarbons of Biological Origin from a One-Billion-Year-Old Sediment," *Science* 145:263-264, 1964.

85. G. Eglinton and M. Calvin, "Chemical Fossils," *Scientific American* 261:32-43, 1967.

86. *Proceedings of the Biosignature Workshop,* University of California, San Diego, March 3-5, 2000.

87. K.E. Peters and J.M. Moldowan, *The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments,* Prentice Hall, Englewood Cliffs, New Jersey, 1993.

88. See, for example, R.E. Summons, L.L Jahnke, J.M. Hope, and G.A. Logan, "2-Methylhopanoids as Biomarkers for Cyanobacterial Oxygenic Photosynthesis," *Nature* 400:554-557, 1999.

89. M. Rohmer, P. Bouvier, and G. Ourisson, "Molecular Evolution of Biomembranes: Structural Equivalents and Phylogenetic Precursors of Sterols," *Proc. Natl. Acad. Sci. USA* 76:847-851, 1979.

90. T.M. McCollom, G. Ritter, and B.R.T. Simoneit, "Lipid Synthesis Under Hydrothermal Conditions by Fischer-Tropsch-type Reactions," *Orig. Life Evol. Biosph.* 28:1-14, 1998.

91. F. Kenig, J.S. Sinninghe Damsté, A.C. Kock-van Dalen, W.I.C. Rijpstra, A.Y. Huc, and J.W. de Leeuw, "Occurrence and Origins of Mono, Di, and Trimethylalkanes in Modern and Holocene Cyanobacterial Mats from Abu Dhabi, United Arab Emirates," *Geochim. Cosmochim. Acta* 59:2999-3015, 1995.

92. G. Flesch and M. Rohmer, "Prokaryotic Hopanoids: The Biosynthesis of the Bacteriohopane Skeleton. Formation of Isoprenic Units from Two Distinct Acetate Pools and a Novel Type of Carbon/Carbon Linkage Between a Triterpene and D-Ribose," *Eur. Journal Biochem.* 175:405-411, 1988.

93. G. Ourisson and P. Albrecht, "Hopanoids I. Geohopanoids: The Most Abundant Natural Products of Earth," *Chem. Res.* 25: 398-402, 1992.

94. G. Ourisson, P. Albrecht, and M. Rohmer, "The Microbial Origin of Fossil Fuels," *Scientific American* 251:44-51, 1984.

95. B. Chappe, P. Albrecht, and W. Michaelis, "Polar Lipids of Archaebacteria in Sediments and Petroleums," *Science* 217:65-66, 1982.

96. See, for example, R.E. Summons, L.L Jahnke, J.M. Hope, and G.A. Logan, "2-Methylhopanoids as Biomarkers for Cyanobacterial Oxygenic Photosynthesis," *Nature* 400:554-557, 1999.

97. J.J. Brocks, G.A. Logan, R. Buick, and R.E. Summons, "Archaean Molecular Fossils and the Early Rise of Eukaryotes," *Science* 285:1033-1036, 1999.

98. T.-M. Han and B. Runnegar, "Megascopic Eukaryotic Algae from the 2.1-billion-year-old Negaunee Iron-formation, Michigan," *Science* 257:232-235, 1992.

99. A.H. Knoll, "The Early Evolution of Eukaryotes: A Geological Perspective," *Science* 256:622-627, 1992.

100. N. Woolf, "Finding/Studying the Pale Blue Dot," in *Pale Blue Dot 2 Workshop: Habitable and Inhabited Worlds Beyond Our Own Solar System,* L.I. Caroff and D.J. Des Marais (eds.), NASA/CP 2000-209595, Ames Research Center, Moffett Field, California, 2000, pp. 33-46.

101. See paper by J.M. Moldowan, Session 4, in this appendix.

102. J.D. MacDougall, G.W. Lugmair, and J.F. Kerridge, "Early Solar System Aqueous Activity: Sr Isotopic Evidence from the Orgueil CI Meteorite," *Nature* 307:249-251, 1984.

103. B. Zellner and E. Bowell, "Asteroid Compositional Types and their Distributions," in *Comets-Asteroids-Meteorites,* A.H. Delsemme (ed.), University of Toledo Press, Toledo, 1977, pp. 185-195.

104. G. Flynn, "The Delivery of Organic Matter from Asteroids and Comets in the Early Surface of Mars," *Earth, Moon, and Planets* 72:469-474, 1996.

105. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

106. M.N. Fomenkova, S. Chang, and L.M. Mukhin, "Carbonaceous Components in Comet Halley Dust," *Geochim. Cosmochim. Acta* 58:4503-4512, 1994.

107. E. Anders and E. Zinner, "Interstellar Grains in Primitive Meteorites: Diamond, Silicon Carbide, and Graphite," *Meteoritics* 28:490-514, 1993.

108. S. Pizzarello, personal communication.

109. G. Cooper, personal communication.

110. J.R. Cronin, S. Pizzarello, and D.P. Cruikshank, "Organic Matter in Carbonaceous Chondrites, Planetary Satellites, Asteroids and Comets," in *Meteorites and the Early Solar System,* J.F. Kerridge and M.S. Matthews (eds.), University of Arizona Press, Tucson, 1988, pp. 819-857.

111. J.R. Cronin, S. Pizzarello, and D.P. Cruikshank, "Organic Matter in Carbonaceous Chondrites, Planetary Satellites, Asteroids and Comets," in *Meteorites and the Early Solar System,* J.F. Kerridge and M.S. Matthews (eds.), University of Arizona Press, Tucson, 1988, pp. 819-857.

112. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

113. See paper by J.M. Moldowan, Session 4, in this appendix.

114. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The*

Chemistry of Life's Origins, J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

115. L. Becker, R.J. Poreda, and T.E. Bunch, "Fullerenes: An Extraterrestrial Carbon Carrier Phase for Noble Gases," *Proc. Natl. Acad. Sci. USA* 97:2979-2983, 2000.5

116. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

117. W.A. Bonner, "Homochirality and Life," in *D-Amino Acids in Sequences of Secreted Peptides of Multicellular Organisms,* P. Jollès (ed.), EXS Volume 85, Birkhäuser Verlag AG, Switzerland, 1998, pp. 159-188.

118. See, for example, K. Kvenvolden, J. Lawless, K. Pering, E. Peterson, J. Flores, C. Ponnamperuma, I.R. Kaplan, and C. Moore, "Evidence for Extraterrestrial Amino Acids and Hydrocarbons in the Murchison Meteorite," *Nature* 228:623-626, 1970.

119. M.H. Engel and B. Nagy, "Distribution of Enantiomeric Composition of Amino Acids in the Murchison Meteorite," *Nature* 296:837-840, 1982.

120. J.L. Bada, J.R. Cronin, M.-S. Ho, K.A. Kvenvolden, J.G. Lawless, S.L. Miller, J. Oro, and S. Steinberg, "On the Reported Optical Activity of Amino Acids in the Murchison Meteorite," *Nature* 301:494-497, 1983.

121. M.H. Engel, S.A. Macko, and J.A. Silfer, "Carbon Isotope Composition of Individual Amino Acids in the Murchison Meteorite," *Nature* 348:47-49, 1990.

122. J.R. Cronin and S. Pizzarello, "Enantiomeric Excesses in Meteoritic Amino Acids," *Science* 275:951-955, 1997.

123. M.H. Engel and S.A. Macko, "Isotopic Evidence for Extraterrestrial Nonracemic Amino Acids in the Murchison Meteorite," *Nature* 389:265-268, 1997.

124. J.A. Bailey, A. Chrysostomou, J.H. Hough, T.M. Gledhill, A. McCall, F. Menard, and M. Tamura, "Circular Polarization in Star-formation Regions: Implications for Biomolecular Homochirality," *Science* 28l:672-674, 1998.

125. E. Rubenstein, W.A. Bonner, H.P. Noyes, and G.S. Brown, "Supernovae and Life," *Nature* 300:118, 1983.

126. W.D. Langer, E.F. van Dishoeck, E.A. Bergin, G.A. Blake, A.G.G.M. Tielens, T. Velusamy, and D.C.B. Whittet, "Chemical Evolution of Protostellar Matter," in *Protostars and Planets IV,* V. Mannings, A.P. Boss, and S.S. Russell (eds.), University of Arizona Press, Tucson, 2000, pp. 29-57.

127. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

128. E. Zinner, "Interstellar Cloud Material in Meteorites," in *Meteorites and the Early Solar System,* J.F. Kerridge and M.S. Matthews (eds.), University of Arizona Press, Tucson, 1988, pp. 956-983.

129. E. Anders and E. Zinner, "Interstellar Grains in Primitive Meteorites: Diamond, Silicon Carbide, and Graphite," *Meteoritics* 28:490-514, 1993.

130. S. Chang, D. Des Marais, R. Mack, S.L. Miller, and G.E. Strathearn, "Prebiotic Organic Synthesis and the Origin of Life," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983, pp. 53-92.

131. C.C. Kung, R. Hayatsu, M.H. Studier, and R.N. Clayton, "Nitrogen Isotope Fractionations in the Fisher-Tropsch Synthesis and in the Miller-Urey Reaction," *Earth Planet. Sci. Lett.* 46:144-146, 1979.

132. R. Summons, "Abundance and Composition of Extractable Organic Matter," in *The Proterozoic Biosphere,* J.W. Schopf and C. Klein (eds.), Cambridge University Press, New York, 1992, pp. 101-115.

133. J.M. Hayes, I.R. Kaplan, and K.W. Wedeking, "Precambrian Organic Geochemistry: Preservation of the Record," in *Earth's Earliest Biosphere: Its Origin and Evolution,* J.W. Schopf (ed.), Princeton University Press, Princeton, New Jersey, 1983, pp. 93-134.

134. H. Strauss, D.J. Des Marais, J.M. Hayes, and R.E. Summons, "The Carbon Isotopic Record," in *The Proterozoic Biosphere,* J.W. Schopf and C. Klein (eds.), Cambridge University Press, New York, 1992, pp. 117-127.

135. G. Yuen, N. Blair, D.J. Des Marais, and S. Chang, "Carbon Isotopic Composition of Low Molecular Weight Hydrocarbons and Monocarboxylic Acids from Murchison Meteorite," *Nature* 307:252-254, 1984.

136. G.W. Cooper, M.H. Thiemens, T.L. Jackson, and S. Chang, "Sulfur and Hydrogen Isotope Anomalies in Meteorite Sulfonic Acids," *Science* 277:1072-1074, 1997.

137. K. Biemann, J. Oro, P. Toulmin III, L.E. Orgel, A.O. Nier, D.M. Anderson, P.G. Simmonds, D. Flory, A.V. Diaz, D.R. Rusneck, J.E. Biller, and A.L. Lafluer, "The Search for Organic Substances and Inorganic Volatile Compounds in the Surface of Mars," *Journal of Geophysical Research* 82:4641-4658, 1979.

138. S.J. Mojzsis, G. Arrhenius, and K.D. McKeegan, "Evidence for Life on Earth Before 3,800 Million Years Ago," *Nature* 384:55-59, 1996.

139. B.L. Beard and C.M. Johnson, "High-precision Iron Isotope Measurements of Terrestrial and Lunar Materials, *Geochim. Cosmochim. Acta* 63:1653-1660, 1999.

140. C.N. Marechal, P. Telouk, and F. Albarede, "Precise Analysis of Copper and Zinc Isotopic Composition by Plasma-source Mass Spectrometry," *Chem. Geol.* 156:251-273, 1999.

141. J. Barling, G.L. Arnold, and A.D. Anbar, "Natural Mass-Dependent Variations in the Isotopic Composition of Molybdenum," *Earth Planet. Sci. Lett.* 193:447-457, 2001.

142. X.K. Zhu, R.K. O'Nions, and Y. Guo, "Secular Variation of Iron Isotopes in North Atlantic Deep Water," *Science* 287:2000-2002, 2000.

143. X.K. Zhu, R.K. O'Nions, Y. Guo, N.S. Belshaw, and D. Rickard, "Determination of Natural Cu-Isotope Variation by Plasma-source Mass Spectrometry: Implications for Use as Geochemical Tracers," *Chem. Geol.* 163:139-149, 2000.

144. M. Rehkamper, "The Precise Measurement of Tl Isotopic Compositions by MC-ICPMS: Application to the Analysis of Geological Materials and Meteorites," *Geochim. Cosmochim. Acta* 63:935-944, 1999.

145. T.D. Bullen, A.F. White, C.W. Childs, D.V. Vivit, and M.S. Schulz, "A Demonstration of Significant Abiotic Iron Isotope Fractionation in Nature," *Geology* 194:39-51, 2001.

146. A.D. Anbar, J.E. Roe, and J. Barling, "Nonbiological Fractionation of Iron Isotopes," *Science* 288:126-128, 2000.

147. B.L. Beard, C.M. Johnson, and L. Cox, "Iron Isotope Biosignatures," *Science* 285:1889-1892, 1999.

148. S.L. Brantley, L. Liermann, and T.D. Bullen, "Fractionation of Fe Isotopes by Soil Microbes and Organic Acids," *Geology* 29:535-538, 2001.

149. T.D. Bullen, A.F. White, C.W. Childs, D.V. Vivit, and M.S. Schulz, "A Demonstration of Significant Abiotic Iron Isotope Fractionation in Nature," *Geology* 194:39-51, 2001.

150. K.W. Mandernack, D.A. Bazylinski, and W.C. Shanks III, "Oxygen and Iron Isotope Studies of Magnetite Produced by Magnetotactic Bacteria," *Science* 285:1892-1896, 1999.

151. B.L. Beard, C.M. Johnson, and L. Cox, "Iron Isotope Biosignatures," *Science* 285:1889-1892, 1999.

152. K.H. Nealson and D. Saffarini, "Iron and Manganese in Anaerobic Respiration: Environmental Significance, Physiology, and Regulation," *Annu. Rev. Microbiol.* 48:311-343, 1994.

153. K.H. Nealson and C. Myers, "Iron Reduction by Bacteria: A Potential Role in the Genesis of Banded Iron Formations," *Amer. Journal Sci.* 290A:35-45, 1990.

154. B.L. Beard and C.M. Johnson, "High-precision Iron Isotope Measurements of Terrestrial and Lunar Materials," *Geochim. Cosmochim. Acta* 63:1653-1660, 1999.

155. X.K. Zhu, R.K. O'Nions, and Y. Guo, "Secular Variation of Iron Isotopes in North Atlantic Deep Water," *Science* 287:2000-2002, 2000.

156. B.L. Beard and C.M. Johnson, "High-Precision Iron Isotope Measurements of Terrestrial and Lunar Materials," *Geochim. Cosmochim. Acta* 63:1653-1660, 1999.

157. X.K. Zhu, R.K. O'Nions, and Y. Guo, "Secular Variation of Iron Isotopes in North Atlantic Deep Water," *Science* 287:2000-2002, 2000.

158. X.K. Zhu, R.K. O'Nions, and Y. Guo, "Secular Variation of Iron Isotopes in North Atlantic Deep Water," *Science* 287:2000-2002, 2000.

159. B.L. Beard and C.M. Johnson, "High-Precision Iron Isotope Measurements of Terrestrial and Lunar Materials," *Geochim. Cosmochim. Acta* 63:1653-1660, 1999.

160. X.K. Zhu, R.K. O'Nions, and Y. Guo, "Secular Variation of Iron Isotopes in North Atlantic Deep Water," *Science* 287:2000-2002, 2000.

161. B.L. Beard, C.M. Johnson, and L. Cox, "Iron Isotope Biosignatures," *Science* 285:1889-1892, 1999.

162. T.D. Bullen, A.F. White, C.W. Childs, D.V. Vivit, and M.S. Schulz, "A Demonstration of Significant Abiotic Iron Isotope Fractionation in Nature," *Geology* 194:39-51, 2001.

163. S.L. Brantley, L. Liermann, and T.D. Bullen, "Fractionation of Fe Isotopes by Soil Microbes and Organic Acids," *Geology* 29:535-538, 2001.

164. K.W. Mandernack, D.A. Bazylinski, and W.C. Shanks III, "Oxygen and Iron Isotope Studies of Magnetite Produced by Magnetotactic Bacteria," *Science* 285:1892-1896, 1999.

165. A.D. Anbar, J.E. Roe, and J. Barling, "Nonbiological Fractionation of Iron Isotopes," *Science* 288:126-128, 2000.

166. T.D. Bullen, A.F. White, C.W. Childs, D.V. Vivit, and M.S. Schulz, "A Demonstration of Significant Abiotic Iron Isotope Fractionation in Nature," *Geology* 194:39-51, 2001.

167. E.A. Schauble, G.R. Rossman, and H.P. Taylor, "Theoretical Estimates of Equilibrium Fe-Isotope Fractionations from Vibrational Spectroscopy," *Geochim. Cosmochim. Acta* 31: 2487-2497, 2001.

168. V.B. Polyakov and S.D. Mineev, "The Use of Mössbauer Spectroscopy in Stable Isotope Geochemistry," *Geochim. Cosmochim. Acta* 64:849-865, 2000.

169. B.L. Beard, C.M. Johnson, and L. Cox, "Iron Isotope Biosignatures," *Science* 285:1889-1892, 1999.

170. X.K. Zhu, R.K. O'Nions, and Y. Guo, "Secular Variation of Iron Isotopes in North Atlantic Deep Water," *Science* 287:2000-2002, 2000.

171. S.L. Brantley, L. Liermann, and T.D. Bullen, "Fractionation of Fe Isotopes by Soil Microbes and Organic Acids," *Geology* 29:535-538, 2001.

172. A.D. Anbar, J.E. Roe, and J. Barling, "Nonbiological Fractionation of Iron Isotopes," *Science* 288:126-128, 2000.

173. T.D. Bullen, A.F. White, C.W. Childs, D.V. Vivit, and M.S. Schulz, "A Demonstration of Significant Abiotic Iron Isotope Fractionation in Nature," *Geology* 194:39-51, 2001.

174. K. Biemann, J. Oro, P. Toulmin III, L.E. Orgel, A.O. Nier, D.M. Anderson, P.G. Simmonds, D. Flory, A.V. Diaz, D.R. Rushneck, J.E. Biller, and A.L. Lafleur, "The Search for Organic Substances and Inorganic Volatile Compounds in the Surface of Mars," *Journal of Geophysical Research* 82:4641-4658, 1977.

175. B.P. Weiss, Y.L. Yung, and K.H. Nealson, "Atmospheric Energy for Subsurface Life on Mars?" *Proc. Natl. Acad. Sci. USA* 97:1395-1399, 2000.

176. D.P. Glavin, M. Schubert, O. Botta, G. Kminek, and J.L. Bada, "Detecting Pyrolysis Products from Bacteria on Mars," *Earth and Planetary Science Letters* 185(1-2):1-5, 2000.

177. S.A. Benner, K.G. Devine, L.N. Matveeva, and D.H. Powell, "The Missing Organic Molecules on Mars," *Proc. Natl. Acad. Sci. USA* 97:2425-2430, 2000.

178. See, for example, D.P. Glavin, J.L. Bada, K.L.F. Brinton, and G.D. McDonald, "Amino Acids in the Martian Meteorite Nakhla," *Proc. Natl. Acad. Sci. USA* 96:8835-8838, 1999.

179. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

180. P.A. Bland and T.B. Smith, "Meteorite Accumulations on Mars," *Icarus* 144:21-26, 2000.

181. A.S. MacKenzie, S.C. Brassell, G. Eglinton, and J.R. Maxwell, "Chemical Fossils: The Geological Fate of Steroids," *Science* 217:491-504, 1982.

182. L.J. Allamandola, G.G.M. Tielens, and J.R. Barker, "Interstellar Polycyclic Aromatic Hydrocarbons: The Infrared Emission Bands, the Excitation/Emission Mechanism, and the Astrophysical Implications," *Astrophys. Journal* 71:733-775, 1989.

183. J.R. Cronin and S. Chang, "Organic Matter in Meteorites: Molecular and Isotopic Analyses of the Murchison Meteorite," in *The Chemistry of Life's Origins,* J.M. Greenberg, C.X. Mendoza-Gómez, and V. Pirronello (eds.), Kluwer Academic Press, Boston, 1993, pp. 209-258.

184. L.J. Allamandola, G.G.M. Tielens, and J.R. Barker, "Interstellar Polycyclic Aromatic Hydrocarbons: The Infrared Emission Bands, the Excitation/Emission Mechanism, and the Astrophysical Implications," *Astrophys. Journal* 71:733-775, 1989.

185. D.P. Glavin and J.L. Bada, "Isolation of Amino Acids from Natural Samples Using Sublimation," *Anal. Chem.* 70:3119-3122, 1998.

186. J.L. Bada and G.D. McDonald, "Detecting Amino Acids on Mars," *Anal. Chem.* 68:668A-673A, 1996.

187. J.L. Bada and G.D. McDonald, "Amino Acid Racemization on Mars: Implications for the Preservation of Biomolecules from an Extinct Martian Biota," *Icarus* 114:139-143, 1995.

188. L.D. Hutt, D.P. Glavin, J.L. Bada, and R.A. Mathies, "Microfabricated Capillary Electrophoresis Amino Acid Chirality Analyzer for Extraterrestrial Exploration," *Anal. Chem.* 71:4000-4006, 1999.