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The following text is proposed to replace the current description of Goal I in the document:

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Goal 1: Determine if life ever arose on Mars

As embodied in the new mantra, “seeking signs of life”, the search for life is a key driver of the Mars exploration program. The general notion that Earth and Mars may have been relatively similar worlds during their early histories, combined with the relatively early emergence of life on Earth, has long led to speculation about the possibility for life on Mars. Current and emerging technologies will enable us to evaluate this possibility with scientific rigor.

The implications of such an investigation are far reaching, and finding life on another world would have great impact at both social and scientific levels. Importantly, life-related investigations would not halt upon an affirmative or negative finding (although the negative can never be definitively established). Demonstration of extant or past life on Mars would motivate a variety of sequel investigations to determine how that life functions or functioned; which attributes of structure, biochemistry, and physiology may be shared with terrestrial life and which are addressed via alternative strategies; and whether Mars preserves evidence relating to the origin of that life. Apparent absence of life in systems that could clearly have both supported and preserved evidence of it would raise questions about the differences in the nature, extent, and duration of habitable conditions on Mars compared to Earth that may underlie this absence; and whether Mars preserves evidence of prebiotic chemistry. Life-related investigations also serve as a unifying theme for Mars system science: to understand the context for the emergence, proliferation, and fate of life requires an integrated understanding of the factors – ranging from geophysical to climatological – that shape the planetary environment.

While the search for life will ultimately take the form of dedicated life-detection missions, it should be based on a series of missions – both landed and orbital – that develop a detailed and global perspective on where and how to conduct those dedicated missions. The purpose of this document is to lay out such a strategy.

Challenges Inherent in a Search for Extraterrestrial Life: The Need for a Working Model

Any effort to search for life beyond Earth must confront the potential for bias and “tunnel vision” that arises from having only one example – terrestrial life – on which to base our concepts of habitability and biosignatures. Such efforts should accommodate the possibility for exotic organisms that may differ in biochemistry or morphology, by conceiving life, habitability, and biosignatures in the most general terms possible. Nonetheless, the design and implementation of search-for-life strategies and missions requires concreteness, and therefore a working model of what is being sought.

Many definitions for “Life” have been posited – an often referred-to example is “life is a chemical system capable of Darwinian evolution” – although no consensus version exists. Exceptions can be cited for nearly any definition, and it has been suggested that science presently lacks the capability to develop a comprehensive definition. For the purposes of formulating a search strategy, however, it is largely suitable, and perhaps of more practical use, to consider life’s apparent properties – what it needs, what it does, and what it is made of – without attempting to define what it is. To this end, the NRC Committee on an Astrobiology Strategy for the Exploration of Mars assumed that hypothetical Martian life forms would exhibit the following characteristics¹:

- They are based on carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, and the bio-essential metals of terran life.
- They require water.
- They have structures reminiscent of terran microbes. That is, they exist in the form of self-contained, cell-like entities rather than as, say, a naked soup of genetic material or free-standing chemicals that allow an extended system (e.g., a pond or lake) to be considered a single living system.
- They have sizes, shapes and gross metabolic characteristics that are determined by the same physical, chemical, and thermodynamic factors that dictate the corresponding features of terran organisms. For example, metabolic processes based on the utilization of redox reactions seem highly plausible. But the details of the specific reactions, including the identities of electron donors and electron acceptors, will be driven by local conditions and may well not resemble those of their terran counterparts.
- They employ complex organic molecules in biochemical roles (e.g., structural compounds, catalysis, and the preservation and transfer of genetic information) analogous to those of terran life, but the relevant molecules playing these roles are likely different from those in their terran counterparts.

This set of characteristics is adopted here as a working model. The bearing of this model on the approach to characterizing habitability and seeking biosignatures is discussed briefly below, and in greater detail in the Appendix to Goal 1.

Delineating Objectives: Past versus Extant Life

Finding evidence of *either* past or extant life on Mars would be a watershed event. However, significant differences exist in the strategies, technologies, target environments, and forms of evidence that are most appropriate in searching for ancient versus extant life. For example, it is generally thought that definitive evidence of life in ancient samples might only be obtained through return of samples from Mars to Earth, whereas some investigations for extant life may be best, or obligately, conducted *in situ*. Likewise, a presumable need to access the Martian subsurface in order to find presently habitable environments yields significant differences, relative to past-life investigations, in the possibilities to perform remote screening, the types of observations that can be made, and the possibilities for obtaining samples. For this reason, separate Objectives are delineated for ancient and extant life (Objectives A and B, respectively), with associated investigations that are specifically tailored to each search type. Ancient systems are given higher priority here based on a majority view that deposits formed in various ancient habitable environments are presently more accessible to characterization at the level of detail needed to constitute a viable search for evidence of life. However, recent findings (e.g., detection of methane on Mars, and an expanding understanding of the potential for extant photosynthesis-independent subsurface life on Earth) emphasize the significance of potential subsurface habitable niches on Mars. The possibility should thus remain to reverse the order of priority depending upon emerging evidence, technology, or a changing consensus with respect to the accessibility of presently habitable environments.

Delineating Investigations: Habitability, Biosignatures, and Preservation Potential

Mars presents a diverse array of environments that may vary widely in the type, abundance, and quality of biosignature evidence they could or do preserve. The targeting of life-detection missions should thus be strongly informed by assessment of (a) habitability, i.e., how much and what sorts of evidence of life a given environment could expectedly have accumulated when/if it was inhabited, and (b) preservation potential, i.e., how well that evidence may have been preserved, and what information may have been lost, to the point in space and time at which we could access it. The structure of Objectives A and B below reflects this notion, with separate investigations for characterizing habitability and preservation potential that serve as precursors to life-detection investigations. Within the context of Objectives A and B, the chief purpose of the habitability and preservation potential investigations is to inform life detection, and they should be conducted in this spirit, rather than as ends unto themselves. A third Objective (C) recognizes the stand-alone importance of investigating the long-term evolution of habitability in the context of planetary processes. The concepts of habitability, biosignatures, and preservation potential, as they bear on Goal 1 and Mars exploration, are discussed in detail in the appendix. Key considerations are as follows:

Habitability:

In the context of Mars exploration, “habitability” has been previously defined as the potential of an environment (past or present) to support life of any kind, and has been assessed largely in reference to the presence or absence of liquid water. To support site selection for life-detection missions, additional metrics should be developed for resolving habitability as a continuum (i.e., more habitable, less habitable, uninhabitable) rather than a one-or-zero function, and this will require that additional determinants of habitability be characterized. Based on the working

model above, the principal determinants of habitability for life on Mars would be: the presence, persistence, and chemical activity of liquid water; the presence of thermodynamic disequilibrium (i.e., suitable energy sources); physicochemical environmental factors (e.g., temperature, pH, salinity, radiation) that bear on the stability of covalent and hydrogen bonds in biomolecules; the presence of bioessential elements, principally C, H, N, O, P, S, and a variety of metals. An expanded discussion of the bearing of these factors on habitability is included in the appendix.

Preservation Potential:

Once an organism or community of organisms dies, its imprint on the environment begins to fade. Understanding the processes of alteration and preservation related to a given environment, and for specific types of biosignatures, is therefore essential. This is true not only in the search for fossil traces of life, but also for extant life. For example, metabolic end-products that are detected at a distance, in time and space, from their source, may be subject to some level of alteration. Degradation and/or preservation of physical, biogeochemical and isotopic biosignatures is controlled by a combination of biological, chemical and physical factors, and a combination that would best preserve one class of features may not be favorable for another. These factors include diagenetic processes, radiation and oxidation degradation, and physical destruction by impact shock and dissolution. These factors might have varied substantially from one potential landing site to the next, even among sites that had all maintained habitable environments sometime in the past. *Characterization of the environmental features and processes on Mars that preserve specific lines of biosignature evidence is a critical prerequisite in the search for life.* Accordingly the selection of landing sites should assess the capacity for any candidate sites to have preserved such evidence. Further discussion of preservation potential may be found in the Appendix.

Biosignatures:

Biosignatures can be broadly organized into three categories: physical, biomolecular, and metabolic. Physical features range from individual cells to communities of cells (colonies, biofilms, mats) and their fossilized counterparts (mineral-replaced and/or organically preserved remains) with a corresponding range in spatial and temporal scale. Molecular biosignatures relate to the structural, functional, and information-carrying molecules that characterize life forms. Metabolic biosignatures comprise the unique imprints upon the environment of the processes by which life extracts energy and material resources to sustain itself – e.g., rapid catalysis of otherwise sluggish reactions, isotopic discrimination, biominerals, and enrichment or depletion of specific elements. Significantly, examples can be found of abiotic features or processes that bear similarity to biological features in each of these categories. However, biologically-mediated processes are distinguished by speed, selectivity, and a capability to invest energy into the catalysis of unfavorable processes or the handling of information. It is the imprint of these unique attributes that resolves clearly biogenic features within each of the three categories. A detailed discussion of biosignatures appears in the Appendix.

Ordering and Prioritization of Objectives, Investigations, and Sub-investigations

Objectives are listed in priority order, based on the rationale outlined above (see “***Delineating objectives...***”). Within Objectives A and B, Investigations are listed in preferred order of execution (not priority), based on the rationale outlined above (see “***Delineating***”).

investigations...). More specifically, the habitability and preservation potential Investigations within Objectives A and B are considered prerequisite “screening” to support the life detection Investigation, which has overall highest priority within each Objective. Priority is implied in the ordering of Sub-investigations within Objectives A and B, and Investigations within Objective C. However, but it should be noted that an Investigation will not be “complete” without the conduct of each Sub-investigation. In this case, priority implies a sense of which Sub-investigations will yield the greatest “partial progress” with respect to a given Investigation.

Objective A: Characterize past habitability and search for evidence of ancient life

1. Characterize the prior habitability of surface environments, with a focus on resolving more habitable vs. less habitable sites.

Sub-investigations are focused on establishing overall geologic context and constraining each of the factors thought to influence habitability. Importantly, it must be noted that the purpose of such investigations is to constrain *ancient* conditions by inference, based on the presently available record of such conditions. Data relevant to each sub-investigation can potentially be obtained by orbital measurements – in particular, by characterizing morphology and mineralogy in concert. Such measurements should be heavily utilized as a “screening” tool, with which to target landed platforms capable of more detailed measurements.

- 1.1. Establish overall geologic context.
 - 1.2. Constrain prior water availability with respect to duration, extent, and chemical activity.
 - 1.3. Constrain prior energy availability with respect to type (e.g., light, specific redox couples, etc.), chemical potential (e.g., Gibbs energy yield), and flux.
 - 1.4. Constrain prior physicochemical environment, emphasizing temperature, pH, and water activity and chemical composition.
 - 1.5. Constrain the abundance and characterize potential sources of bioessential elements.
2. Assess the potential of various environments and processes to enhance preservation or hasten degradation of biosignatures. Identify specific environments having high preservation potential for either individual or multiple types of biosignatures.
 - 2.1. Determine the major processes that degrade or preserve complex organic compounds, focusing particularly on characterizing oxidative effects in surface and near-surface environments (including determination of the “burial depth” in regolith or rocks that may shield from such effects, if at all), the prevalence, extent, and type of metamorphism, and potential mechanisms and rates for obscuration of isotopic or stereochemical information.
 - 2.2. Identify the processes and environments that preserve or degrade physical structures on micron to meter scales.

- 2.3. Characterize processes that preserve or degrade environmental imprints of metabolism, including obscuration of chemical or mineralogical gradients and loss of stable isotopic and/or stereochemical information.
3. Search for evidence of ancient life in environments having high combined potential for prior habitability and preservation of biosignatures (as determined by A.1 and A.2).
 - 3.1. Characterize organic chemistry, including (where possible) stable isotopic composition and stereochemical information. Characterize co-occurring concentrations of possible bio-essential elements.
 - 3.2. Seek evidence of possibly biogenic physical structures, from microscopic (micron-scale) to macroscopic (meter-scale), combining morphological, mineralogical, and chemical information where possible.
 - 3.3. Seek evidence of the past conduct of metabolism, including stable isotopic composition of prospective metabolites, mineral or other indicators of prior chemical gradients, localized concentrations or depletions of potential metabolites (especially biominerals) and evidence of catalysis in chemically sluggish systems.

Objective B: Characterize present habitability and search for evidence of extant life

1. Identify and characterize any presently habitable environments.

Sub-investigations are built on the assumption that, because liquid water is not presently stable at the surface of Mars, any modern habitable environments will be in the near- to deep-subsurface. Sub-investigations are focused (and priorities based) on the sorts of information needed to fully characterize habitability in such environments, without reference to the present ability/difficulty in obtaining such information. The purpose of this approach is to accommodate future missions/technologies that may enable direct measurements by virtue of direct access to the subsurface. Importantly, however, orbital platforms may be capable of providing some information in each category, either by direct measurement (e.g., radar soundings to search for possible aquifers) or by inference (e.g., trace gas emissions that may imply a source region having liquid water and specific redox conditions). Heavy use should be made of such orbital measurements in providing global screening-level constraints on subsurface habitability.

- 1.1. Identify areas where liquid water presently exists, placing particular emphasis on reservoirs that are relatively extensive in space and time.
- 1.2. Establish general geologic context (e.g., rock-hosted aquifer or sub-ice reservoir; host rock type; etc.)
- 1.3. Identify and constrain the magnitude of possible energy sources (e.g., water-rock reactions, radiolysis) associated with occurrences of liquid water.

- 1.4. Assess the variation through time of physical and chemical conditions in such environments. Of particular importance are temperature, pH, and fluid composition.
- 1.5. Identify possible supplies of bioessential elements to these environments.
2. Assess the potential of various environments and processes to enhance preservation or hasten degradation of biosignatures of extant life.
 - 2.1. Evaluate the physico-chemical conditions of actual surface regolith/rock habitats in terms of the potential for degrading or preserving biosignatures, and the effects of these processes on specific types of biosignatures. For example, whereas biomolecules are likely to be destroyed in surface materials, physical biomarkers such as fossil (mineralized) cells or communities of cells, or biominerals, could be preserved.
 - 2.2. Evaluate the physico-chemical conditions at depth in regolith, ice or rock habitats in terms of the potential for degrading or preserving biosignatures.
3. Search for extant life at localities identified by Investigations B.1 and B.2.
 - 3.1. Seek evidence of ongoing metabolism, in the form of rapid catalysis of chemically sluggish reactions, stable isotopic fractionation, and strong chemical gradients. A particularly important sub-class of such features is possibly biogenic gases, which have potential to migrate from (currently habitable) deep subsurface environments to surface environments where they may be accessible to remote or *in situ* characterization.
 - 3.2. Characterize organic chemistry and co-occurring concentrations of possibly bio-essential elements, including stable isotopic composition and stereochemistry. Analyses may include but should not be limited to known molecular markers of terrestrial life, such as membrane lipids, proteins, nucleic acid polymers, and complex carbohydrates.
 - 3.3. Seek evidence of organic and mineral structures or assemblages that may be associated with life. Seek evidence of mineral transformations bearing evidence of biological catalysis (e.g., depletion of possibly bio-essential elements in mineral surfaces).

Objective C: Determine how the long-term evolution of Mars affected prebiotic chemistry and habitability

In Objectives A and B, the principal aim of characterizing habitability is to inform the selection of sites for subsequent life-detection missions. However, understanding the factors and processes that give rise to habitable conditions at planetary and local scales, and how those conditions change in concert with planetary and stellar evolution, is an important stand-alone

pursuit for Mars science. Investigations below focus on constraining the major planetary processes that collectively affect habitability through time.

Investigations:

1. Characterize the evolution of the Martian hydrological cycle, emphasizing likely changes in the location and chemistry of liquid water reservoirs.
2. Constrain evolution in the geological, geochemical, and photochemical processes that control atmospheric, surface, and shallow crustal chemistry, particularly as it bears on provision of chemical energy and recycling and mobilization of bioessential elements.
3. Constrain the nature and abundance of possible energy sources as a function of changing water availability, geophysical and geochemical evolution, and evolving atmospheric and surface conditions.
4. Evaluate the presence and magnitude of oxidative or radiation hazards at the surface and in the shallow crust.

Appendix to Goal 1

The specific approach and methods involved in any search for life beyond Earth depend critically on how the concepts of life, habitability, and biosignatures are conceived. Below, these concepts are discussed in specific reference to Mars exploration and the strategy outlined in this document.

Life

The NRC Committee on the Limits of Organic Life noted that the only unquestionably universal attribute of life is that it must exploit (and therefore requires) thermodynamic disequilibrium in the environment, in order to perpetuate its own state of disequilibrium. Beyond this absolute, the Committee cited a set of traits that it considered likely be common to all life²:

- It is chemical in essence, and most probably consists of interacting sets of molecules having covalently bonded atoms, including a diversity of “heteroatoms” (such as N, O, P, etc. in terrestrial organisms) that promote chemical reactivity.
- It probably requires a liquid solvent to support such molecular interactions.
- It probably employs a molecular system capable of Darwinian evolution.

Reference to the known characteristics of life on Earth can serve to add detail and constraint within each of these categories, but heavy reference to this single example carries the risk of “terracentricity” – a potential to overlook life that may be unlike our own. A key challenge for Mars astrobiology is thus to find a point of balance between the all-encompassing generality of

the descriptions above and the specificity and concreteness that comes from reference to life on Earth. The NRC Committee on an Astrobiology Strategy for the Exploration of Mars developed a working set of characteristics of life (as quoted earlier) that reflects such a balance, and which serves as the basis for the approach outlined here. This approach generally corresponds to the following logic:

The relative similarity of Earth and Mars (in comparison to, for example, gas giants or icy moons) suggests that differences in life forms that originated independently on the two bodies would likely occur at a secondary, rather than first-order level. That is, notions of life that differ at the fundamental levels of biochemical scaffolding (alternatives to carbon) or required solvent (alternatives to water) require planetary conditions and chemistries that differ dramatically from that of either Earth or Mars. However, differences from terrestrial life become increasingly possible, and ultimately probable, with increasing levels of biochemical specificity. These considerations bear differently on the conceptualization of the habitability and life detection objectives. For the most part, habitability relates to the core needs and attributes of life, so a presumed first-order similarity between terrestrial and Martian life allows terrestrial notions of habitability to be applied, with somewhat relaxed boundary conditions, to Mars. On the other hand, as developed in studies of terrestrial systems, biosignatures (especially molecular/organic biosignatures) frequently represent extremely specific attributes of biochemistry (e.g., specific lipids or particular sequences of amino or nucleic acids), morphology, or process. While such specific markers of life would be unquestionably valuable if detected on Mars, the likelihood that the *same* markers (the same specific choices of biomolecules) would arise through an independent origin and elaboration of life seems low. Thus, while life detection strategies for Mars should ideally allow for the detection and characterization of Earth-like biosignatures, highest priority should be given to approaches and methods that define and seek biosignatures in a broader sense. Strategies for framing and applying concepts of habitability and biosignatures are addressed in greater detail below.

Defining and Quantifying Habitability

In the context of Mars science, habitability has thus far been defined (for example, in the NRC “An Astrobiology Strategy for the Exploration of Mars”) as the potential of an environment to support life. Assessment of this potential has focused to a very large degree on determining whether liquid water was or is present in the environment in question. These constitute an inherently “binary” approach to habitability – liquid water was either present or was not; life could either be supported, or could not – that has served to identify a wide spectrum of apparently water-formed (nominally habitable) environments. Reference to life on Earth suggests that significant variability could exist within this set, with some environments being vibrantly inhabited, others sparsely so. As described above, the main purpose of Habitability Investigations A.1 and B.1 is to narrow and prioritize the search space for life detection efforts. Investigations and methodologies capable of resolving “more habitable” environments from “less habitable” ones should therefore be emphasized. A key challenge for the coming decades of Mars exploration is thus to augment the liquid water metric that has served as a guide to habitability with additional metrics that will aid in prioritizing sites for life detection missions. Although a consensus approach for characterizing “relative habitability” does not yet exist within the Mars community, it is clear that additional resolving power in virtually any model will

depend on the ability to resolve (by measurement or inference) variations in each of the parameters thought to underpin habitability:

- A solvent capable of supporting complex biochemistry. For terrestrial life, liquid water (above minimum chemical activity levels) is an absolute requirement.
- A source of energy to drive metabolism. Organisms on Earth require energy availability to meet discrete minimum flux and Gibbs energy requirements. Light (from the near infrared to visible range) and chemical energy are known to be utilized by life on Earth; the viability of alternative energy sources has yet to be seriously explored or validated.
- Raw materials for biosynthesis. All life on Earth requires the elements C, H, N, O, P, and S, and also variously requires many “micronutrients” (typically, transition metals).
- Physicochemical (environmental) conditions that allow for the assembly, persistence, and function of complex structures and biomolecules (especially biopolymers, like proteins and nucleic acid polymers, whose backbones contain relatively labile bonds). Extremes of temperature, pH, radiation, and salinity can, individually or in combination, render an environment uninhabitable.

Given the working model and rationale described above, habitability shall be considered to correspond closely to the parameters known to constrain life on Earth. While environments that could be habitable for exotic organisms may be missed by this approach, it is appropriately conservative. Conditions that could support terrestrial life can be said to be definitively habitable. Some level of divergence from a strictly Earth-centric view of habitability can also be adopted by (a) focusing more on “core requirements” (e.g., water, carbon, and energy) than on requirements that underpin the more specific attributes of biochemistry (e.g., micronutrient requirements), and (b) allowing for the possibility, at least at a screening level, that Martian organisms might conceivably transcend the currently known physicochemical boundaries (e.g., the biologically-tolerated temperature range) of life on Earth.

Whatever models emerge for resolving habitability may differ in parameterization of, and sensitivity to, each of these basic factors that underpin habitability. Yet all will be supported by an effort to constrain “degree” in reference to each parameter: How long liquid water was available, at what chemical activity level, and whether intermittently or continuously. How much energy was available, in what forms, and how fast it could have been delivered into a system. What concentrations or fluxes of bioessential elements were present, and what processes may have served to mobilize or cycle them. And what range of temperature, pH, radiation level, and other relevant environmental parameters an environment may have experienced. All such measurements should be placed, to the greatest extent possible, within geologic and environmental context.

While the ability to resolve almost any of these parameters will likely be greater with landed platforms and instruments, a key aspect of the habitability investigations is the capability of orbital measurements to yield several lines of “screening level” information, beyond evidence of liquid water. Of particular interest is the ability of combined morphological and mineralogical evidence to establish geologic context and place screening-level constraints on possible energy sources and physicochemical regimes; and of trace gas and other measurements to infer conditions of formation in subsurface source regions. Such measurements should serve as a key

initial step in resolving habitability among the variety environment types that could be targeted for life-detection investigations.

Biosignature types and contamination challenges

Biosignatures can be broadly organized into three categories: biomolecular, metabolic, and structural. Significantly, examples can be found of abiotic features or processes that bear similarity to biological features in each of these categories. However, biologically-mediated processes are characterized by speed, selectivity, and a capability to invest energy into the catalysis of unfavorable processes or the handling of information. It is the imprint of these unique attributes that resolves clearly biogenic features within each of the three categories. Most of the biosignatures can be, to a certain degree, imitated by non biological processes. Robust identification of traces of life therefore requires a variety of evidence, ideally from the three categories.

1. Biomolecular. Life invests energy into the synthesis of complex structural, functional, and information-carrying molecules. Identifying terrestrial versions of these molecules (e.g., membrane lipids, proteins, and nucleic acid polymers, respectively) on Mars would aid in attributing a biological origin, but would likewise increase the importance of ruling out terrestrial contamination. Likewise, because these represent specific biochemical “choices”, our search must allow for alternative possibilities. Accordingly, the methods employed should be as inclusive as possible with the broad spectrum of organic compounds, and should seek to capture information about structure, complexity, and organization. In synthesizing the suite of biomolecules that constitute a functional organism, life also concentrates key elements (e.g., C, N, P, S, and various micronutrients, in terrestrial life) in stoichiometric ratios, and evidence of such co-occurring elements (particularly in organic form) should be sought. Finally, the enzymatic processes that synthesize biomolecules frequently also impose significant kinetic isotope fractionation effects and exhibit high stereochemical or enantiomeric selectivity. These additional layers of information within the basic organic chemistry should be sought when possible.

2. Metabolic. In constructing and maintaining itself, life extracts energy and material resources from its surroundings, and may leave unique overprints on the environment in the process. Photosynthetic energy harvesting is evident in light-absorption by pigments (for example, characteristic deep absorption features in the NIR to visible) and may confer on organisms an ability to build up significant redox disequilibrium in their surroundings (as with the strong oxidizing effect of oxygenic photosynthesis). Chemosynthetic metabolism extracts energy from chemical reactions that are thermodynamically favored to proceed even in the absence of life. Life distinguishes itself in these reactions by speed (catalysis 10^6 -fold or greater, in many terrestrial examples) and selectivity (as expressed in kinetic isotope effects and, sometimes, stereoselectivity). Catalytic speed may be evident in progress toward equilibrium in chemical reactions that are abiotically sluggish under ambient conditions, concentration or depletion of specific elements or chemical species, or strong chemical gradients or zonation (including in redox and pH). The latter can sometimes be recorded in biomineralization, which may be an important class of evidence for ancient systems. Selectivity may be evident in isotopic fractionation between candidate substrate and product pairs (noting that abiotic processes may

also fractionate), or in deposition of structurally or chemically distinctive mineral forms. Where possible, chemical information (e.g., analysis of potential metabolic product/reactant pairs) should be coupled with isotopic and other information, to capture combined evidence of life's catalytic and selective effects. An important aspect of the metabolic class of biosignatures is that, unlike biomolecular markers, life's role in imposing an imprint on the environment is simply catalytic. Hence, special allowance need not be made, in this category, for "alternative" or exotic biochemical machineries – it is the reactants and products of catalyzed reactions (and the imprints of speed and selectivity thereon) that constitute the biosignature, and not the catalyst (organism) itself.

3. Physical structures. Life imposes organization and order on its physical environment at many levels, from the structure and sub-structures within a cell to community-level structures formed by trillions of individuals (e.g. microbialites and microbial fabrics). The structural components, cells, colonies, biofilms, mats and EPS, may be preserved in fossilized form in a number of ways. Cells may leave organic walled impressions, mineral-coated or impregnated structures, or empty casts in a mineral precipitate. Biofilms and mats may also be preserved as organic impressions in sediments or mineralized structures.

Cells walls can be preserved as organic impressions in fine-grained, anaerobic sediments. This kind of preservation can be aided by the fixation of metals, such as Fe, on cell envelopes, which may retard lysis. The most common form of preservation of microbial structures is mineral-assisted fossilization. In this process, minerals bind to the organic surfaces of the cells and/or their polymers in a passive reaction resulting in encrustation or permeation of the organic structure. The microbial surfaces and exopolymers therefore act as "mineralizing templates". Depending upon the availability of the minerals in solution, the microorganisms may be completely entombed in a mineral precipitate. Many mineral phases can bind to microbial cell walls including silica, carbonates (Ca, MgCa, Fe, Mn), metal oxides/hydroxides (Fe/Mn and magnetite), sulfates (Ca, Sr, Ba, Fe), sulfides (Fe, Ni, Pb, Zn, CuFe), phosphates (Ca), clays, and zeolites. In anaerobic environments, the macromolecules can be entombed within the mineral precipitate. However, in order for the fossilised cells or cell communities to be preserved in the rock record, the mineral-coated/permeated microbial structure needs to become encased in a mineral cement or by fine-grained sediments. Here, further diagenetic changes may take place, including changes in mineralogy (e.g. transformation of oxyhydroxides to oxides), replacement (complete or partial) of one mineral by another (e.g. silicification of carbonate mineralized remains), or dissolution. The final mineral or sediment-encased microbial fossils may exhibit different morphological preservation modes.

On a cautionary note, abiological mineral precipitates can be notoriously confused with fossilized microorganisms. Many minerals, for instance silica, may form simple spherical, oval, elongated and even twisted morphologies.

The problem of contamination

Any of the classes of biosignature evidence that may be sought in our investigations is potentially subject to contamination. However, this is perhaps most critical for the "biochemical" class, where any of a broad range of organic contaminants have potential to be introduced by the spacecraft itself. Investigations targeting biochemicals must therefore include appropriate controls against terrestrial contamination. To this end, new techniques and

instruments are presently being developed for cleaning and monitoring of spacecraft contamination. In searching for life on Mars, sample handling and analytical procedures must include procedural blanks that allow for the tracking and quantification of contamination introduced by the spacecraft and its processes, for any analytes that may serve as evidence of life. Planning along these lines should also address the potential that the aging of the spacecraft, or its exposure to different environments, could alter its potential to introduce contamination over the course of a mission.

Preservation of features related to assessing habitability or biosignatures

Once an organism or community dies, its imprint on the environment, in any of the classes of features described above, begins to fade. Preservation/degradation of the different types of biosignatures is controlled by the combination of biological, chemical and physical factors, and a combination that would best preserve one class of features may not be favorable for another. *Characterization of the environmental features and processes on Mars that preserve specific lines of biosignature evidence is a critical prerequisite in the search for life.* Along with an assessment of relative habitability, assessment of preservation potential should serve as a key criterion in selecting sites for life detection missions. It should not, however, have high priority as a *stand-alone* enterprise, since life detection is the ultimate and highest priority objective of Goal 1.

It will be important to consider an environment's potential to preserve evidence in each of the three categories of biosignatures. Often, preservation within the biochemical category is given the most attention, because such molecules (in undegraded form) may present the most diagnostic evidence of life, but may also be among the most labile forms of evidence. However, obtaining clear evidence of life on Mars will likely require multiple biosignatures in different categories. Thus, recognizing physical structures in context, identifying associated biominerals, and finding the chemical and isotopic imprints of metabolism will be no less important. Investigations of ancient communities on Earth may provide a preliminary guide for understanding preservation potential on Mars. However, it should be noted that the differing histories and surface environments of those two worlds may translate into quite significant differences in the processes that degrade or preserve specific lines of evidence. For example, metamorphic alteration represents a major destructive mechanism for biosignatures from early Earth environments, while radiation and oxidation may present the greater challenge to biosignatures on Mars.

Preservation of biochemicals

Organic molecules in sediments are rapidly degraded in natural environments by a number of chemical and biological processes during early diagenesis and rock lithification, as well as during low temperature burial metamorphism to high temperature metamorphism (on Mars this will be equated with impact shock and/or volcanism). Chemical and radiolytic degradation on the surface of Mars would include the effects of UV and ionizing radiation, radionuclide decay, oxidation in the presence of liquid water and certain minerals, such as Fe(III), and exposure to oxidants, such as H₂O₂. Furthermore, in the presence of liquid water, racemization of chiral organic molecules could occur within a couple of million years. The ideal locality for searching

for biomolecules on Mars would therefore be in the subsurface in materials that have not been exposed to liquid water since their burial and preservation. Molecules that have a greater chance of long-term preservation are those that have undergone restructuring to become resistant cross-linked aliphatic or aromatic macromolecules and that have been preserved by association with certain lithologies and minerals, such as clays, silica, sulfates, carbonates, and ices. The isotopic composition of organic compounds is relatively stable, to the extent that basic molecular skeletons are preserved. On Earth, the effect of thermal metamorphism on organic matter is to degrade it chemically, typically forming isotopically lighter volatile species and isotopically heavier residual refractory solids.

Preservation of physical structures

On Earth, long-term preservation of physical microbial structures depends upon several factors, in particular the following: (1) The rapid burial of organic structures in anaerobic conditions by fine-grained impermeable siliceous sediments, such as clays, where they are protected from oxidizing fluids. This preserves the structures as flattened organic compressions between sediment layers. (2) Replacement or coating by a wide range of minerals. However, different microorganisms have different susceptibilities for mineral fossilization and those that are particularly delicate may not fossilize at all, thus the microfossils preserved in a rock will not necessarily represent the original microbial community.

The preservation of larger scale biological constructs (biolaminated deposits or stromatolites) is aided by the association with sediments and carbonate precipitation. Such physical biosignatures may be mechanically destroyed by erosion (including impact erosion). As mineralogical structures, they can be corroded, for instance by acidic ground waters if they have a carbonate composition. The complicated post-diagenetic history of aqueous alteration of the sediments at Meridiani Planum is illustrative of the processes that could have affected potential Martian microbial structures. Changes to the rock encasing the physical structures brought about by different types of metamorphism (shock, thermal), will induce gradual destruction of the structures depending upon the degree of metamorphism. For example, Early Archaean terrestrial rocks that have undergone little more than burial metamorphism (prehnite-pumpellyite to lowermost greenschist facies) contain well preserved physical biosignatures. In the long term, because the degradation of organic biosignatures over time is inevitable, physical biosignatures have a greater chance of preservation than complex organic markers.

Preservation of biominerals

The range of minerals passively formed as a result of microbial metabolism is very large. As with fossilized microbial structures (as above), the preservation of biominerals will depend on the history of alteration (metamorphic, chemical, physical) of the rock after formation.

References

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