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Organic matter and biomarkers: Why are samples required?

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The search for evidence of past prebiotic or biotic activity on Mars will be enhanced by the return of samples to Earth laboratories. While impressive analytical feats have been accomplished by in situ missions on the red planet, accessing the capabilities of Earth's global laboratories will present a step change in data acquisition. Highly diagnostic markers of past life are biomarkers, organic molecules whose architecture can be attributed to once living organisms. Similar organic molecular structures can also be used to identify the prebiotic steps that preceded any emergence of life. The style of modification or degradation of such organic structures indicates their agents of change, including oxidants, radiation, heating, water, and pressure. For biomarker analysis, sample return provides enhanced opportunities for sample preparation and analyte isolation. The augmentation of biomarker data with spatial information provides the opportunity for confirmatory data but is a multistep and multitechnique process best achieved here on Earth. Efficient use of returned samples will benefit from lessons learned on Earth's ancient records and meteorites from Mars. The next decade is a time when analytical capabilities can be improved as we prepare for the delivery of carefully selected and collected extraterrestrial samples containing potential evidence of the development or even emergence of past life on Mars.

Mars | astrobiology | organic | sample return

Mars Sample Return aims to retrieve samples of the Martian atmosphere, regolith, and rock from carefully chosen and recorded sites on Mars and return them to Earth for detailed analysis (1, 2). Robotic missions to Mars have previously analyzed samples from orbit or in situ in a fashion that precludes the level of analysis possible to achieve in laboratories here on Earth. The NASA Mars 2020 Perseverance rover mission will cache samples for return to Earth and represents the first leg of Mars Sample Return (3). The objectives of Mars Sample Return have been deliberated extensively, and the search for evidence of past or present life is one of the most exciting opportunities (1, 2).

Mars is the prime target for the search for life elsewhere in our solar system. Numerous missions have orbited and landed on the red planet. Observations have revealed that Mars had an early history characterized by an atmosphere far more dense than at present and with liquid water at or near the surface (4). Surface operations employing sophisticated instruments have revealed the presence of carbon-containing organic compounds (5–8). Yet, despite numerous investigations on or around Mars, conclusive evidence of past or present life has proved elusive.

Increasing certainty of evidence of life can be provided by the detection of organic carbon and its molecular specificity that may be diagnostic of life (9). A particularly information-rich type of carbon-based biosignature are biological markers or “biomarkers” (10). The biochemistry of an organism is a chemical adaptation to its habitat. The resulting organic structures reflect, therefore, both the organism and its environment. The science of biomarker analysis is well established for rocks on Earth (11). Combinations of evidence also influence definiteness, and multiple mutually confirmatory observations are more convincing than single observations. For Earth rocks, a complete ecosystem can be reconstructed using the fossil organic signatures contained within a single drop of organic extract, and similar approaches can be used for Mars.

Our ability to detect, recognize, and interpret organic biomarkers in Martian samples is enhanced back on Earth where access to instruments with maximal sensitivity, resolution, and diagnostic power is available. The detection of past or present life on Mars would be an extraordinary discovery and would require corresponding extraordinary evidence (12), most likely to be obtained using the combined power of Earth's state-of-the-art analytical laboratories.

Analytical Justification of Sample Return

Minerals on Mars are confounding the search for organic matter on in situ missions to the red planet (13–15). The effects were unpredicted and unexpected because some Mars minerals are not commonly associated with organic accumulations on Earth. Most in situ analyses on Mars have used thermal extraction techniques during which organic contents are volatilized or dissociated and thereby liberated from their mineral matrices. When juxtaposed organic matter and minerals are heated, some minerals can oxidize the organic matter or decompose to release oxygen (13–15).

Perchlorate salts were discovered on Mars by the Phoenix lander in 2008 (16). Perchlorates decompose under thermal stress to produce chlorine which combines with carbon to

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produce chlorohydrocarbons and oxygen that can facilitate combustion reactions (13). Other reactive minerals are also present on Mars. Sulfate salts decompose to release oxygen and contribute to combustion reactions using any organic matter present (15). Ferric iron oxides are another reactive mineral that can cause oxidation of organic matter while it transforms to its less oxidizing ferric state (17). The temperature at which mineral decomposition or reaction takes place is different for each mineral, providing the potential for degradation to obscure useful organic responses across a wide span of temperatures (18). Furthermore, resource restrictions associated with in situ analyses can lead to relatively enhanced analytical difficulties such as a comparably high instrument background that impairs trace detection of compounds, sample-to-sample carryover, and the inherent ambiguity of compound identification using evolved gas analysis by pyrolysis–gas chromatography-based techniques. State-of-the-art techniques such as compound-specific stable isotope analysis, position-specific isotope measurements, or clumped isotope analysis require more sophisticated equipment that is available in Earth-based laboratories.

The return of samples to Earth allows the detailed preparation of samples before analysis. For example, samples can be disaggregated in numerous ways before separation into fragments of certain physical and chemical composition. Once the target material is identified, there are several ways of isolating organic material (19). Soxhlet extraction and ultrasonic extraction are two common methods and great care is taken to avoid heating the samples or extracts to avoid degrading temperature-sensitive signals. Supercritical fluid extraction (20, 21) or solid phase microextraction (22, 23) can also be employed when the tailored extraction protocol is suitable for the analyte of interest. A multitude of solvents can be selected to dissolve particular analytes, ranging from water to supercritical carbon dioxide. Standards can be added for quantification to assess extraction efficiency. Standards can be introduced either to the rock samples prior to extraction, to the extract immediately before analysis, or after the analysis to test the responses of the instrument.

Conventionally molecular identification is assisted by preparative techniques that purify the analyte. Spectrometric responses are most convincing when the target analyte signal is at its greatest strength relative to background noise or contributions from overlapping organic responses. Once an extract is obtained, preparative chromatography can fractionate and isolate chemical classes of compound for bespoke treatment and analysis (19). The separation of compound classes exploits differences in dynamic equilibria for extracted compounds when presented with liquid and solid phases. Insoluble organic matter is commonly liberated by online or offline thermal extraction, either applied in isolation with oxygen excluded or accompanied by water or hydrogen (24). Insoluble organic matter can also be isolated by dissolution of mineral phases using specific acids (25).

Sample Return allows the removal of problematic minerals prior to analysis. For example, water leaching of Atacama Desert soil samples, which also have perchlorate contents thought to preclude normally expected detection of organic contents by pyrolysis–gas chromatography–mass spectrometry, was shown to be effective at removing the oxidizing salts

present (26). Pyrolysis–gas chromatography–mass spectrometry of the dried mineral residues following leaching generated biomarkers associated with indigenous microbes while, by contrast, the same samples analyzed without leaching by water failed to show detectable organic matter (27).

The organic compounds in returned samples may be diverse. Clues from terrestrial rock samples (28) and meteorites from asteroids (29) suggest that we must be prepared to accommodate a variety of compound types. Molecular weight variations are a common feature with a range of compound sizes extending from small units to highly networked structures. Polarity of compounds also varies, with nonpolar hydrocarbons and polar carboxylic or amino acids potentially present in the same sample. Martian meteorites (30) and in situ data from Mars (5) suggest that we must plan for at least some organic diversity in the returned samples and therefore provide access for multiple techniques back on Earth. These methods should be optimized for trace level detection of the analytes.

In Earth laboratories, sizable instruments can be brought to bear on small returned samples. Large electron microscopes can be employed for studying organic matter to provide high-resolution images and detailed structural information at small scales (31). The morphology, texture, and spatial distribution of organic materials can be imaged. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can be used to characterize the size, shape, and composition of organic materials; surface analysis techniques, such as atomic force microscopy (AFM) and environmental SEM (ESEM) can reveal fine details of organic materials and their interfaces with matrix minerals; energy-dispersive X-ray spectroscopy (EDS) coupled with electron microscopy allows for the mapping of the elemental composition and spatial distribution of organic matter.

The return of samples to Earth introduces important samples to large scientific facilities such as synchrotrons where analyses can be performed using intense beams of electromagnetic radiation across a broad range of wavelengths, from infrared light to X-rays (32). A high photon flux allows for sensitive detection and characterization of trace levels of organic molecules. Selection of optimal wavelengths and ideal techniques for the organic compounds under investigation alongside detailed imaging and mapping of organic materials at high spatial resolution, provide the potential to reveal organic–inorganic associations. Importantly, the returned sample set will be of finite mass and therefore highly valuable, making the nondestructive or minimally invasive nature of some synchrotron techniques ideally suited.

Another promising technique linking molecular imaging to element mapping on micrometer scales is the use of high-resolution imaging mass spectrometry (IMS) coupled to μ XRF (33). While the laser desorption ionization (LDI) technique of this method is destructive to the surface of samples, it only requires the use of minimal sample amounts and allows a volumetric reconstruction of the distribution of elements and organic molecules in a sample by using stacked thin slices. It could therefore aid in the identification of potential terrestrial contaminants in samples brought back to Earth. Current research also focuses on utilizing ultrahigh-resolution mass spectrometry for stable isotope analysis of larger molecules

(34) providing the possibility for reconstruction of position-specific and multiply substituted (clumped) isotope abundances, which can give critical insights into the origins of organic materials.

Abiotic Features

Carbonaceous chondrites are ancient meteorites that contain percentage levels of organic matter. They provide two important opportunities for Mars Sample Return. Firstly, they represent a bona fide example of abiotic organic matter (29) that can be a benchmark for interpretations of Mars samples. Secondly, they provide direct access to the organic materials that must fall, and may accumulate, on the surface of Mars. Abiotic organic materials in carbonaceous chondrites have a number of characteristic features that distinguish them from biological organic matter (9). Organic compound classes, often associated with biology, such as amino acids, are found in carbonaceous chondrites but display a number of nonterrestrial versions that have not been generally selected for use by Earth biochemistry. Enantiomers of amino acids also display nonterrestrial features, with both left and right-handed isomers present in near racemic mixtures, an observation that contrasts with the homochirality displayed by life on Earth.

In the aliphatic hydrocarbon and carboxylic acid fractions, there is structural diversity and a corresponding dominance of branched isomers, alongside a decrease in abundance with increasing carbon number (29). For life, enzymatically directed synthesis produces high proportions of specific molecular structures, exemplified by the fatty acids. In the aromatic fractions, the thermodynamically stable forms are more abundant, and polycyclic aromatic hydrocarbons are present. In life, there is no known synthetic route to polycyclic aromatic hydrocarbons. Nevertheless, polycyclic aromatic hydrocarbons in very old sample material can be generated from biological derived organic matter during geologic processes involving extended periods of exposure to elevated temperatures and pressures (35).

A very unique characteristic feature of extraterrestrial sourced material is enrichments in the heavier stable isotopes compared to terrestrial materials (9). Measured carbon and oxygen isotope ratios for evolved carbon dioxide and oxygen of aeolian deposits and sedimentary rocks analyzed by the Curiosity rover's Sample Analysis at Mars instrument (SAM) are in line with those observed in most Martian meteorites (36). Yet, it should be noted that there are also biological systems on Earth that can result in the generation of biomolecules that are strongly enriched in the heavy isotope of carbon (10).

Diagnostic molecular architectures can be augmented with stable isotopic compositions via compound-specific analysis. The systematic variation of stable isotopes between organic molecules is a useful way to recognize abiotic organic matter and this approach has been demonstrated on carbonaceous chondrites (37). For example, indigenous abiotic organic matter has been distinguished from biological contamination in carbonaceous meteorites (38). However, applying the systematic isotopic ordering known from Earth's biological systems to extraterrestrial biomass may not be straightforward, as the carbon source for Martian biomass may be isotopically heavier and biosynthetic routes and

enzyme activities during biomolecule synthesis may differ from that in terrestrial biology.

Even though organic molecules have unambiguous features that identify them as biologically produced, there are various geologic, cosmogenic, or diagenetic pathways that can obscure these original molecular features. Interrogating the information encoded in isotopes could help reveal the various processes that were evolved in formation of the respective organic molecules. The collected understanding of the stable isotopic compositions of indigenous abiotic organic matter in carbonaceous chondrites has been developed by a multitude of techniques, many that required preparation steps for compound extraction, isolation, and derivatization. Understanding of indigenous organic matter in samples returned from Mars should benefit from similar optimized techniques in Earth laboratories.

Biotic Features

In contrast to the structural diversity displayed by abiotic organic compounds, the compounds associated with life are structurally specific owing to enzyme-directed reactions that generate compounds for particular biochemical roles. Characteristics of life include particular structural isomers, the selected synthesis of homochiral amino acids, a lack of decline in abundance with carbon number, and the lack of thermally stable polycyclic aromatic hydrocarbons (9). The low statistical probability of the selected organic isomers found in life originating by alternative nonbiological means provides a highly convincing argument for source. For example, a 10 carbon atom alkane has over 70 potential isomers, a 12 carbon alkane can have over 350 isomers, and a 20 carbon alkane can have over 350,000 isomers (39).

Environmental Indicators

Organic biomarkers can provide insights into a range of past environmental conditions (Fig. 1). The distribution and abundance of biomarkers can reveal past modifications of organic compounds that are sensitive to the presence or absence of oxygen (40). For instance, certain lipid biomarkers, such as unsaturated fatty acids, are relatively susceptible to oxidation. By analyzing the abundance and preservation of these compounds, exposure to oxidants through geological time can be understood (41). Yet, organic biomarkers perhaps work best when present as a confirmatory set of signals. While potentially achievable by *in situ* analyses, multiple indicators of past oxidation are perhaps best achieved in Earth laboratories with greater analytical flexibility.

Organic records can also act as an indicator of past exposure to radiation. Studies of radioelement-containing sedimentary grains indicate that small molecules can be polymerized by radiation (42) while higher molecular weight organic matter will be degraded faster than smaller molecules (42, 43). Concurrent changes in irradiated high molecular weight organic materials include an increase in abundance of oxygen-containing compounds and an enrichment in the heavy stable isotope of carbon (42). The nature of irradiated organic materials is understood using multidisciplinary techniques (42, 44) and access to the various analytical instruments and microscopes needed to obtain

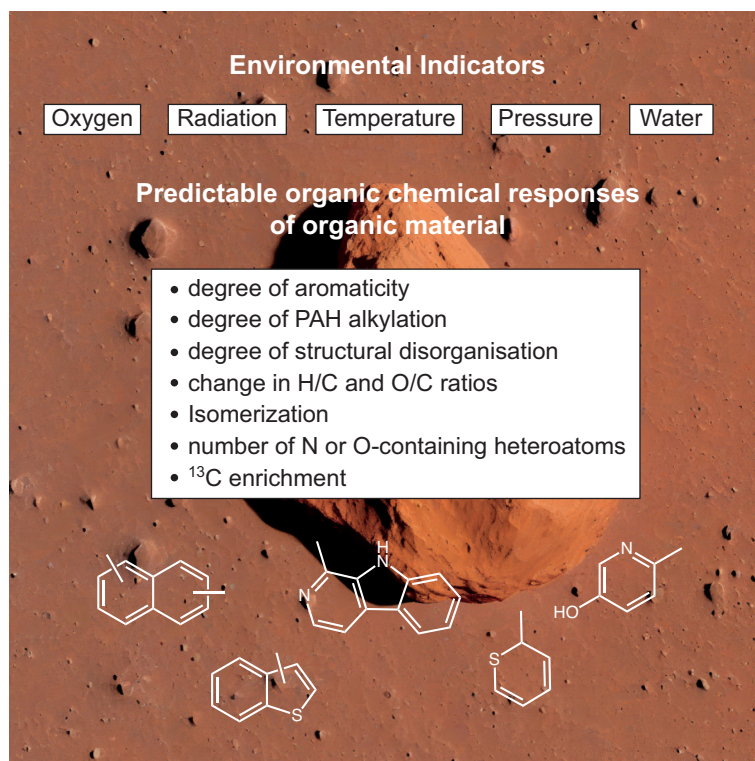


Fig. 1. Various characteristics of organic compounds that can be used for paleoenvironmental reconstructions.

complementary datasets to allow interpretation of intractable organic radiation residues is more realistic in Earth laboratories than in situ on Mars.

Organic maturity parameters (heat-sensitive ratios of isomers of organic structures) are useful indicators of the thermal history and maturity level of organic matter (45). These parameters help in understanding the degree of organic matter transformation and therefore the fidelity of the organic materials to their sources. If information is desired on the synthetic, or possibly biosynthetic, source then a low degree of thermal transformation is ideal. If the degree of transformation is more extensive, then the organic maturity parameters can be used to reveal the geological thermal history of the sample. Common organic maturity parameters on Earth utilize membrane lipids or their hydrogenated forms with straight chains or cyclic structures (28). Substituted and parental aromatic hydrocarbon structures are also used as maturity parameters (28). Because maturity parameters are based on the fundamental thermodynamic stability of organic compounds, the same principles can be applied to both abiotic and biotic organic matter (46). Although separation and identification of organic compounds and their isomers is achievable by in situ instruments, the separation of coeluting compounds with similar chemical structures is most easily achieved in highly resourced terrestrial laboratories using state-of-the-art technologies in isomer separation such as ion mobility mass spectrometry.

The molecular configuration of organic matter can contain information that reflects the influence of water. For compounds that are reasonably similar to their original biological chemical structure, a certain amount of chemical treatment may be required prior to analysis, e.g., derivatization procedures that require humidity-controlled conditions, and treatment in Earth

laboratories is optimal. Examples include the use of molecular inventories to discriminate between microbial life that has been preserved with high fidelity (47), the organic remains of microbial life that have experienced hydrothermal processing (48), or organic transformation products that reflect organic-inorganic reactions that occur in hydrothermal vents or serpentinizing systems (49). Organic indicators of aqueous processing include the presence of temperature parameters which are suppressed relative to other indicators of thermal evolution. These effects have been observed in both fossil biological organic matter (50) and the abiotic organic matter in meteorites (46). The identification of potentially contradictory information, and therefore recognising the correct interpretation of geochemical signals of past conditions, requires the type of multi-disciplinary studies that are best achieved in collaborating laboratories on Earth where a range of skills can be employed.

Organic matter can contain information that indicates the past influences of pressure. The organic chronicle of pressure can be read in a number of ways. In analogous fashion to isomeric responses to heat, certain isomers of small molecules respond to pressure. Substituted polycyclic aromatic hydrocarbons have been shown to act as cosmobarometers (51) and can be used to reveal the influence of pressure from organic materials that have experienced a complex geological history. Polycyclic aromatic nitrogenated heterocycles also display pressure responses that differ from polycyclic aromatic hydrocarbons with similar numbers of rings demonstrating barometry potential (52). Larger organic molecules may also contain pressure information. The pressures associated with impact ejection from the Martian subsurface appear to selectively degrade long-chain hydrocarbon structures that are normally associated with microbes (53). The residual aromatic hydrocarbon-dominated organic networks

would superficially, and potentially incorrectly, indicate a dominant abiotic input from meteoritic materials. Correctly interpreting the signals of Martian samples requires an appreciation of the geological history of the samples. Organic materials are, therefore, capable of misinterpretations and multidisciplinary investigations in Earth laboratories can at least partly mitigate against any loss in organic record fidelity.

Biomarker Distributions

Alongside and augmenting bulk analysis techniques, spatial techniques are also crucial to understanding the provenance and formation characteristics of organic material. It is at a high spatial scale that sample preparation becomes difficult to accomplish in any other setting than Earth-based laboratories. A most comprehensive understanding of the provenance and formation mechanisms of organic material requires analysis from the centimeter to the nano-scale by techniques that can identify both the mineralogy and its association with organic materials. Typically, these techniques attain datasets that are complementary to the molecular identifications undertaken by bulk extraction techniques.

Organic matter can be distributed so that it correlates with certain minerals or textures in geological samples (54). The distribution of organic matter and its correlation with other components depends on the environment in which it formed, the processes to which it has been subjected, and the geological history of the rock. Examination of the distribution and composition of organic matter within samples can help in reconstructions of past environmental, depositional and alteration histories. Once the correlation of organic matter with other components is understood the presence of organic biomarkers can support interpretations of past conditions.

In the water column on Earth, mineral surfaces can be rapidly coated by a conditioning film of biologically sourced macromolecular organic matter (55). These organic molecules originate from dissolved and particulate matter in the seawater. On the present-day Earth, the conditioning film serves as a substrate for the subsequent (rapid) adherence of microbial cells (56–58). In detrital sediments, the organic components are distributed within sediments as either fine-grained particulate matter or, if in dissolved form, eventually bound to mineral surfaces, such as clay minerals (59). Life itself may be associated with minerals with epilithic microbial colonies common on submarine volcanic glass (60, 61), and partially fossilized cells have been observed on modern glasses (62). In certain sedimentary situations, both abiotic and biotic organic matter may be present in the same sample. Organic carbon of both abiotic and biogenic origin may contribute to the detrital fraction of a sediment, or they may be formed in situ, for example, as remnants of microbial colonization of abiotic organic matter-containing sediment surfaces, sediment particles, or fractures in sediments and rocks as suggested by studies on ancient terrestrial samples.

The presence of organic matter of extraterrestrial origin, probably from micrometeorites and in the form of fine particles, has been recognized in 3.33 Ga coastal volcanic sediments from the Josefsdal Chert, Barberton Greenstone Belt

(63), and is intermixed with organic matter of biogenic origin (Fig. 2A–C). Detrital carbonaceous matter was observed intermixed with similar aged volcanic sediments from the 3.45 Ga Kitty's Gap Chert, Pilbara Greenstone Belt. The detrital fragments were of both biogenic origin (torn fragments of microbial biofilms), as well as other, solid fragments that were clearly reworked from buried crustal material that was probably abiotic (Fig. 2D) (64–66). Fossilized epilithic colonies, similar to those observed in modern settings have been observed in volcanic sediments in the Kitty's Gap Chert, 3.45 Ga, from the Pilbara (Fig. 2E–H) (64, 66)

Importantly, the Kitty's Gap Chert findings were only possible by combining multiple techniques in a fashion not currently possible without operation in Earth-based laboratories. The great age of the samples (the oldest cellular microfossils documented to date) alongside their simple coccoidal morphologies made confirmatory techniques necessary. Further investigations using state-of-the-art instrumentation, e.g., synchrotron deep ultraviolet and FTIR (Fig. 1I–L), as well as numerous other techniques (micro-Raman, micro-Particle Induced X-Ray Emission Spectroscopy, and in situ Laser Ablation Inductively Coupled Plasma Mass Spectrometry), were necessary to prove biogenicity through analysis of the distribution, composition, and structure of the organic matter (66). This example demonstrates the necessity for sample return in order to ensure the most robust characterization of organic materials. The Perseverance rover is collecting a variety of samples, ranging from volcanic and chemical sediments to volcanic rocks, all of which should contain a background signature of abiotic molecules of meteoritic origin (5, 8), as well as of mantle/crustal origin (68–70). In addition, the samples may contain organic traces of past life. The distribution of both abiotic and potentially biogenic components in the returned rocks and sediments will depend on the origin of the organic matter.

Degraded Organic Records

Planetary surfaces provide multiple opportunities for the degradation of organic biomarkers. Degradative processes include radiation of various types, a range of oxidants, physical attrition and, if life ever existed, possible microbial biodegradation. Under degradative conditions, the ability to interpret organic signals becomes more difficult. Distinguishing between abiotic and biotic organic materials can become more challenging as degradation proceeds, as indicated by comparisons of Jurassic paleosols fed with charcoals from forest fires and the Murchison meteorite (Fig. 3) (26). Although free small molecules may be subject to degradation, greater relative preservation often occurs in the polar fractions of organic extracts (71). Larger organic networks may also contain relatively high-fidelity organic structures, protected by their bound or occluded nature (72). Under these conditions, accessing the vestiges of an organic source mechanism requires liberation of the preserved and protected organic entities by chemical and/or thermal pretreatment prior to analysis (71, 73, 74). Many of these more complex procedures are multicomponent or multistep and at the current level require Earth laboratories. Returning materials to Earth can raise the chances of detecting what signals still remain in degraded samples.

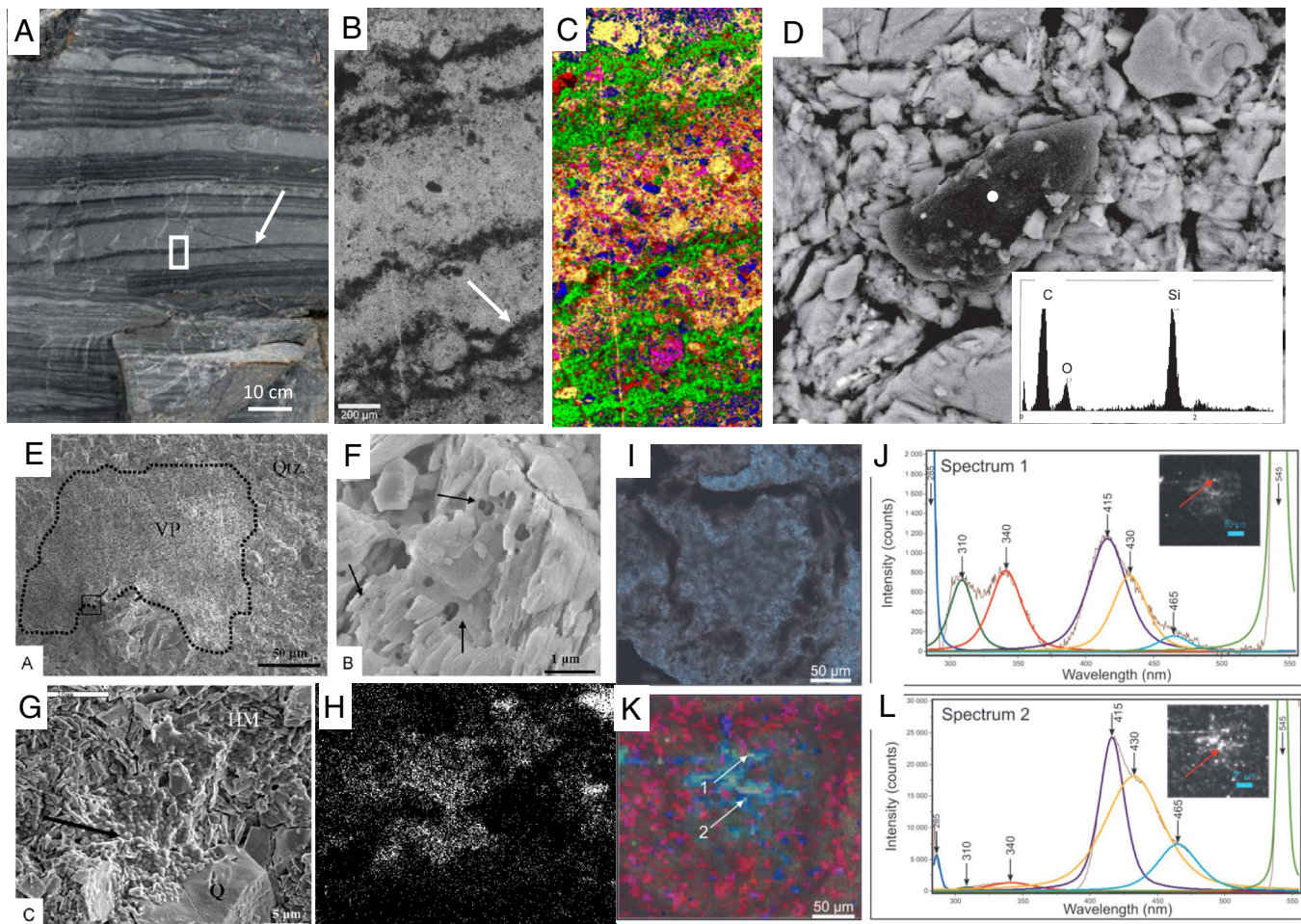


Fig. 2. Organic biosignatures in Paleoproterozoic volcanic sediments. (A–C) Layers of ashfall into shallow, coastal waters, the darker layers enriched in lighter, fine-grained, particulate detrital organic matter of biogenic as well as extraterrestrial origin: 3.33 Ga Josefsdal Chert, Barberton Greenstone Belt, South Africa (63) (A–C: Reprinted from ref. 63, with permission from Elsevier). (A) Field photograph with layer of interest highlighted by the white arrow and white rectangle. (B) Thin section of the layer (in fact, a triple layer) in the white rectangle, highlighting the organic-enriched layers. The white arrow points to a particular layer that contains extraterrestrial organics. (C) Raman spectral map of the thin section in (B); yellow = quartz matrix; green = carbonaceous matter; blue = anatase; pink = muscovite, the latter two are alteration products of the volcanic detrital component. (D) Angular fragment of solid detrital carbonaceous matter; SEM backscatter image with corresponding EDX spot analysis (white spot) of the carbonaceous particle. 3.45 Ga Kitty's Gap Chert, Pilbara Greenstone Belt, Australia (64) (D: Used with permission of the Geological Society of America, from ref. 64; permission conveyed through Copyright Clearance Center, Inc.). (E–H) Fossilized epilithic microbial colonies on the surface of a phyllosilicate-altered volcanic detrital particle in the Kitty's Gap Chert. (E) SEM image of a fine-grained, phyllosilicate-altered particle (VP) embedded in a quartz cement matrix (Qtz) (E: Used with permission of the Geological Society of America, from ref. 64; permission conveyed through Copyright Clearance Center, Inc.). (F) Fine organic fibrils (black arrows) stretched between the phyllosilicate leaflets (now muscovite) (F: Reprinted from ref. 65, with permission from Elsevier.). (G) Fossilised colony of silicified chemolithotrophic at edge of volcanic particle [black rectangle in (E)] (67) (G and H: Used with permission of the Geological Society of America, from ref. 64; permission conveyed through Copyright Clearance Center, Inc.). (I–L) In situ DUV analysis of the organic and mineral composition of a volcanic particle (I: optical image; K: DUV spectral map) in the Kitty's Gap Chert, documenting aromatic groups (Spectrum 1), as well as mineral peaks (spectrum 2) (66) (I–L: Reprinted from ref. 66. The publisher for this copyrighted material is Mary Ann Liebert, Inc.).

Organic Controversies

The history of organic analysis in extraterrestrial objects, the controversies that developed, and the resolution of those controversies by fresh sample and state-of-the-art analytical techniques provide strong justification for Mars Sample Return. Straight chain hydrocarbons (*n*-alkanes) were observed in the Orgueil carbonaceous chondrite in the 1960s and their similarity to “the products of living things and sediments on earth” used to infer a biogenic origin (75). Interpretation of biological inputs appeared to be supported by the presence of “organized elements” believed to be microfossils indigenous to the meteorite (76). Other authors noted the increased concentration of the straight chain hydrocarbons on the surface of the samples and an association of these alkanes with branched versions (isoprenoidal hydrocarbons) indicative of Earth life (77, 78). Eventually

more modern analytical techniques including compound-specific isotope analysis revealed a terrestrial origin (38).

A similar story emerged in the 1990s, when the Martian meteorite ALH84001 was reported to contain organic compounds and potential microfossils alongside other potentially biological observations (79). Yet, the polycyclic aromatic hydrocarbons detected have no known biochemical synthetic route, they are simply a common transformation product of any organic matter. Moreover, the smallest potential microfossils would be too small to contain all the biochemical components required by life (80). Furthermore, terrestrial contamination was found in the meteorite (81). More recently, ALH 84001 has been shown to contain organic material produced by abiotic processes including serpentinization (70).

In a cautionary comment that is as relevant to returned Mars samples in the 2030s as it was to carbonaceous chondrites in the 1960s, J.D. Bernal encouraged scientists to avoid

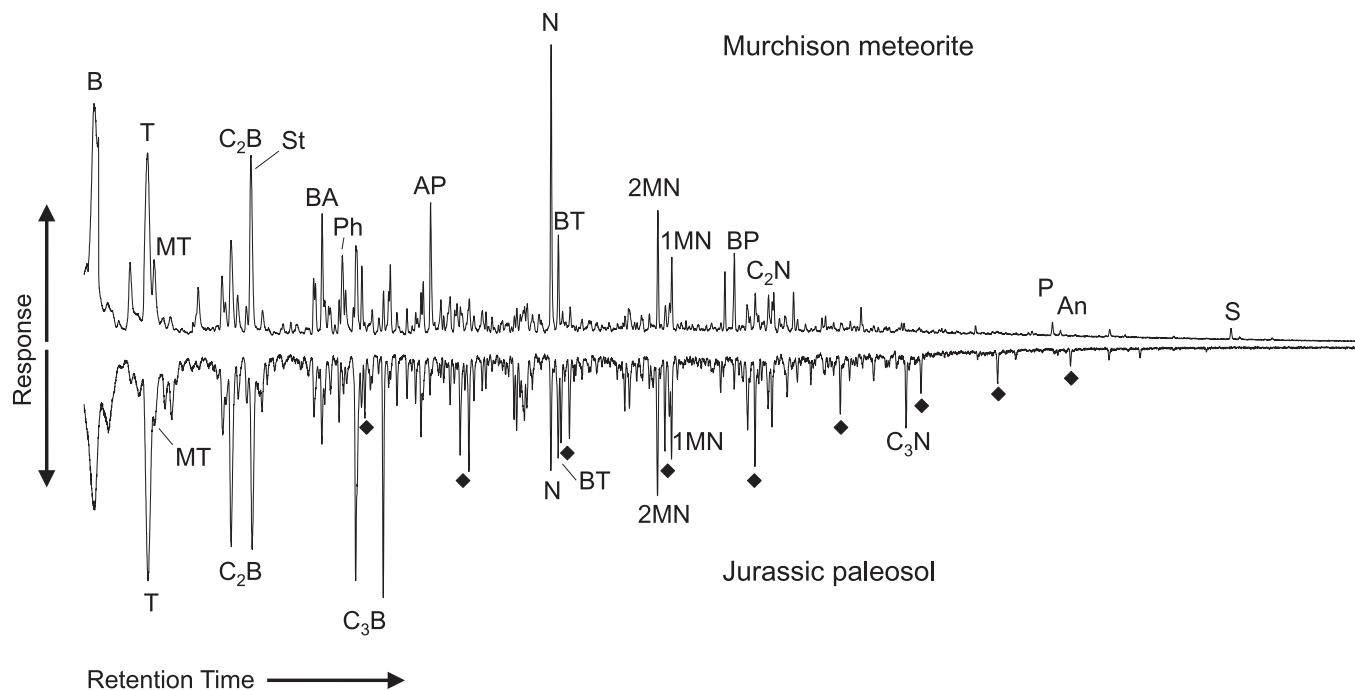


Fig. 3. All roads lead to Rome. A comparison of total ion currents of pyrolysis products (600 °C) from a Jurassic paleosol containing reworked charcoal and the Murchison carbonaceous chondrite (26). The relative responses of all samples have been scaled to allow comparison. It can be seen that the key responses from the meteorite are also present in the pyrolysis products of the degraded materials in the paleosol. Preservation is key because degradation reduces the diagnostic potential of biomarkers. Some biogenic character still remains in the paleosol, evidenced by the exaggerated responses for certain C_2 - and C_3 -alkylbenzenes (C_2B and C_3B) and the presence of *n*-alkene/*n*-alkane pairs (filled diamonds). Other assignments are B = benzene; T = toluene; MT = methylthiophene; C_2B = C_2 -alkylbenzenes; BA = benzaldehyde; Ph = phenol; C_3B = C_3 -alkylbenzenes; AP = acetophenone; N = naphthalene; BT = benzothiophene; 2MN = 2-methylnaphthalene; 1MN = 1-methylnaphthalene; BP = biphenyl; C_2N = C_2 -alkylnaphthalenes; C_3N = C_3 -alkylnaphthalenes; P = phenanthrene; An = anthracene; S = sulfur; and filled diamonds = *n*-alkene/*n*-alkane pairs. Data modified from ref. 26.

being ensnared by these "jokes of nature" (82). Encouragingly, these controversies were somewhat settled by the application of an understanding of the curatorial history of the samples and modern analytical techniques. Mars Sample Return provides us with the opportunity to prepare ourselves so that we keep discoveries maximized and controversies minimized.

Martian Meteorite Lessons

When preparing for organic analysis in returned Martian samples, the Martian meteorites we have on Earth and have previously analyzed are sensible analogues to examine. Studying Martian meteorites has already provided valuable insights into the nature of organic matter on Mars. Martian meteorites have been found to contain significant amounts of organic matter, some of which is present as small molecules and some of which is present as large organic networks dominated by aromatic structures. Martian meteorite organic matter appears to have an observable association with certain mineral phases. As with any sample containing relatively trace amounts of organic matter, excluding or recognizing terrestrial organic contamination is a persistent challenge.

Even with the difficulties of terrestrial contamination, *in situ* techniques have been used to reveal that Mars has an inventory of organic material formed through abiotic synthesis mechanisms, including electrochemical reduction of carbon dioxide, serpentinization, and carbonation (30, 68–70, 83). By marrying nano-scale organic characterization with *in-situ* hydrogen isotope analysis, an inventory of organic material in several Martian meteorites has been shown to be definitively Martian in origin. This has allowed studies using bulk extraction

techniques to begin to elucidate the definitive molecular makeup of Martian organic material, (5, 30, 84, 85). Indeed, recent studies have revealed close to 5,000 separate organic species showing N, O, S, Cl, and Mg species. All but the Mg functionality has previously been detected by *in situ* techniques in Martian meteorites and all but the N functionality have been confirmed by observations on the Curiosity rover.

Future Directions

Ambitious targets lead to advances as humanity strives to meet expected challenges. An example is provided by the preparation of laboratories for the return of samples from Earth's moon by the Apollo missions. It was into these laboratories that two new carbonaceous chondrite falls (Murchison and Allende) were fed to revolutionize our understanding of meteoritic organic matter (86, 87). The combination of new techniques and new samples settled controversies that had persisted for decades. We can expect similar advances as scientists await the return of samples from Mars. With a preliminary understanding of the nature and composition of the samples destined for return, analytical laboratories will have a short period to optimize and improve their techniques for an unprecedented and historic arrival of deliberately collected materials from another planet. Advances will be realized in the analytical field as smaller sample sizes are accommodated and greater diagnostic potential sought. Already, frameworks and protocols are being developed that involve organic analysis for planetary protection purposes. It can be hoped that as the returned samples are allocated to collaborating researchers and institutions on a global scale,

the sharing of knowledge, resources, and expertise will promote accelerated progress in analytical capabilities. Inevitably, the analytical advances achieved can be transferred immediately to other areas of science, such as environmental monitoring, medical diagnostics, and the careful use of Earth's resources, to prompt new discoveries and more efficient applications, as was the case during the Apollo Era (88).

Data, Materials, and Software Availability. All study data are included in the main text.

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