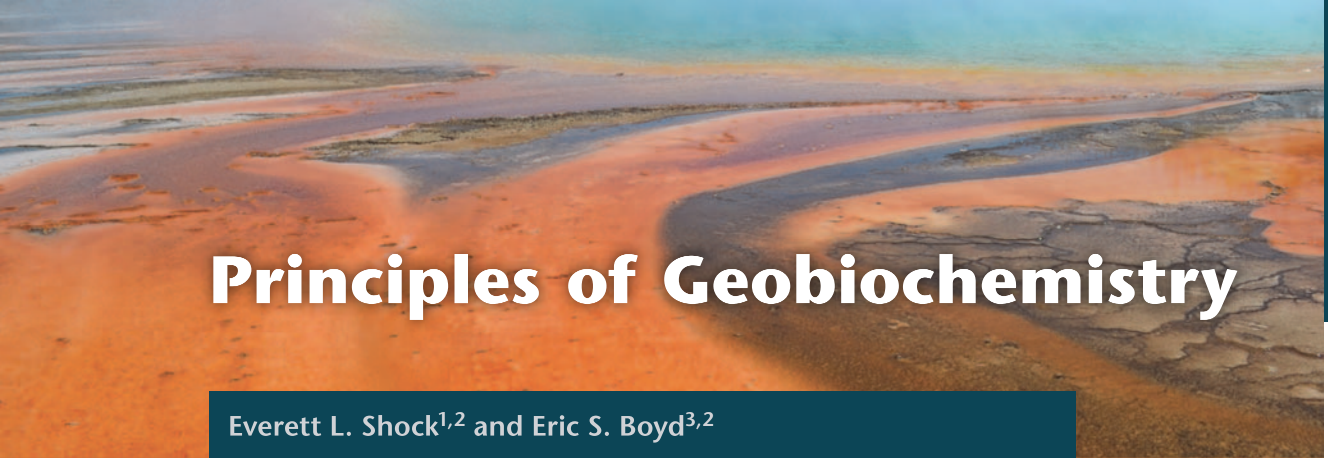

Principles of Geobiochemistry

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The basic premise of geobiochemistry is that life emerged on Earth where there were opportunities for catalysis to expedite the release of chemical energy in water–rock–organic systems. In this framework, life is a planetary response to the dilemma that cooling decreases the rates of abiotic processes to the point that chemical energy becomes trapped. Catalysis via metabolism releases the trapped energy, and life benefits by capturing some of the energy released. Out of necessity, biochemical processes have geochemical origins, and geobiochemistry asserts that these origins can be revealed by mapping reaction mechanisms onto deep time. We propose five principles that should help guide research in the emerging field of geobiochemistry.

KEYWORDS: geobiochemistry, geochemistry, biochemistry, thermodynamics

INTRODUCTION

The venerable field of biogeochemistry has the goal of understanding how biology affects geochemistry. The complementary emerging field of geobiochemistry aims to explain how geology affects biochemistry. This is a postgenomic topic, fueled by the huge supply of new data emerging from molecular biology and the efforts of geoscientists to incorporate molecular methods into their field, laboratory, and theoretical work (Woese et al. 1990). It benefits from efforts to predict the thermodynamic properties of organic compounds, including biomolecules, at all temperatures and pressures where life exists, as well as from recent analytical advances that allow the characterization of lipids, proteins, nucleic acids and other large biomolecules in geologic samples. Geobiochemistry also harbors a perspective from deep time that links biological evolution recorded in genes with geologic history recorded in rocks (Lyons et al. 2015 this issue), as seen through the lens of evolutionary analyses.

The tools are now in place to study how geologic processes affect biochemistry, both today and in Earth's past. Extraction of DNA and RNA from geological samples is increasingly routine, although fully exploiting the data locked in diverse sediment and rock compositions will require further analytical developments. Nevertheless, there are growing databases of gene sequences from complex soil, sediment, and rock samples that are ushering in an era of genomics and transcriptomics (Dick and Lam 2015 this issue). Armed with these new data, researchers can explicitly link evolutionary relations, as well as changes in protein-encoding genes and their transcripts, with the temperatures, pressures, and compositions of geochemical processes. Likewise, obtaining lipid extracts from geological samples is increasingly common, and recent developments indicate that massive amounts of lipid data may soon rival data from gene sequencing efforts (Wörmer et al. 2014). Given their long lifespans once they reach the geologic record, lipids are rich stores of preserved information about how major developments in geologic processes are reflected in biochemistry and vice versa.

Advances in mass spectrometry and in the identification of the genes involved in lipid biosynthesis are accelerating the rate by which links are forged between the distribution of lipids and taxonomic and functional information (Welander et al. 2010; Briggs and Summons 2014). This information will provide valuable new approaches to link geological change with major biological innovations in deep time. We can also anticipate making far greater use of the molecular transformations of lipids throughout diagenesis and use these transformations as tracers of geologic history subsequent to lipid burial. Substantial progress is also being made in linking stable isotope compositions to taxonomy and/or metabolism in extant microorganisms to provide new constraints on the evolution of biogeochemical cycles. Lagging somewhat behind are methods to extract proteins from complex environmental matrices, which, when such methods become possible, will allow the development of new proteomic approaches to define far more intimate links between the dynamic changes of geochemical compositions and shifts in gene expression and metabolic activity.

Owing to progress made in the field of theoretical geochemistry it is now possible to calculate the standard state thermodynamic properties of thousands of organic compounds (Amend et al. 2013), including proteins and other biomolecules. As a consequence, we can now estimate the energy required to make the biomolecules found in fluids of diverse geochemical compositions at temperatures and pressures where life is known to exist, and beyond. This means that biomolecules and biochemical processes can be fully integrated into theoretical models of water–rock reactions, which allows tests of geobiochemical hypotheses that are independent from observations of natural systems. Hydrothermal experiments, long-used in

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the fossil fuel industry to study how organic compounds are transformed during geologic processes, are now being used to answer outstanding questions in geobiochemistry (McCollom and Seewald 2003; Seewald 2003; Shipp et al. 2013; Shock et al. 2013). Although still at an early stage, these efforts are revealing which reactions are reversible and which primarily undergo irreversible transformations during geologic processes.

The convergence of these streams of research makes it possible for geochemists, microbiologists, and biochemists to interact in previously unforeseen ways. As we witness the development of geobiochemistry as a new field of investigation it is propitious to consider some general principles that can help guide hypothesis generation.

The goal of this communication is to articulate five of these principles. By no means are these inclusive of all the possibilities that geobiochemistry offers, and they are certainly unable to articulate insights that will become possible owing to new advances.

PRINCIPLE 1: LIFE EMERGES AS A PLANETARY RESPONSE

If we are to work from the assumption that "the biochemistry we have is one that the Earth allows" (Craig Manning, pers. comm.), then we are also implicitly assuming that—alongside differentiation, mantle convection, volcanism, tectonics, metamorphism, hydrothermal alteration, weathering, and climate change—life is a planetary process. Constructing evolutionary trees of life has blossomed over the past 30 years as part of the revolution in molecular biology. These trees suggest that the use of chemical energy preceded the use of light energy to drive metabolism (Decker et al. 1970). Life on Earth appears to have emerged in response to the flow of energy through a water–rock–organic system (Martin et al. 2008). Why is this the case?

At the high temperatures and pressures of most magmatic, metamorphic, and hydrothermal processes, the approach to thermodynamic equilibrium is rapid enough that it is commonly attained. In these areas of research, measuring or predicting thermodynamic properties of substances and their solutions has afforded considerable experimental and theoretical progress. Modeling high-temperature/pressure planetary processes by assuming thermodynamic equilibrium often proves useful for describing differences between initial and final states. Computational models also make it possible to delve into the details of how equilibrium occurs, or to examine closely occasions where the underlying drive toward equilibrium is unrequited. At lower temperatures and pressures the drive toward equilibrium persists, but the avenues for attaining equilibrium become increasing convoluted. It is not uncommon for states of metastable equilibrium to develop if some reactions are reversible (Shock et al. 2013); but attainment of a truly stable equilibrium in low-temperature/pressure water–rock–organic systems is rare. The problem is that the duration of planetary processes can be insufficient to attain equilibrium owing to the sluggish rates of chemical reactions. As a result, many natural systems persist at stages of partial reaction progress between initial and final states. Models of low-temperature/pressure processes are inherently complicated by kinetic rate laws, which account for the failure of natural systems to attain stable equilibrium due to disruptions in the flow of energy. Experimental and theoretical work is dominated by testing reaction mechanisms to account for these rate laws, with the hope of developing predictive methods.

When reactions that are thermodynamically favorable meet profound mechanistic or kinetic barriers, catalysis emerges as a major factor in determining what actually happens. In essence, life is a collection of complex adaptive catalysts tuned to energy supplies that persist unexpended owing to abiotic complications that inhibit reaction progress. Without those abiotic mechanistic difficulties, life would not be possible. It follows that life is "allowed" by the mechanistic complications of low-temperature geochemistry, and life owes its existence to interferences and hurdles in the relentless drive for the release of chemical energy. If life is generated as a planetary response to expediting the release of energy, then biochemistry is the manifestation of how catalysis is accomplished. Familiar biochemistry emerged, in large part, to dissipate Earth's energy, which would otherwise be tied up due to mechanistic or kinetic inhibitions. The extent to which biochemistry on another world (exobiochemistry) might be familiar depends on whether the same mechanistic complications to abiotic chemical energy dissipation exist. The ranges of compositional variability inferred by the ongoing search for exoplanets suggest that diverse and potentially unfamiliar planetary processes are possible. How chemical energy dissipation is thwarted in these processes may differ from the way energy is dissipated on Earth. It is expected that exobiochemistry will differ accordingly. Geobiochemistry offers the potential of predicting those differences.

PRINCIPLE 2: BIOCHEMICAL PROCESSES HAVE GEOCHEMICAL ORIGINS

Biochemical processes emerged within a system of existing abiotic geochemical processes. It is a theme of geobiochemical research that processes can be traced through the transitions from geochemistry to biochemistry. Furthermore, ongoing research will eventually reveal how such transitions occurred. As an example, mixing of hydrothermal fluids and seawater at submarine hydrothermal systems can create geochemical conditions that favor the synthesis of the aggregate biological monomers that compose biomolecules (Shock and Canovas 2010; Amend et al. 2013). The thermodynamic drives of systems hosted in both peridotite and basalt are such that biosynthesis *releases* energy available in the seawater–hydrothermal fluid mixture. The fact that a geochemical consequence of an unavoidable geologic process of fluid mixing is to generate conditions where biosynthesis is thermodynamically favored is often cited as supporting evidence for geochemical explanations for the emergence of life. It also reminds us that conditions in other parts of the Earth differ radically from conditions required by humans and that assumptions based on Earth's surface conditions, including the pervasive notion that there are always energetic costs to biosynthesis, should be avoided (Amend and Shock 1998).

The tricarboxylic acid cycle (also termed the citric acid cycle, or Krebs cycle) consists of a series of enzyme-directed chemical reactions that involve a handful of C_4, C_5, and C_6 organic acid anions (Fig. 1A). This cycle plays a central role in the metabolism of many microorganisms. The citric acid cycle is described as proceeding forward (clockwise in Fig. 1A) when it operates in the direction familiar from introductory biology courses, i.e. when citrate is progressively altered through several steps to ultimately generate oxaloacetate and then regenerated again when oxaloacetate and acetate (from pyruvate, and in the activated form of acetyl CoA) combine to form citrate. The function of the forward citric acid cycle is to convert organic compounds into CO_2 and H_2O and capture the energy released, as well as to provide intermediates for the biosynthesis of amino acids and other compounds. The cycle is thought of as operating in reverse (counterclockwise in Fig. 1A) when CO_2 and H_2O are combined into organic compounds starting with oxaloacetate and ultimately yielding citrate,

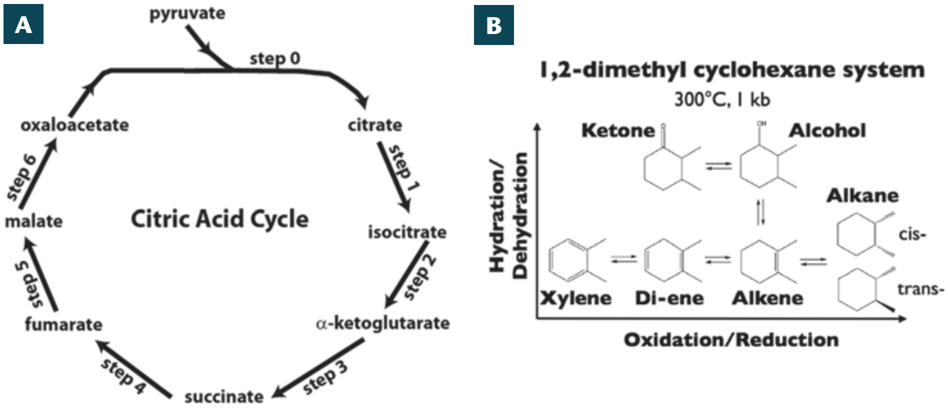

FIGURE 1 (**A**) Steps in the tricarboxylic acid cycle (also termed the citric acid cycle, or Krebs cycle) are reversible when managed by enzymes (see BOX 1), in much the same way that hydration/dehydration and oxidation/reduction reactions (**B**) are reversible in hydrothermal experiments. This could be an example of how high-temperature geochemical reaction pathways were taken over by enzymatic processes in biochemistry.

which then reacts to form acetyl-CoA and oxaloacetate, so allowing the cycle to continue (Fuchs 2011). The reversibility of reactions in the citric acid cycle is heavily managed by enzymes, and it is that very reversibility that makes the citric acid cycle such a versatile system of reactions for energy production or biosynthesis, depending on the needs of various types of organisms.

As described in BOX 1, the citric acid cycle consists of hydrogenation/dehydrogenation, hydration/dehydration and coupled redox–carboxylation/decarboxylation reactions. Hydrothermal experiments show that many of the underlying fundamental reactions are also reversible at high temperatures and pressures. As shown in FIGURE 1B, analogous hydrogenation/dehydrogenation reactions between ketones and alcohols, as well as between alkenes and alkanes, and hydration/dehydration reactions between alcohols and alkenes, are central to the reversible hydrothermal transformation reactions that link ketones, alcohols, alkenes, and alkanes (McCollom and Seewald 2003; Seewald 2003; Shipp et al. 2013). Evidence from natural systems supports the involvement of these reversible reactions in establishing metastable equilibria among organic compounds in sedimentary basins and hydrothermal systems (Shock et al. 2013). Evidently, the reversibility inherent to the usefulness of the citric acid cycle is a normal feature of aqueous organic reactions at high temperatures and pressures. This leads us to the hypothesis that the occurrence of the citric acid cycle is a geobiochemical fossil. In effect, the role of the enzymes in managing and expediting the steps in the cycle is like heating these compounds to the temperatures and pressures where these reversible reactions happen rapidly. Perhaps the citric acid cycle results from life taking advantage of organic reaction mechanisms that are already in place, but that are slow to operate at lower temperatures and pressures.

Box 1

There are only a few types of reactions in the citric acid cycle. There are hydrogenation–dehydrogenation (redox) reactions like that between oxaloacetate and malate, which can be written as

$$C_4H_2O_5^{-2} + H_2 = C_4H_4O_5^{-2}$$

where a carbonyl group is converted to an alcohol and vice versa, or that between fumarate and succinate, which can be written as

$$C_4H_2O_4^{-2} + H_2 = C_4H_4O_4^{-2}$$

where the double bond in fumarate is converted to the single bond in succinate and vice versa. There are also hydration–dehydration reactions like that between fumarate and malate, which is given by

$$C_4H_2O_4^{-2} + H_2O = C_4H_4O_5^{-2}$$

and involves transitions between a hydroxyl group and a double bond. In addition, there are coupled redox and carboxylation/decarboxylation reactions like that between isocitrate and alpha-ketoglutarate, which can be written as

$$C_6H_5O_7^{-3} + H^+ = C_5H_4O_5^{-2} + CO_2 + H_2$$

PRINCIPLE 3: ENZYMATIC PROCESSES RECAPITULATE MINERAL CATALYSIS

Similarities in the structure and reactivity of metal-bearing minerals and of the metal clusters that form the active sites of metalloenzymes has led to the hypothesis that the metal centers in many enzyme cofactors are vestiges of mineral catalysts (Russell and Martin 2004). These similarities are particularly pronounced when iron–sulfur (Fe–S) minerals and enzymes are compared (FIGS. 2A AND 2B). For example, the common Fe–S minerals pyrrhotite ($Fe_{1-x}S$) and pyrite (FeS_2) participate in numerous redox reactions, including those involving hydrogen, nitrogen, and carbon dioxide—reactions which are considered by many to have been integral to the origin of life (Fuchs 2011). Similarly, the Fe–S enzymes hydrogenase, nitrogenase (Nif), and carbon monoxide dehydrogenase catalyze interconversions of hydrogen, nitrogen, and carbon dioxide, respectively, with the key redox reactions occurring at the active site Fe–S metal clusters of each enzyme (Boyd et al. 2014b) (FIG. 2A).

The reactions catalyzed by Fe–S minerals, and the fact that these minerals are similar in structure and reactivity to many Fe–S metal clusters in enzymes, have compelled many to think that Fe–S minerals may have played a central role in the origin of life (Boyd et al. 2014a). Key unanswered questions include how such mineral-based reactivity was emulated by primitive proteins, what the selective pressures were that led to these events, and when and where these events might have taken place. Clues to answering these questions may be obtained by comparing and contrasting (i) abiotic and biotic catalysis and (ii) mineral structures and the active-site metal clusters of modern day enzymes.

The near-universal occurrence of Fe–S enzymes in the three domains of life (Archaea, Bacteria, and Eukarya) and the fact that these enzymes play a central role in a variety of ancient biogeochemical processes strongly suggest that they were present in the last common ancestor (LCA) of Archaea and Bacteria (Boyd et al. 2014b). It then follows that simple Fe–S clusters, such as those illustrated in FIGURE 2A, may have been synthesized for use by biology early in life's history, perhaps prior to 3.8 Ga, through what could be described as a form of convergent mineralogical and biological evolution. If true, this would imply that the earliest forms of life, those that predate the LCA, may have lived vicariously through reactions catalyzed by metal-bearing minerals in their local environment and evolved proteins capable of ligating metal clusters derived from solubilized minerals. What was the selective pressure that drove the integration of these metal clusters into enzymes? One potential explanation is that conversions of inorganic nutrients (e.g. H_2O, CO_2, N_2) into organic forms capable of supporting early life by abiotic, mineral-based mechanisms

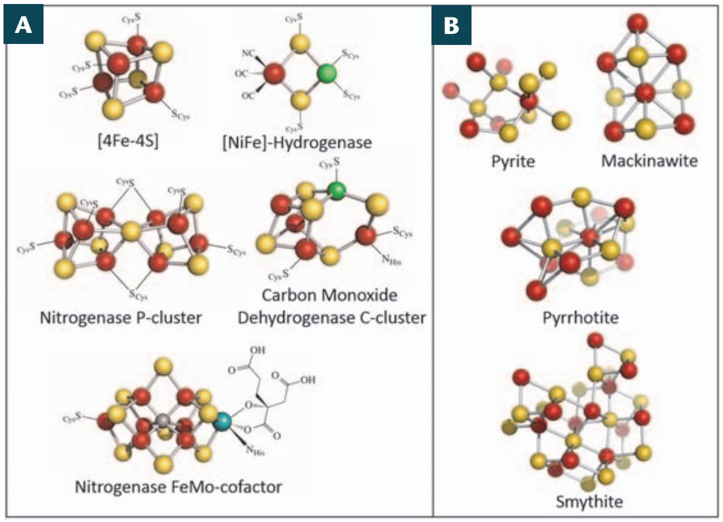

FIGURE 2 (**A**) Simple and complex Fe–S clusters present at the active sites of several enzymes hypothesized to have played key roles in the origin and evolution of life. Sulfur (red); iron (golden); nickel (green); carbon (grey); molybdenum (light blue). Organic ligands to the clusters including cyanide (CN), carbon monoxide (CO), cysteine (Cys) and histidine (His) are indicated. Protein database coordinates are as follows: [4Fe–4S] cluster (3C8Y), Nitrogenase P-cluster and FeMo cofactor (1M1N), carbon monoxide dehydrogenase C-cluster (1SU8), [NiFe]-hydrogenase (1H2R). (**B**) Structures of a variety of Fe–S minerals (not necessarily unit cells). Mineral structures were compiled from the Inorganic Crystal Structure Database (ICSD). The ICSD coordinates for pyrite (53529), mackinawite (42977), pyrrhotite (35009), and smythite (44885) were used in the generation of structures. Element colors as for 2A.

were accompanied by mechanistic or kinetic barriers (i.e. high activation energies) that may have limited the flux of substrates available to support life. Thus, in addition to focusing the reactivity and specificity for certain functions, the impetus to integrate metal clusters into biology may have been to lower the activation barriers of these reactions through the evolution of protein catalysts, which had the added benefits of releasing life from dependency on mineral surfaces and allowing diversification into new environment types (Boyd et al. 2014b).

As life diversified into oxic environments, how was it able to cope with the decreased availability of metals (e.g. Fe ions or FeS_2)? Insights into this question may come from the organic compounds (i.e. ligands) that tune the redox properties of Fe–S clusters in enzymes, including the carbon monoxide and cyanide ligands on the [NiFe]-hydrogenase active site cluster and the (homo)citrate ligand in the active site FeMo-cofactor of Mo-nitrogenase (Fig. 3A). Today, the low solubility of some minerals in many types of environments (e.g. oxic, sulfidic) and the limitations that this places on their use by biology is often overcome by chelators that have large equilibrium constants for metal complexation. Early life may have utilized similar, albeit less highly evolved, approaches to acquire metals to meet its biosynthetic needs. The low solubility of some minerals that contain Ni or Mo may have been overcome through chelation by simple compounds, such as carbon monoxide or cyanide, or by organic acids, such as (homo)citrate. Thus, the cyanide and carbon monoxide and (homo)citrate ligands present in the active site Fe–S cluster of [NiFe]-hydrogenase and Mo-nitrogenase (Fig. 3A), respectively, may be relics of the early need to increase the solubility of these metals for use in the biosynthesis of these enzymes' active sites (Boyd et al. 2014b).

The parallels in the structure and reactivity of minerals and enzyme metal clusters, as explored above, can be extended to other rock types, in particular those rich in ferrous iron. Reduction of water to hydrogen (H_2) occurs during serpentinization by oxidation of the ferrous iron component of mafic and ultramafic rocks, and the reaction of H_2 with the CO_2 that is dissolved in the ocean at hydrothermal systems favors the synthesis of reduced carbon compounds (Shock 1990), such as formate (HCOOH) and methane (CH_4) (McDermott et al. 2015) Biology has evolved, perhaps again mimicking abiotic mineral catalysis, a suite of hydrogenase enzymes with active sites comprising iron ([FeFe]-hydrogenase) or iron and nickel ([NiFe]-hydrogenases) that are involved in interconversion of H_2 and protons. It seems probable that [NiFe]-hydrogenase first evolved in an ancestor to the H_2-dependent methanogens (Boyd et al. 2014a), which use the primitive reductive acetyl CoA pathway for both ATP generation and CO_2 fixation (Martin 2012). The reductive acetyl CoA pathway is the only core CO_2 fixation pathway present in both Archaea and Bacteria, suggesting that it originated early in the evolution of life (Fuchs 2011). In this pathway, electrons from the oxidation of H_2 are sequentially transferred to CO_2, which in methanogens ultimately forms CH_4. The reductive acetyl CoA reaction pathway is chemically similar to that associated with abiotic synthesis reactions that accompany serpentinization (Martin 2012), namely, the initial reduction of CO_2 to formate/CO, followed by further reduction to CH_4. Thus, in addition to evolving enzymes that may recapitulate mineral-based catalysis, biology may have also evolved metabolic pathways that emulate abiotic reaction pathways.

PRINCIPLE 4: BIOLOGICAL INNOVATIONS OCCUR NEAR TO WHERE FIRST NEEDED

Evolutionary analyses of taxonomic (e.g. small subunit rRNA) genes (see Dick and Lam 2015 this issue), in the context of the environment from which those organisms were recovered, reveals that early branches in the archaeal and bacterial taxonomic domains are represented by organisms that inhabit high-temperature environments (Fig. 3A). This indicates that life may have its evolutionary roots in a high-temperature environment. What characteristics of such environments would be favorable for the origin of life and what metabolisms might have sustained this life? Many origin-of-life researchers prefer to think about early life as being fermentative, essentially to overcome the problem of limited availability of oxidants capable of supporting respiration. However, this primitive fermentative life would be dependent on abiotic sources of fixed carbon, which may not have been sufficient in supply or of appropriate composition to meet the needs of primitive biosynthetic pathways (Martin 2012).

The appeal of H_2-dependent organisms operating the reductive acetyl CoA pathways is that they only require CO_2 as a carbon source/oxidant and H_2 as reductant (Martin 2012), both of which are presumed to have been abundant in certain settings on early Earth, such as in hydrothermal systems. Methanogens, which are commonplace in many hydrothermal vent environments, use the reductive acetyl CoA pathway to generate energy in the form of ATP at the expense of reducing CO_2—in essence grabbing a free lunch that the organisms are paid to eat (Shock et al. 1998; Martin 2012). Thus, primitive methanogens operating the reductive acetyl CoA pathway overcome organic carbon limitation and at the same time generate ATP for metabolic purposes. To this end, life and autotrophic pathways appear to have evolved where they were first needed and most favorable, perhaps in high-temperature hydrothermal settings.

There is a growing interest among geobiologists in defining the environmental conditions that promoted the evolutionary innovations that in turn influenced the diversification of life. For example, evolutionary analysis indicates that the enzymatic machinery involved in the detoxification of mercury (Hg) ions has its evolutionary roots in high-temperature, volcanically heated hydrothermal environments (Barkay et al. 2010). To the ore geologist, this observation is perhaps not surprising: hydrothermal fluids can be enriched in Hg and, over geological time, concentrate Hg into economically important ore deposits. Thus, the enzyme responsible for the biological detoxification of Hg, the most toxic metal to biology because of its high affinity for sulfhydryl groups present in proteins (Barkay et al. 2010), appears to have evolved where it was first needed: hydrothermal environments where elevated concentrations of Hg naturally occur.

Molybdenum nitrogenase (Nif) catalyzes the reduction of dinitrogen (N_2) to ammonia (NH_3) and also appears to have evolved where it was first needed. Nif has modulated the availability of NH_3 in the biosphere since early in Earth's history (Boyd et al. 2011). Today, the availability of NH_3 often limits ecosystem productivity, which is overcome by the widespread application of industrially produced fertilizers. The metabolic costs associated with fixing N_2 may lead one to imagine an origin for this process in an NH_3-limited organism operating a high energy-yielding reaction, such as photosynthesis or respiration using oxygen. However, evolutionary analysis of Nif reveals that the earliest evolving organisms, those which branch at the base of the tree, were H_2-dependent and thermophilic methanogens (Fig. 3B) (Boyd et al. 2015). Because methanogens are poisoned by oxygen (they are strict anaerobes), this finding implies an origin for Nif in a high-temperature, anoxic environment, which is consistent with the prevalence of Nif from anaerobic organisms that branch closer to the base of the tree than aerobic organisms (Fig. 3B). Energetically, H_2-dependent methanogenesis

$$4H_2 + CO_2 \rightarrow 2H_2O + CH_4 \qquad (1)$$

is one of the lowest energy-yielding reactions capable of sustaining life, with the energetic yield being highly dependent on temperature as well as on the activities of H_2, CO_2, and CH_4 in the local environment (Amend and Shock 2001). For these reasons, it seems contradictory that selective pressure to evolve the energy-intensive process of N_2 fixation would act on an ancestor of H_2-dependent methanogens, leading to the emergence of this enzymatic machinery.

Three possible explanations are proposed to resolve this paradox. The first explanation considers the limitation of NH_3. Methanogens living near hydrothermal vents may have had ample supplies of fixed NH_3 created through cooling and reequilibration of volcanic gases and mineral-based catalysis (Dörr et al. 2003). However, the flux of NH_3 would have been finite, and, as biology became more productive, NH_3 may have become limiting and provided the selective pressure to evolve this enzyme complex. The second explanation draws on Darwinian selection to act on functional variants within a population, thereby allowing diversification to take place over time. The evolutionary precursor or ancestor of Nif is a set of proteins that are likely involved in cofactor F_{430} biosynthesis; this cofactor and its biosynthetic pathway are only known to be present in methanogens and several recently evolved bacterial taxa. From this perspective, it would be surprising if Nif evolved in any organism other than in an ancestor of the methanogens. The third explanation hinges on the reduction of the inert N≡N triple bond in N_2 having a high activation energy (~200–400 kJ mol^{-1}). At low temperatures without catalysts, this activation energy favors preservation of the reactants (i.e. N_2 and H_2). At increased temperature (>150 °C), this activation energy is lowered, which would allow the reaction to proceed if not for the accompanying temperature-dependent shift in the equilibrium position of the reaction toward the reactants.

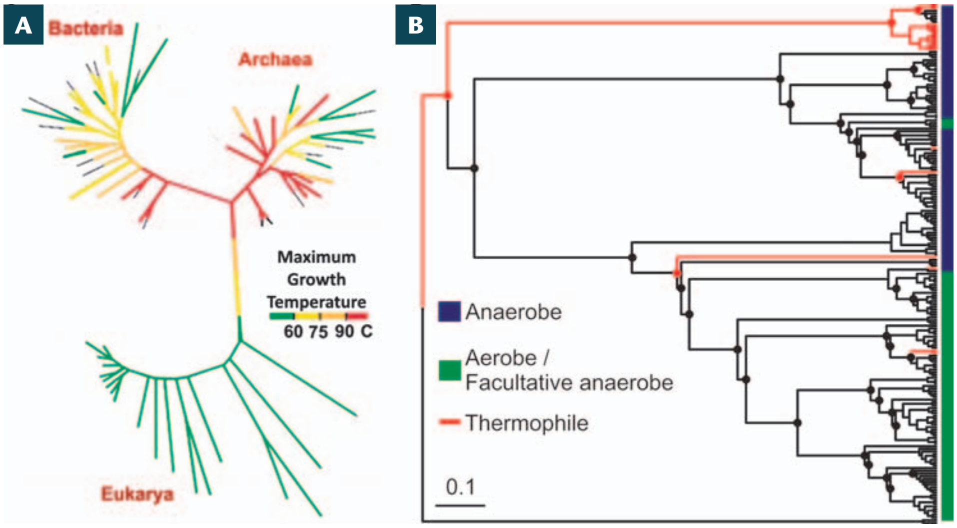

Figure 3 (**A**) Universal tree of life with an overlay of the maximum growth temperatures of the strains used to generate this tree. The presence of numerous branches near the base of the tree (trifurcation point at the center of the diagram) from thermophilic (optimal growth of ~ 45–80 °C) organisms and the presence of numerous branches at the tips of the tree from mesophilic (optimal growth of ~25–45 °C) organisms suggests that the ancestor of life most likely inhabited a high temperature environment. Figure adapted from Lineweaver and Schwartzman (2004). (**B**) Evolutionary analysis of nitrogenase (Nif) proteins from a number of Bacteria and Archaea, where the root or base of the tree is the vertical line on the left-hand side of the figure. Lineages from thermophilic organisms are colored red. The ability for an organism to utilize oxygen (green = aerobe or facultative anaerobe; blue = anaerobe) is depicted by the bars to the right of the tree. The presence of branches near the base of the tree from thermophiles and from anaerobes indicates that nitrogenase likely evolved in a high-temperature anoxic environment. Figure adapted from Boyd et al. (2015).

The Haber–Bosch process of industrial N_2 fixation overcomes the high activation energy for N_2 reduction by increasing the temperature while at the same time running the reaction at elevated pressure, at high reactant concentrations, and in the presence of iron-based catalysts in order to keep the equilibrium position favoring product formation. Intriguingly, at biologically relevant temperatures (<121 °C) and in the presence of elevated pressure and high concentrations of reactants, the equilibrium for the reaction should also permit product formation if appropriate catalysts (e.g. Fe–S compounds) are available. Indeed, abiotic experiments in the presence of Fe–S minerals under conditions similar to those present at hydrothermal vents were shown to promote reduction of N_2 to ammonia, albeit with meager yields (Dörr et al. 2003). Such conditions may have provided the impetus for a H_2-dependent methanogen to evolve an inefficient and primitive nitrogenase with an Fe–S-containing active site by which to overcome the activation barrier for N_2 reduction and to push the reaction toward product formation and relieve NH_3 limitation. Selection and refinement of this enzyme complex over evolutionary time would have further improved its catalytic efficiency to the point where organisms with this functional capacity could diversify into lower-temperature and lower-pressure environments (Boyd et al. 2015).

PRINCIPLE 5: "THINGS THAT BURST INTO FLAME ARE NOT GOOD TO EAT"

This statement (originally by Melanie Holland, pers. comm.) appears among these "Principles of Geobiochemistry" to help frame explanations for natural distributions of microbial metabolisms and biochemical strategies. If a reaction can proceed abiotically at a rapid rate, there is no need for catalysis, and no option for that reaction to supply energy in support of microbial metabolism. Rates typically increase with increasing temperature, which is why one of the limits of the biosphere is assumed to be at high temperatures. If a reaction reaches equilibrium faster than microbes can intervene to take advantage of it, that reaction cannot support life.

Reaction rates constrain habitability and depend on numerous compositional factors. Those especially relevant to energy supplies are the abundances of electron acceptors and donors, which depend on reactions that are governed by geochemical processes involving mineral dissolution or precipitation, gas transfer into or out of aqueous solutions, adsorption/desorption, and organic transformations. Sources of solutes may be proximal to microbial habitats or distantly removed, with transport pathways triggering the emergence of energy supplies. As energy supplies become available, abrupt transitions appear within habitats that indicate the appearance of life with metabolic strategies to take advantage of this energy. Each of these transitions can be studied from a geobiochemical perspective to determine how geochemical changes drive biochemical responses. These then can become case studies of how geology shapes biochemistry, ultimately leading to a new set of geobiochemical principles that can enhance the five proposed here.

Let's take, as an example, the element sulfur, because its various forms are well integrated into metabolism and apparently have been since early in Earth's history. Transitions in the utilization (or lack thereof) of specific forms of sulfur are often not evident to the naked eye, but can be determined by integrating geobiochemical methods and perspectives. As our example, we focus on the biological utilization of elemental sulfur (S_8^0) and how abiotic reaction kinetics involving numerous intermediate sulfur compounds (see Hansel et al. 2015 this issue) potentially influence the distribution of microbial metabolisms. In circumneutral to slightly acidic environments, sulfur-reducing microorganisms are thought to reduce polysulfide (S_x^{2-}), which is a soluble form of S_8^0 produced through the reduction of insoluble S_8^0 by sulfide (H_2S). However, in acidic ($pH < 6.0$) environments S_x^{2-} molecules are unstable and rapidly hydrolyze to produce H_2S and soluble S_8^0 rings

$$2S_5^{2-} + 4H^+ \rightarrow \alpha\text{-}S_8^0 + 2H_2S \qquad (2)$$

thereby placing constraints on the utilization of S_x^{2-} by acidophiles (organisms that preferentially grow in acidic environments). Further complicating its use by biology, S_8^0 rings rapidly aggregate (coarsen) due to hydrophobic attraction to produce S_8^0 nanoparticles of decreasing solubility; the rate of particle coarsening is inversely related to the pH of the aqueous solvent (Boyd and Druschel 2013). Sulfur-reducing organisms, which tend to be enriched in many acidic volcanic environments, apparently overcome these kinetic limitations by increasing the solubility of S_8^0 by driving the reverse of reaction 2 through the production of H_2S. This then promotes the formation of S_x^{2-} and, ultimately, soluble nanoparticulate S_8^0, which serves as an electron acceptor (Boyd and Druschel 2013). Similar constraints likely exist for the utilization of thiosulfate as an electron donor or acceptor in acidic environments, given the instability of $S_2O_3^{2-}$ in low pH environments, and can be extended to numerous other elements, including iron and manganese. Moreover, reactions between elements (e.g. Fe and S) are also likely to determine the availability of such elements for use by biology. Observations like these lead us to believe that, within the confines of environmental compositional space where reactions are energy yielding, abiotic reaction kinetics play key roles in further delineating the distribution of metabolisms and the microbial populations that they sustain.

CONCLUSIONS

Decades of interest in the merging of disciplines and the development of multidisciplinary research strategies to address societally relevant geoscience problems have resulted in substantial outcomes in terms of research and educational goals. However, these strategies have largely been in the form of binary fusions of concepts: biology and chemistry (biochemistry), chemistry and geology (geochemistry), or geology and biology (geobiology). The emerging field of geobiochemistry seeks to stimulate new conversations and to forge new research and educational strategies through integration of concepts borrowed from geology, chemistry, *and* biology in a ternary strategy that differs from biogeochemistry by placing emphasis on the influence of geological/chemical processes on biological processes. In doing so, geobiochemists will further erode traditional boundaries in teaching and research and accelerate new technological and intellectual advances in the geosciences. Acknowledging that the "Principles of Geobiochemistry" as outlined above are not all-encompassing, it is our hope that they will illustrate the breadth of new possibilities in this field as it advances to meet the myriad challenges and societally demanding questions confronting 21st century geoscientists.

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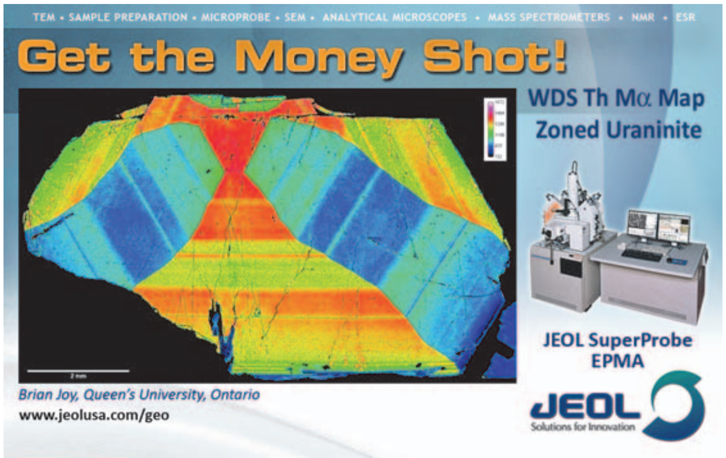

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