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INTRODUCTION AND OVERVIEW: WHAT DO WE KNOW FOR SURE?

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ABSTRACT. In the midst of an intellectual feeding frenzy with regard to interdisciplinary science, the area of microbial biogeochemistry (or subtle variations such as geomicrobiology, geobiology, and others) has emerged as an exciting way of thinking with regard to understanding our planet. So much so that it sometimes seems that we are actually beginning to understand the complex interactions between the biota and the highly modified planet that it occupies. The past two decades have seen dramatic advances in techniques used to analyze biological and geological samples, as well as advances in computing and information technology that have allowed modeling and simulations that would have been hardly dreamt of twenty years ago. Despite these advances it seems that, as often happens, the more we learn, the less we know. Some things have clearly emerged, however, and in this introduction we will attempt to assess what is known, what is thought, and to some extent, where the challenges lie.

DEFINING AND WORKING WITH THE LIVING WORLD

In the 1700's, with the work of Linnaeus, the classification of life was codified and organisms given binomial names (genus and species) based on morphological characters. These methods, which worked well with organisms large enough to be seen by the unaided eye ultimately led to the establishment of four kingdoms of Eukaryotes (fig. 1, plants, animals, fungi, and protists), so-called because they were all composed of cells with a true nucleus. The fifth kingdom, which for many years went largely unnoticed because of their single celled mode and microscopic size was called the monera or prokaryotes (so called because of the lack of organized chromosomes and a nuclear membrane) (Margulis, 1974, 1981; Whittaker and Margulis, 1978). For our purposes, the distinction between these groups is that the eukaryotes (plants, animals, fungi, and protists) form a metabolically coherent group with regard to both electron acceptors (their need for oxygen), and electron donors (their carbon-based heterotrophic metabolism), while the prokaryotes display considerably more versatility, being able to grow anaerobically via the respiration of electron acceptors other than oxygen, and using many different energy sources other than organic carbon or visible light (fig. 2). In recent years, our knowledge and appreciation of the extent of prokaryotic diversity has greatly expanded in three fundamental ways: 1) the revelation of the use of previously unknown electron acceptors and donors; 2) the respiratory versatility of eukaryotes that disobey the rules by living in symbiosis with prokaryotes; and 3) new prokaryotic assemblages that drive previously unknown reactions. But, before proceeding, it is appropriate to add some perspective on the definition and organization of the living part of the world as we know it.

In the early 1980's, it became apparent through the work of Carl Woese and his colleagues, that nucleic acids (specifically the genes coding for ribosomal RNA molecules) had considerable utility in the identification and classification of earthly life (Olsen and others, 1986; Pace and others, 1986; DeLong and others, 1989; Woese and others, 1984, 1990; Woese, 2004). While the approach of molecular taxonomy had

Fig. 1. The old and new tree of life.

Fig. 2. The diversity of electron donors and electron acceptors used by life.

been suggested and tried previously (Zuckerlandl and Pauling, 1965), it was primarily with proteins, most of which were not universally distributed throughout the living world. Woese pointed out that ribosomes were universal, and in particular, the 16S ribosomal RNA subunit sequence could be used to compare all life on the planet (with the exception of viruses, which use their hosts' ribosomes). Using such sequence comparison, it became apparent that the previously divided 4 eukaryotic kingdoms were sufficiently closely related to each other that they could be placed into one kingdom (the Eukarya). In contrast, large genetic diversity was seen among the "monera" or prokaryotes; so much so that they were divided into two kingdoms called the Archaea and the Bacteria (Woese and others, 1984, 1990). In addition to redefining the taxonomy and phylogeny of the microbial world, this approach also revealed an embarrassingly large depth of ignorance with regard to the microbial communities in the environment: seldom were more than 1 percent of the microbes that were visible (and detectable by their rRNA gene signatures) possible to obtain as growing cultures. The "up-side" of this, was that specific probes could be made to the 16S rRNA genes (even for non-cultivated microbes), and then used to quantify the number of cells in samples via fluorescence microscopy (Pace and others, 1986; DeLong and others, 1989; Amann and others, 1992). It thus became possible, for the first time to begin to quantify natural populations of prokaryotes, even those that had not yet been cultivated. In addition to adding immense insight into the diversity and evolution of the prokaryotic world via sequence comparisons, the impact of this seemingly simple step forward had a secondary impact on both microbial ecology and biogeochemistry, allowing one to answer with some conviction, the question, "Who and how many are there?" for the prokaryotes in a variety of geochemical settings (Olsen and others, 1986; Pace and others, 1986).

Concurrent with this advance in microbial genetic diversity has been a growth in the appreciation of the diversity at the metabolic level. This diversity clearly delimits the prokaryotes from the eukaryotes, and forms a major connection of the prokaryotes with biogeochemistry: one that is quite distinct from that of the eukaryotes, who express their own impressive diversity in terms of structure and behavior. What is stressed here is the remarkable metabolic diversity that characterizes and distinguishes the prokaryotic world (fig. 3): a diversity that reveals many of the energetic connections that knit together prokaryotic metabolism and planetary geology. Some notable examples, with regard to this are: (a) the use of inorganic energy sources is found only in the prokaryotes; (b) the process of anaerobic respiration (that is, using electron acceptors other than oxygen) is, with few exceptions, a process done only by, prokaryotes; (c) the oxidation and reduction of these inorganic compounds forms a strong link with planetary geology, as many of the reactions either form or dissolve minerals during the process (fig. 3). An additional point relates to the universal nature of respiration and redox chemistry by living systems. While "all" eukaryotes engage in oxygen respiration, even this process was "invented" by prokaryotes and arose in the eukaryotes via symbiosis (Margulis, 1981), making respiration a hallmark trait of the prokaryotes, and symbiosis perhaps one of the most important parts of eukaryotic evolution.

While focusing on all of the metabolic diversity above, it is easy to miss the fact that there is an impressive uniformity to the metabolism of life as well. Virtually all present day metabolism on Earth operates in a similar way, with environmental redox equivalents being harvested and used for the reduction of cellular electron or hydrogen carriers which are similar throughout all of life (Nealson, 1997; Nealson and Conrad, 1999). These reduced carriers are then used directly for biosynthesis, or for the generation of a membrane potential (combination of a pH and electrical potential) that can be used for a variety of functions (including ATP formation, transport, and

Fig. 3. The general characteristics of biomineralization in the three kingdoms of life.

motility). Thus, despite apparent rampant metabolic diversity, the central energy processing systems of all life, including those of structurally complex large eukaryotes, operates in a similar way. With regard to this issue, there are really only two metabolic means of extracting energy from the environment: chemiosmosis and fermentation. Almost without exception the interactions between the living world and the mineral world are of the chemiosmotic type (that is, redox chemistry). While it is difficult to specify exactly when the ability to respire arose in time, one imagines that it is indeed one of the earliest inventions of life (Nealson and Rye, 2003), and once invented, became a component of virtually all subsequent successful experiments in evolution.

We end the introduction with a look at the definition and usage of the word "prokaryote", which has been questioned by Carl Woese (Woese, 2004) and others (N. Pace, personal communication). In the absence of a suitable substitute, we will use the word here, with the major connotation being the remarkable difference that it implies in metabolic versatility—eukaryotic oxygen to carbon respiration on one hand, and prokaryotic diversity of electron donors and acceptors, on the other.

EATING, BREATHING, AND MAKING ROCKS: THE GEOBIOLOGY CONNECTION

This volume deals with issues related to processes, scaling, and interfaces in biogeochemistry. The processes and interfaces discussed herein occur over spatial scales that range from angstrom to global, and most involve components from both the animate (biological) and inanimate (geological) worlds. It is taken as a given that the rates of many geochemical processes at low temperatures are sufficiently slow that without life, they would hardly occur over time scales we can measure experimentally, and that present day earthly processes are thus a unique blend of biologically driven catalysis and geochemically mediated reactions. For the sake of discussion, we focus here on the reactions that are involved with rock and mineral formation and dissolution, making the point of the "how and why" of biological involvement. Our focus will be heavy towards the lithosphere, not because we feel it is more important, but because

Fig. 4. The redox activity of some bacteria (such as Fe-reducers) results in the formation of various minerals (vivianite, magnetite, siderite); their formation controlled mostly by the chemistry of the environment.

it is the one on which we work—similar things can be said about the biogeochemical cycles of other elements in the atmosphere and/or hydrosphere.

Prokaryotic mineral formation.—As discussed briefly above, one of the things that characterizes the prokaryotic world is the remarkable diversity of electron donors and acceptors that are utilized. In figure 2, some of this diversity was presented to make this point, and to emphasize that many of the components used in this diverse metabolism are components of minerals. Oxidation or reduction of these components often results in a change of state of the component (for example, insoluble to soluble) leading to formation or dissolution of minerals. In this sense, the metabolism of the prokaryotes, designed for the purpose of harvesting energy from the environment, is inadvertently linked to the formation and/or dissolution of many minerals on the planet.

This has been addressed by studies of the dissimilatory iron reducing bacterium *Shewanella algae* CN-32, in which the reduction of hydrous iron oxide can be shown to produce any of several mineral products depending on the environment in which the bacteria are grown (fig. 4) (Roden and Zachara, 1996). Thus, to some extent, one can view the formation of minerals by some (perhaps most) of the prokaryotes as metabolic “accidents”, dependent on the metabolic chemistry, but not directed by it. Insofar as can be seen, there is no direct advantage to the bacterium to forming a given mineral in comparison to any other: this is determined in fact by the chemistry of the environment in which the bacteria are living. This leaves us in the interesting position of viewing prokaryotic minerals as metabolic by-products that may be valuable as indicators of microbial metabolism, but without the morphological clues usually associated with biominerals or fossils.

This being said, while specific mineral formation may have no advantage for prokaryotes, it may well be that mineral formation in general serves a generally useful

thermodynamic/kinetic purpose. From the point of view of the bacteria, removing one of the soluble products as an insoluble mineral could have a dynamic effect on the chemical abilities of the bacteria. This is a strategy often employed by bacteria living in symbiosis with other bacteria, in which a product of one is the substrate for the next, and chemical reactions proceed at rates far beyond what might be predicted if the reaction products were disposed of by abiotic processes alone. A recently discovered example is that of anaerobic methane oxidation, in which the process is apparently driven by sulfate reducing bacteria, that consume the hydrogen produced via methane oxidation, pushing the reaction forward (Boetius and others, 2000; Orphan and others, 2001a, 2001b).

Eukaryotic mineral formation.—In marked contrast to the prokaryotic mineral producers, the eukaryotes, which are characterized by “simple” metabolism and complex structures and life styles, have perfected the art of mineral fabrication, making a wide array of biominerals with many different uses (Lowenstam, 1981; Lowenstam and Weiner, 1989; Dove and others, 2003). In contrast to the diverse prokaryotic metabolism discussed above, which is responsible for early mineral deposits, the true biominerals produced by eukaryotes (teeth, shells, bones, diatom frustules, sponge spicules, *et cetera*) did not appear in the rock record until ~500 million years ago, when the first sponge spicules and carbonate biominerals can be seen: that is, the processes are geologically young (Li and others, 1998; Nealson and Rye, 2003). Biomineralization is extremely common among the multicellular eukaryotes, many of which need structural elements to grow and function in three dimensions, providing advantages for predation (teeth, bones, *et cetera*) and protection from behavior (shells, frustules, cell coverings) (Lowenstam, 1981; Lowenstam and Weiner, 1989). These biominerals are often ornate and recognizable structures that provide us with a visible fossil record in recent times, allowing the calibration of the molecular record (discussed below), something that is extremely difficult prior to the “invention” of biominerals.

The mechanisms by which the eukaryotic biominerals are produced (specific protein templates, *et cetera*) are only beginning to be appreciated (Dove and others, 2003), and while much more is waiting to be learned, it is already evident that situation is quite different from that seen in the prokaryotes (fig. 5). The eukaryotic biominerals are pre-ordained and reproducible: genetically directed protein templates are used to catalyze and direct the synthesis of specific proteins on which mineral synthesis occurs. The products of these template-directed mineralizations are sufficiently constrained that they can be used to identify the organism that produced them, often to the level of genus or even species. As opposed to the prokaryotes, which form minerals while gaining metabolic energy, the formation of eukaryotic biominerals is “costly” in the sense of requiring specific templates, energy for synthesis, often auxiliary systems for transport and assembly. This is in marked contrast to the prokaryotic mineral formation and dissolution, which is primarily a function of environment rather than genetics (fig. 4). In addition, redox chemistry is not a fundamental part of eukaryotic mineral formation: for the most part biominerals formed by prokaryotes involve redox-active compounds, while that formed by eukaryotes do not. Given that the eukaryotes are unable to view minerals as either viable electron donors or acceptors, their ability to form or dissolve redox-active minerals is extremely limited.

A final note with regard to the biominerals constructs produced by prokaryotes and eukaryotes: because protein templates and other structural aids are not utilized by most prokaryotes, these minerals are often “pure”—sulfides, carbonates, *et cetera*, while those of eukaryotic mineral are “hybrids” composed of biological material interspersed with crystalline “minerals” (figs. 4 and 5). These adaptations add structural integrity

Fig. 5. Biomineralization of shells in Mollusks is a complex process involving an extracellular organic matrix. (A) Empty organic shell; (B) Organic shell filled with organic matrix (grid) and nucleation points (black circles); (C) Growth of aragonite nuclei in the matrix (yellow ovals); (D) Aragonite plate filling the compartment; (E) Many hexagonal plates of aragonite (yellow) surrounded by an organic framework (blue) and by vertical canals (red); (F) Pile of hexagonal plates of aragonite.

and strength to the minerals, and make them chemically distinct from the prokaryotic minerals (Dove and others, 2003).

The enigmatic bio-magnetite.—Dissimilatory iron-reducing prokaryotes (of which both Bacteria and Archaea are known) can produce high levels of extracellular reduced (Fe^{2+}) iron, and under the appropriate conditions, form copious amounts of extracellular magnetite (Lovley and Phillips, 1988; Roden and Zachara, 1996). Whether there are any recognizable signals to distinguish such magnetite from abiotically formed magnetite is not yet clear. To some degree, these extracellular products are examples of prokaryotic mineral formation—they are metabolic products of no known use, and they are produced when extracellular conditions favor their production.

In marked contrast, many strains of magnetotactic Bacteria (no magnetotactic Archaea are known) produce highly ordered intracellular crystalline magnetite inclusions called magnetosomes (fig. 6). These magnetosomes are single domain, mineralogically nearly perfect, highly magnetic crystals, and often, but not always, arranged in chain-like structures (Blakemore, 1975; Bazylinski and Frankel, 2000). They provide the cells that contain them with the ability to sense and respond to a magnetic field: the response being that these highly motile cells move swiftly towards one magnetic pole or the other. It appears that each bacterial strain produces a characteristic magnetite pattern (size, shape, and arrangement of the magnetosomes), and that specific genes (and thus, specific proteins) are involved in the production of the magnetosomes (Schuler, 1999; Matsunaga and Okamura, 2003; Schuler, 2004). Furthermore, while the function(s) of the magnetosome is not yet proven, nearly everyone agrees that there must be an advantage to the cells containing the magnetosomes, and most agree that it is connected with environmental sensing and location (Frankel and others, 1997).

Fig. 6. Magnetosomes in *Magnetospirillum magnetotacticum* AMB1.

This brings to light the question of whether there may be more examples of such highly structured biominerals produced by bacteria. Recently it was reported that highly crystalline forms of metal sulfides were produced by sulfate reducing bacteria (Suzuki and others, 2003). Whether these are in fact structured with a “purpose” is not known, but their highly crystalline structure is an intriguing finding, and perhaps more prevalent than is now appreciated (Fortin, 2004).

As a final point, many eukaryotes are known to form intracellular magnetite, which is thought to be involved in magnetic sensing of the environment (that is, navigation in birds and bees), and perhaps in other functions such as biorhythms (Lowenstam, 1981; Lowenstam and Weiner, 1989). Almost certainly redox processes are used to produce the intracellular magnetite crystals, but whether this is done by the eukaryotes, by prokaryotic symbionts, or simply acquired by the eukaryotes still remains unknown.

ORGANIC GEOCHEMISTRY

While it is not part of this introductory chapter, one should not forget the powerful approach that organic geochemistry has added to our appreciation of present and past events. This is reviewed in many places in great detail, and in general provides a powerful tool to the appreciation of the past via the identification of well-preserved biomolecules (see Nealson and Rye, 2003, and references therein). As discussed below, well-preserved key biomarkers can be used to calibrate molecular clocks. However, as with all methods, the limitations must be stressed. The identification of almost all organic molecules involve assumptions that it is known which group of organisms contain them, and have contained them over time. Given that only a small percentage of microbes have been cultured (and thus studied with regard to their molecular components), these are often assumptions that are difficult to confirm.

Fig. 7. Phylogeny and biochemical data can be used to some extent to reconstruct the evolutionary history of the metabolism. This highly speculative diagram is designed to suggest the very important role that symbiosis has played in evolution (the single stars denote where apparent exceptions to the designation occur because of symbioses between prokaryotes and eukaryotes). The double star denotes a special kind of photosynthesis that occurs in the halobacteria, in which halorhodopsin uses sunlight to power proton translocation). The column entitled calcium metabolism is denoted to emphasize the many ways that eukaryotes deal with calcium (in comparison to prokaryotes).

SCALING

Biologically formed minerals can be scaled temporally and spatially. With regard to temporal scaling, two important issues arise. The first relates to geological time scales, and the search for indicators of past life, while the second relates to short time scale kinetic processes, primarily catalyzed by enzymes—processes that can not only be directly measured, but which lead to the formation of nutrient gradients and interfaces.

Long term temporal scaling.—One of the great hopes of molecular phylogeny approaches is that it would be possible to look back in time using sequence data: to use these data to estimate when major metabolic “inventions” occurred in the past. Such inventions would include not only structural innovations visible in the fossil record, but metabolic inventions like respiration and photosynthesis, and other prokaryotic specialties like nitrogen fixation, denitrification, sulfur oxidation, *et cetera* (fig. 7).

To some extent, there is hope that such an approach can work, but probably only for relatively short time scales, and with systems that can be cross-calibrated. For example, Benner and others (2002) discussed the issue of the use of various types of data to understand the evolution of metabolism, concluding that within the last 50

million years one can do this with some confidence, utilizing a combination of fossil, isotopic, organic geochemical, and molecular evolutionary clock records to infer past patterns of evolution (in this case, the evolution of certain sugar fermentation abilities).

Prior to the formation of “true” biominerals (that is, recognizable fossils), the signatures that exist are primarily geochemical in nature. Thus, while prokaryotically formed minerals may not be readily recognizable by their morphologies or unique crystal structures, many can be judged to be of biological origin via the fractionation of isotopes during the “supply” of chemical components for their formation. Kinetic fractionation occurs as a function of enzymatic catalysis, leading to biological materials preferentially being composed of the “lighter” isotopes (Hayes, 1983; Madigan, 1989; Schidlowski, 1992; Orphan and others, 2001a). Thus light carbon is preferentially used by living organisms during carbon fixation, and accumulates in the resulting biomass, and light sulfide is produced during sulfur or sulfate reduction, and accumulates in sulfide minerals. These isotopic tracers have been of value in tracing the metabolic activities of modern organisms in both the laboratory and the field, and provide a major tool for looking for indicators of metabolic activity in ancient samples. That is, it is possible, using C and S isotopes to see in the ancient rock record the appearance of processes that result in fractionation of these isotopes (Schidlowski, 1992). Herein lies an important distinction that can be easily missed: while the isotopes may strongly suggest the existence of a process leading to fractionation, they in no way can tell us (for sure) which process, and can absolutely not be used to tell (for sure) which organism or even group of organisms accounted for the fractionation. Unless we accept this tenet, we can easily fool ourselves.

Carbon isotopes have been perhaps the most valuable and widely used of the isotopes with regard to the detection and definition of life and its processes (Hayes, 1983; Madigan, 1989; Schidlowski, 1992; Mojzsis and others, 1996; Orphan and others, 2001). Carbon fractionation occurs during its reduction (fixation) from CO₂ to organic carbon by bacteria, algae, and plants, and to a much greater degree (that is, light carbon) when CO₂ is fixed into methane by methanogenic archaea. However, even for this well known and often used system, deciphering the isotopic signatures from ancient samples is difficult because these pathways are varied and unknown, subsequent diagenetic reactions are not easy to specify, and because the signature of the source of carbon is seldom known with assurance.

It thus seems clear from a variety of studies that biominerals can be traced to the early phases of Earth’s history. Stable isotopic signatures of both carbon and sulfur suggest that metabolic activities were involved with the formation of minerals from very early times. Carbon isotopic ratios (¹³C/¹²C) have been used to suggest that carbon fixation may have existed as early as 3.8 Ga (billion years) ago (Mojzsis and others, 1996). While this number has been challenged, few would argue with 3.5 Ga for convincing evidence of carbon isotopic signals in the ancient record (refs). Similarly, sulfur isotopes (³⁴S/³³S) suggest that sulfur reduction of some kind was occurring 2.5 Ga and perhaps earlier (Canfield and others, 2000; Shen and others, 2001).

Unequivocal evidence for the formation of biominerals prior to 500 million years ago has been difficult to obtain for several reasons. First, the preservation of the materials is often poor, making identification difficult, and second, because virtually none of the putative organisms seen in the samples are alive today. While they have similarities to other organisms, the nature of their behavior, and even their metabolism, can not be specified with certainty. Another discouraging development with regard to molecular evolution methods is that there are rampant examples now appearing in which it is clear that the evolutionary “clock” is neither constant (it can run at different rates for different organisms, and perhaps for different conditions),

nor predictable (Doolittle and others, 1996). Thus, two organisms that would be described as deeply branching, and suspected of being of equal “age” may be quite different because of differences in evolutionary clock speeds. For ancient samples, on the order of hundreds to thousands of millions of years and older, the situation gets even more uncertain.

Another difficulty that is peculiar to the prokaryotes is that the “fossils” used to identify them are reduced to either organic geochemicals (that is, classes of chemicals peculiar to a certain type of cell), or isotopic fractionation patterns indicative of a certain type of metabolism. Both analyses have their limitations. For example, while some classes of compounds can be identified as components of cyanobacteria in the modern day world, there is no way of knowing with certainty that non-cyanobacterial organisms that contained these compounds were not present before the cyanobacteria arose. Similarly, when one sees isotope fractionation, such as the appearance of light sulfur, it is tempting to invoke the appearance of sulfate reduction (and sulfate reducing bacteria), and in fact it is often done (Canfield and others, 2000). While the activity of sulfate reducing bacteria would indeed explain the observed results, it is also true that sulfur can be fractionated by many other organisms, including sulfur and polysulfide, and thiosulfate reducers (Smock and others, 1998).

As a footnote to this discussion, one must remember that much of the work with both organic biomarkers and isotopic fractionations hinges on our knowledge of microbial physiology, and the composition of cultured microbes. We note that it is estimated that less than one percent of the microbial world has been obtained in culture, and thus that we are extrapolating to the world from a very weak data base. In support of this rather depressing-sounding statement, we have assembled in table 1, a list of microbial types that have been discovered in the past 20 years—microbes whose very existence was doubted, or unknown before then. Given that methods are improving with regard to culturing microbes, one can hope that the situation will improve, but surely one must be cautious when trying to extrapolate backwards in time to ancient metabolisms, based on such an incomplete data base.

Finally, we must confront the issue of lateral (horizontal) gene transfer (Doolittle, 2002). With the advent of genome sequencing, it has become apparent that there has been in the past a large amount of mixing of genomes, such that the so called phylogenetic “tree of life” is in fact much more like a cross-hatched bush (Doolittle, 2002). Each of the three “kingdoms” has obtained, and fixed into their genomes, ample information from the other two kingdoms, suggesting that major sharing of genetic information has occurred, especially among the prokaryotes, where this is still happening at rapid rates. Thus, one of the major issues in prokaryotic genomics is that of defining the gene set that characterizes a given group of microbes (as opposed to those genes that can apparently move in and out with non-lethal results).

Before we leave this subject, however, there are aspects of the molecular clock that are of great value especially with regard to understanding the relationship(s) between organisms and functions within the microbes that are alive today. Before beginning the discussion, one of the intellectual “traps” of this approach should be noted—namely that all of the organisms on which the “phylogeny” is based are alive and evolving today. That is, while they may contain “ancient” traits or abilities, these are surely not as they were in the past, so that the phylogenetic approach allows one to look at the most likely sequence of events, but not to accurately date them. Thus, one could argue that we are looking at the living remnants of past microbial evolution, and can have a reasonably good idea of who preceded whom, or what preceded what (within the limitations of uncertainties introduced by horizontal gene transfer), but trying to ascertain when any of these processes arose: that is, when a given process or organism first appeared is very difficult (if not impossible) by this method. As a precaution, one

TABLE 1

“New Microbial Metabolisms”: This table represents some of the metabolic abilities of bacteria that have been revealed since 1988. This list is meant to be illustrative rather than exhaustive

Process	Found in:	Discovered	References
Dissimilatory Fe Reduction	Bacteria & Archaea	1988	Lovley and Phillips, 1988; Myers and Nealson, 1988
Dissimilatory Mn Reduction	Bacteria	1988	Myers and Nealson, 1988
Anaerobic Methane Oxidation	Bacteria/Archaea Consortium	2000	Boetius and others, 2000; Orphan and others, 2001
Anaerobic Ammonia Oxidation	Bacteria	1999	Jetten and others, 1999
Anaerobic Iron Oxidation	Photosynthetic Bacteria	1993	Widdel and others, 1993; Ehrenreich and Widdel, 1994
Anaerobic Iron Oxidation	Heterotrophic Bacteria	1994	Straub and others, 1996
Perchlorate Reduction	Bacteria	1996	Coates and others, 1999
Phosphite Oxidation	Bacteria	2000	Schink and Friedrich, 2000
Dissimilatory Arsenic Reduction	Bacteria	1994	Ahmann and others, 1994
Dissimilatory Selenate Reduction	Bacteria	1994	Oremland and others, 1994

might note that almost none of the organisms seen in the fossil record of 100 million years ago are alive today. If we tried to construct those organisms simply from molecular biology alone, it would almost certainly be a resounding failure. This is the dilemma we are faced with in reconstructing prokaryotic evolution.

Short term temporal scaling.—Temporal scaling can occur over short times as well, and to some extent, this defines one of the major differences between life and non-life – the existence of enzymes that catalyze reactions that would otherwise occur at extremely slow rates. These processes can be studied by direct measurement of chemical reactions, looking at reactant consumption or product appearance, or by a variety of other approaches, including the use of stable or radioactive isotopes as tracers. To some extent, we biologists take this for granted, but when looking at low temperature geochemistry, the ability of life to harvest energy so rapidly is really quite remarkable. Much of the low temperature (that is, less than 100°C) geochemistry on the planet would, in the absence of catalysis, proceed at rates slower than molecular diffusion, so that product accumulation and gradient formation would be minimal processes. In the presence of life, however, reactants are consumed at rates faster than they can be supplied, and products are produced faster than they can diffuse away, leading to the formation of gradients that are indicative of the very life forces that have produced them (Nealson and Berelson, 2003).

Spatial scaling.—Such kinetic processes beget biosignatures that exist over spatial scales of micrometers to 10’s of meters, and perhaps larger—in the absence of life they would not exist. For example, in the Black Sea (fig. 8), a series of redox zones are seen,

nitrate would almost certainly be uniformly distributed in the black sea, as the rates of the processes that might consume it are very slow. Below this are the zones of manganese, iron, and sulfate reduction, all of which would not exist without life catalyzing each process.

These so-called layered microbial communities (LMCs) as we biologists refer to them are thus inferred not from biological measurements, but from the very existence of the chemical layers and non-linear gradients (Nealson and Berelson, 2003). As will be discussed below, they are more complex than is implied above, but the gradients themselves can be used as indicators of specific catalysis, and thus of life. Of interest here is that with regard to spatial scales, we see such LMCs at scales of micrometers in biofilms, to millimeters in algal mats, and centimeters in lake and ocean sediments—they are a universal feature of life on Earth. In environments where rapid mixing occurs, such as the open ocean, mixed lakes, and the atmosphere, such gradients are rapidly dissipated by convection and mixing, but the signals of the biological processes are nevertheless there, as is discussed in many of the following articles.

INTERFACES

Interfaces occur over a wide range of scales: if one looks carefully, interfaces will almost certainly be there. For a microbiologist studying mineral formation, the interface might be at the cell-wall or cell membrane as the microbe interacts directly with a mineral surface. For others who study layered communities, the key interface might be at the oxic/anoxic boundary, where key processes involving oxygen occur, or are inhibited. Similarly, for each of the other redox boundaries shown in figure 8, there is a consumption and production term for each nutrient, terms that are often not well understood, even qualitatively. For those studying carbon cycling, the interfaces might well be those zones where methane is consumed anaerobically and those where it is consumed aerobically. We thus see a very close coupling of the concept of kinetic processes with interfaces and interfacial chemistry. Two points may be relevant with regard to the above discussions of minerals: first, that with regard to what appear to be simple chemical interfaces, they are almost certainly more complex than they appear, and second, with regard to mineral formation, the interfaces that occur in eukaryotic mineral formation result in the production of remarkable biominerals that are in fact quite distinct from anything that might be formed in the absence of life.

With regard to the first issue, it is probably safe to say that every interface in a LMC is the result of several reactions occurring simultaneously, some producing the compound being measured, and other consuming it. Thus each chemical interface or layer is the result of various organisms consuming and producing products related to the gradients either above or below it. In physically stabilized systems, these complex interfaces may someday be used to define the rate of energy flow through the system, and what processes are occurring at what rates.

With regard to the second, it is now clear that eukaryotic biominerals are laid down by, and intercalated with, protein and/or carbohydrate matrices that make them stronger, more resilient, and more robust than nearly any naturally mineral form of the same type. These interfaces, when understood may fundamentally change the way that man-made materials are constructed, but for sure it is a fundamental difference between the prokaryotic minerals, which are really just minerals, and the eukaryotic structures, which are mineral/organic complexes.

The examples we have used here to illustrate issues of scaling and interfaces are derived from our own limited experience. As one reads through this volume, it should become apparent that the processes being discussed are characterized by biologically driven kinetics, catalysis of immense magnitude and specificity that can affect the environment from microscopic, subcellular scales to regional or global scales. Similarly, the interfaces may range from the smallest microbes surface to the “skin of the

Earth,” whatever one imagines that might be. One might give as an example, the processes that involve harvesting light energy and converting it into chemical energy for living systems. Probably no other process has so profoundly affected our planet as photosynthesis, and its scales and interfaces range over the entire gamut discussed above. The intersections of this process with that of mineral formation and LMC formation and stabilization are many, and sometimes they are difficult to separate, as photosynthesis is intimately related to the others. This is the nature of biogeochemistry, and one of the great challenges of biogeochemistry will be unraveling the intricacies of the way that the various element specific processes, scales and interfaces fit together, interact, and make an understandable whole.

WHAT DO WE KNOW FOR CERTAIN?

As one looks over this brief introduction, it may seem at first a bit negative. To the contrary, it was not our intention to be disdainful of the great progress that has been made in recent years. However, one must admit that, if the question is, “what do we know for certain?”—the answer must be, “very little,” when it comes to scaling our processes over both time and space. That being said, one can have considerable optimism that major issues addressed by the articles in this volume will yield to new methods, and new approaches, especially interdisciplinary efforts.

Comfort Zone: Willing to say we know it is so

We might, in fact, look at the areas where we have some comfort in the geobiology realm.

1. **The time of the appearance of an oxic atmosphere**, an event that profoundly shaped the co-evolution of life and Earth is much more constrained than in the past. For example, the time at which the atmosphere of the Earth became oxic has been constrained by the work with mass-independent sulfur isotopes (Farquhar and others, 2000, 2002) such that it appears that there was very little oxygen, if any, prior to about 2.2 Ga.

2. Molecular work with photosynthetic bacteria, cyanobacteria, and eukaryotic photosynthetic algae strongly indicate that the **evolution of photosynthesis** proceeded from two types of anaerobic bacteria photosynthesis, via a fusion event, to cyanobacterial oxygenic photosynthesis, to a symbiotic fusion even to form chloroplast-containing eukaryotes. While it may be difficult to put an exact date on the time of evolution of these processes, the pathway seems clear (Blankenship, 2002).

3. Molecular work with mitochondria indicates that the **evolution of oxygen respiration** in eukaryotes is the result of a symbiotic event between a bacterium and another cell (Margulis, 1981; Woese and others, 1984).

Excitement Zone: Willing to bet we will know in a decade or so

1. **The time of the appearance of life on Earth** remains contentious, not so much because of the nature of the data as because of disagreements with regard to the dating of the samples. The claim that life appeared prior to 3.8 Ga (Mojzsis and others, 1996) will almost certainly be continuously challenged, new samples analyzed, and the issue significantly improved.

2. **The timing of major metabolic innovations**, something that for now remains relatively mysterious, will almost certainly improve drastically in the coming years (Nealson and Rye, 2003). Many new markers, both organic and inorganic (ala stable isotopes, and organic biomarkers) are now being employed, and the application of these techniques, along with better quality samples, and better dating precision should lead to major interpretation with regard to the invention of metabolic pathways with regard to C, N, S, and perhaps many other elements.

3. **Understanding and quantifying the role(s) of the biota in the geochemical cycling of the major elements** may be one of the major accomplishments of the next decade. As one will read in this volume, considerable progress has been made, but with modern approaches to make global measurements along with the ability to understand at the microbial level how organisms work will represent one of the major accomplishments of the next decade.

4. **Understanding the mechanisms of eukaryotic biomineral formation** is an area whose time has come, and the interface between molecular biology, physiology, and mineralogy will see some major developments (Dove and others, 2003).

5. **Understanding the metabolic diversity of life.** As noted in table 1, a great deal of progress has been made in the past decade with regard to deciphering the extent of metabolic diversity among the prokaryotes, and this will continue. The work will almost certainly turn to mechanistic studies on one hand, and ecological studies on the other, attempting to understand how to manipulate prokaryotes for purposes of materials science and bioremediation.

6. One area of great interest will be that of **understanding symbioses across a wide range of organisms**. Organisms seldom live or metabolize alone, whether they be all prokaryotes, or prokaryote/eukaryote assemblages, and the importance of these in community structure and function will emerge as a major theme in the next decade or two.

Twilight Zone: Still hazy after all these years

1. **Putting precise dates on the evolutionary clock** will continue to plague those of us who would like to have such insights available. While advances will be made, they will come from geochemical data (organic and inorganic), which may be useful in calibrating the molecular clock. Other approaches, such as understanding the detailed relationships between DNA (or protein) sequence and the resulting 3-D protein structure may also lend new insights. Without such confidence, however, it is unclear when or how we will be able to move from identifying processes in the ancient rock record (that is, specifying the evolution of metabolism), to identifying the organisms that were responsible for these processes (that is, specifying the evolution of specific microbes or microbial groups). Given the rampant horizontal gene transfer that appears to have occurred, especially among the prokaryotes, it is difficult to have much optimism for relating the presence of an ancient metabolic record to the existence of anything other than a process. Whether the situation is as bad as some suppose (Graur and Martin, 2004) is not clear, but intellectual salvation is not at hand with regard to these issues.

2. Even with a lot of effort, one expects that the **extent of the biosphere that will be possible to cultivate**, and thus to provide useful information for looking at present and past ecosystems, will remain small. This may be particularly true with regard to the large and poorly understood world of the protists (the single celled eukaryotes), which may have many new things to teach us both about the metabolic versatility of eukaryotes and exotic symbioses that may slowly reveal themselves. These will remain particularly challenging because of the need for new approaches as well as the potentially very slow growth rates that will be encountered.

SUMMARY

This introduction has focused to a large degree on the remarkable metabolic diversity of the prokaryotic world, with a view to how it can be used to help understand and interpret the properties of Earth, from ancient times to the present. The frustration of such an effort is several-fold. First, we deal with present day processes that are hard to extrapolate backwards with certainty. Second, the scales and interfaces that we deal with in the laboratory (and even in the field) are often not adequate for

extrapolation (either temporally or spatially) to ancient times and global scales. Finally, while the prokaryotes are of unquestionable importance (and for the first 2 billion years of life, were the major forces driving biogeochemical cycles), we have neglected the eukaryotic community that puts its own remarkable set of impacts on the present-day Earth. No complete understanding of the co-evolved and present day Earth will be complete without both groups—a challenge for the future.

Finally, some philosophy of sorts. While it is true that in the absence of data one may feel free to speculate, there is no assurance that in the presence of overwhelming amounts of data, the situation is much better. The complex world of biogeochemistry is arguably one of the most exciting arenas available to scientists today, but one full of traps where the reductionist may feel irrelevant, and the generalist overwhelmed. We suspect that in another decade, these feelings will lessen, with the tools of molecular biology, information technology, and modeling coming to the rescue. Those studying processes, interfaces, and scaling may truly be brought together to begin to understand the system as a functioning entire unit.

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