

GEOBIOLOGY

CURRENT TECHNOLOGY
and RESOURCE NEEDS



The specific and primary
purposes are to perform
research in the sciences
and in mathematics, to
disseminate the results
obtained therefrom, all to
benefit mankind.

A G O U R O N
I N S T I T U T E

Cover photo:
Phototrophic sulfur and
non-sulfur bacteria in an
enrichment from a microbial
mat reveal part of the enormous
diversity of microorganisms.

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The Agouron Institute

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INTRODUCTION

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With the discovery of recombinant DNA technology in the mid-1970's, came a tremendous set of new opportunities in biology and chemistry. The Agouron Institute (AI), a non-profit research organization, was formed in 1978 by John Abelson, Melvin Simon, and Joseph Kraut, all then Professors at the University of California, San Diego, as a vehicle by which new research frontiers and technologies in biology and chemistry could be investigated in an expeditious manner free from what was perceived then as constraints in the University.

The first scientists working at the Agouron Institute in La Jolla came from the laboratories of the founders. The Institute commenced its activities with a small grant from the Office of Naval Research on the marine fouling problem – the beginning of a long standing program in environmental microbiology. By 1982 the research program

had expanded considerably and had obtained additional funding from the NSF and the NIH. A group of molecular biologists and chemists were collaborating to exploit new technology in which synthetic oligonucleotides were used to direct specific mutations in genes. A crystallography group had been formed and they were collaborating with the molecular biologists to study the properties of the altered proteins. These were among the very first applications of the new technology to form what is now the very large field of protein engineering. In addition a substantial group of computational chemists had formed which had the goal of predicting protein structure and the specificity of protein ligand interactions.

Early successes in the protein engineering and computational groups led to the idea that this technology could be used in the rational design of ligands that interact with and inhibit the activity of proteins, i.e rational drug design. In 1984 a commercial entity, Agouron Pharmaceuticals, was formed to exploit the potential of rational drug design. A commercial cooperation agreement was instituted between Agouron Pharmaceuticals and the Institute and in return the Institute received a major portion of the founding stock. Many of the molecular biologists, chemists and structural biologists shifted to the company and within a few years the two institutions moved in different directions

Agouron

with the Institute specializing in environmental microbiology and molecular biology.

The vision of rational drug design turned out to be a prescient one. Agouron Pharmaceuticals became a major biotechnology company and its first rationally designed drug, Viracept™ is still the leading HIV protease inhibitor. The use of protease inhibitors together with reverse transcriptase inhibitors in a multi-drug therapy regime has led to a dramatic decrease in deaths due to AIDS.

In 1998 Agouron Pharmaceuticals was sold to Warner Lambert which then merged with Pfizer. In the process, the endowment of the Institute increased substantially. As a result, the Board of Directors of the Institute adopted new strategies to achieve the Institute goal of making significant contributions in biology and chemistry. The Institute has decided to change its mode of operation and explore the possibilities of making highly leveraged investments in basic and applied biology and chemistry. To this end the decision was made to carry out a study program in several promising areas of research.

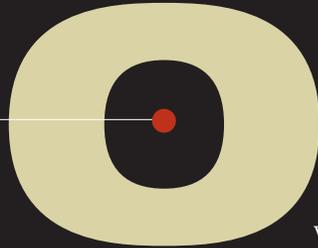
The study of prospects for the determination of the structures of supramolecular complexes was the first such effort. The results of our study have been published in a white paper entitled, "Supramolecular Assemblies, Current Technology and Resource Needs." We have awarded and are continuing

to award grants to institutions here and abroad to support research and facilities in this field. We also have funded a number of postdoctoral fellowships which are administered by the Jane Coffin Childs Memorial Fund for Medical Research and the Helen Hay Whitney Foundation.

Our second study is in the field of Geobiology. In May, 2000 we held a meeting in Pasadena, California. The participating scientists included Gustaf Arrhenius (Scripps), Steven Benner (University of Florida), David DesMarais (NASA Ames Research Center), John Grotzinger (MIT), Christine Guthrie (University of California, San Francisco), Gerald Joyce (Scripps), James Kasting (Penn State), Joseph Kirschvink (Caltech), Andrew Knoll (Harvard), Laura Landweber (Princeton), Elliot Meyerowitz (Caltech), Kenneth Nealson (Jet Propulsion Lab), Dianne Newman (Caltech), Norman Pace (University of Colorado), David Stevenson (Caltech), and Edward Stolper (Caltech). The meeting included research presentations and open-ended discussion about current research and resource needs of the field. This is the white paper that describes the results of that study.

Institute

Current Research



Over the last 5 to 10 years, the interface between biology and geology has grown to become one of the most intellectually stimulating frontiers in science. While it has long been recognized that deciphering the relationship between the history of life on earth and the history of the planet is a profound task, only recently has science evolved to a point where this relationship can be determined (Benner, 2001). Indeed, technological innovations in both the earth and life sciences now allow us to ask and answer questions that simply were beyond our scope before. The emergence of the discipline of geobiology, which can be defined as the study of how organisms have influenced and been influenced by the earth's environment (Knoll and Hayes, 1997), is generating tremendous enthusiasm yet remains surprisingly indeterminate. Highly specialized and separate fields, ranging from paleontology to prebiotic chemistry, can all legitimately claim to belong to this discipline, yet historically they have had minimal overlap. Part of the confusion over what constitutes geobiology springs from its interdisciplinary nature. The fact that geobiology derives from two disparate scientific traditions (those of natural history and experimental science) complicates its description. As C.P. Snow recognized over 40 years ago in his lecture "The Two Cultures", communication between different disciplines often results in "a gulf of mutual incomprehension" that can be difficult to ford (Snow, 1959). Although Snow was referring to the divide that separates the humanities from the sciences, many of his insights can be applied to the divide that until recently has separated biology from geology. With the recognition of the scientific opportunities inherent in geobiology, however, the gulf that separates the earth from the life sciences is rapidly closing. Creative bridges are being built between these fields that promise to significantly advance our understanding of the interactions between the earth and the biosphere in modern and ancient environments.

This introduction highlights some of the conceptual and technological breakthroughs in both biology and geology that have set the stage for a productive fusion between these fields in the 21st century. We can now begin to determine the composition of and the dynamic exchange between the bio-, hydro-, atmo-, and lithospheres, with the specific goal of understanding their co-evolution through time. In this document we summarize some of the current research taking place in geo-biology, and outline particular resources that are needed to advance the discipline.

ADVANCES IN PROBING THE ROCK RECORD

*T*raditionally, geology has tried to explain the nature of our physical environment and how it came to be. Biology, in the form of fossils, has long been used to help decipher the physical and chemical forces that shaped our world. The geologic time scale was first articulated in terms of stratigraphic sequences of particular fossils; the theory of plate tectonics was supported by the near identity of species on widely separated continents; and evidence of bombardment of Earth by meteors came from the study of extinction events. Only recently, however, has the extent of the role of biology in actively shaping the Earth's physical and chemical environment been recognized widely. For example, the genesis of massive iron and manganese ore deposits 2.4 billion years ago is thought to be directly linked to a cyanobacterial bloom (Kirschvink et al., 2000). The key challenge that we now face is to clarify how frequently and in what ways biology has contributed to major events such as this throughout earth history.

Until recently, our ability to identify such episodes has been constrained by our inability to discriminate biological from abiotic signatures in the rock record. Sophisticated techniques to serve this purpose have been developed during the

past two decades in chemistry. These include: synchrotron X-ray scattering methods for determining which elements are present in a sample and defining their coordination and valence at the atomic scale (Koningsberger and Prins, 1988); increased precision in measuring stable isotopes of biologically important elements such as C, O, H, S, and Fe, and an ability to detect them at the microscale; the use of gas chromatography-combustion-isotope ratio mass spectrometry to measure the stable isotopic composition of individual organic compounds (Hayes, 1993); improved techniques for quantifying trace elements in natural waters (Donat and Bruland, 1994); and the development of a new generation of microelectrodes that can measure constituents such as O₂, S(-II), Fe, Mn, and pH (Luther et al., 1999). Since it is generally agreed that one of the defining attributes of life is that it exists in chemical and physical disequilibrium with its environment, the specificity and sensitivity of these techniques make them well suited to detecting specific biosignatures that are preserved in ancient rocks. Biological materials tend to concentrate particular elements and isotopes that distinguish them from their surroundings, as well as promote the growth of distinctive structures (both organic and inorganic,



Rest after an all-day hike through the Hoogland Member, near Zebra River, Namibia. Studies of facies preserved in sequences such as these can tell us much about changes in the Earth's environment over time.

macro and micro) that have no abiotic counterparts. As many of these techniques can be used *in situ*, they likely will improve our understanding of past and present biogeochemical interactions occurring in diverse samples on a variety of scales.

In addition to the increased sophistication of chemical measurement techniques that now enable us to detect traces of life, advances in the earth sciences have given us insights into where to look for such traces. Steady progress in regional mapping and stratigraphy throughout the 19th and 20th centuries has resulted in awareness of the location of world-wide sites of geobiological interest. Advances in geochronology and

paleomagnetism have made it possible to date these sites and group them together with respect to major events in earth history (Bowering and Erwin, 1998; Bowring et al., 1993; Grotzinger et al., 1995; Kirschvink, J.L., 1992; Orth et al., 1981). Investigators can now perform comparative studies on similar facies from locales ranging from Oman to Namibia, providing a truly global perspective on geobiology. Awareness of these locales is essential if we wish to answer questions regarding the distribution (both in time and space) of major physical/chemical changes catalyzed by biology throughout Earth history.

ADVANCES IN MOLECULAR BIOLOGY AND GENOMICS

*I*n parallel with the advances made in probing the rock record, molecular biology has revolutionized the study of evolution. As early as 1965, Zuckerkandl and Pauling recognized that molecular sequences contain a repository of historical information (Zuckerkandl and Pauling, 1965). In the mid 1970s, Carl Woese permanently changed our concept of the universal tree of life by using ribosomal RNA (rRNA) molecules to classify organisms into three separate domains, the Archaea, Bacteria, and Eucarya (Woese and Fox, 1977). Prior to this work, evolutionary study had been largely confined to the metazoa and metaphyta, whose complex morphologies and detailed fossil record provided a sound base for natural taxonomy, yet whose histories at best cover 20% of the total evolutionary time span (Woese et al., 1990). The recognition that rRNA could be used as a molecular chronometer opened up the evolutionary study of deep time, where the history of life is predominantly microbial. Since then, advances in DNA, RNA and protein sequencing have made it increasingly easy to use molecular information to infer phylogenetic relationships between organisms from a diversity of environments, including organisms that have not yet been cultured (Pace, 1997).

The explosion of genome sequencing projects within the past 5 years has made it possible to make a comprehensive comparison of prokaryotes and eukaryotes, extending phylogenetic analyses from comparisons of rRNA to the range of biomolecules encoded by whole genomes (Brown and Doolittle, 1997; Feng et al., 1997; Jain et al., 1999). The microbial genome database web site at the National Center for Bioinformatics currently lists 92 projects either complete or underway around the world, and as the ease of sequencing increases, the availability of new genomic data will rise. Already, much has been learned from genome sequencing projects that has challenged prior notions of the history of life: comparative genomics has revealed phylogenetic incongruities that span the universal tree. For example, the vast majority of gene products from the Archaea most resemble counterparts among the Bacteria and not the Eucarya, yet the rooted phylogenetic tree clearly places the Archaea as specific relatives of the Eucarya (Doolittle, 1998). Such incongruities provide evidence for lateral gene transfer as a primary evolutionary mechanism, and have led to radical rethinking of models for how life evolved (Woese, 1998; Doolittle, 1999).

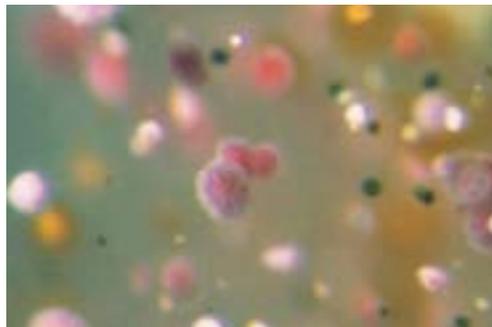
The changes in evolutionary paradigms brought about by molecular sequencing and genomic databases have set the stage for rapid progress in geobiology. The successful culturing of organisms from extreme environments (Stetter, 1996), in addition to molecular techniques for reconstructing genomic information from uncultured organisms (Beja et al., 2000) are rapidly expanding the diversity of organisms that are accessible to molecular scrutiny. An important step forward in the analysis of natural samples has been the combination of gene probes with biomarker structure and molecular level isotopic measurements (Hinrichs et al., 1999). This approach has permitted the direct correlation of biomarkers with likely source organisms, even though the latter may have never before been cultured. Lastly, developments in imaging technologies, genetic manipulation of model organisms, and *in vitro* evolution, are enabling researchers to probe the mechanistic basis of fundamental biological processes that are likely to be ancient in origin.



a



b



c

a: Microbial mats grow in environments such as the Sippewisset salt marsh, near Woods Hole, Massachusetts.

b: Environmental gradients of light, sulfide and oxygen create conditions that favor particular types of organisms: oxygenic Cyanobacteria on top, anaerobic purple sulfur bacteria below and sulfate-reducing bacteria underneath that precipitate black metal sulfides.

c: Phototrophic sulfur and non-sulfur bacteria in an enrichment from this mat reveal part of the enormous diversity of microorganisms.

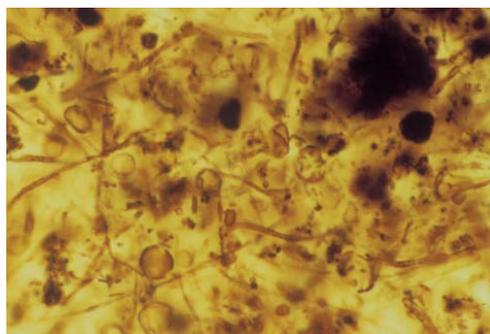
Geobiology

Current Research Directions



In the next sections, we briefly describe our present understanding of a small subset of representative geobiological problems. While by no means inclusive, this subset has been chosen to illustrate the range of topics and research opportunities that fall under geobiological purview. The sections cover research that span global to molecular processes, although we emphasize that neither can stand in isolation. Indeed, the mantra of geobiology—that the history of the evolution of life and the Earth can only be appreciated by deciphering their interdependencies—is the leitmotif of this document.

Questions of What and When: Detecting Life in the Geologic Record



Microorganisms from the 1875 Ma (million years ago) Gunflint Iron formation. The large spheroid in the lower left is 10 microns in diameter.

M

OLECULAR FOSSILS

*B*efore we can ask questions regarding the origin and evolution of life, it is helpful to be able to place life's emergence within the context of geologic time.

Traditionally, geologists named historical intervals in the rock record according to recognizable characteristics of the rocks. Often these characteristics were linked to the presence or absence of particular fossils, such as the extinction event observed in rocks at the K/T boundary that marks the demise of the dinosaurs (Alvarez, 1997). The deeper back in time we travel, however, the more difficult it becomes to rely on fossils to learn about early life. While recent advances in stratigraphy and geochronology have made it possible to elucidate the geobiology of the Precambrian/Cambrian boundary [about 544 million years ago (Ma)], the nature of life in Archean times [> 2500 Ma] is still obscure (Knoll, 1999).

The few well-preserved fossils from this time period are microscopic, and little (if anything) can be concluded about their physiology merely by looking at them. One of the great remaining challenges in geobiology, therefore, is to reveal the nature of life in this remote time period.

Perhaps the most promising approach to understanding life in the Archean lies in interpreting the biogeochemical signatures of

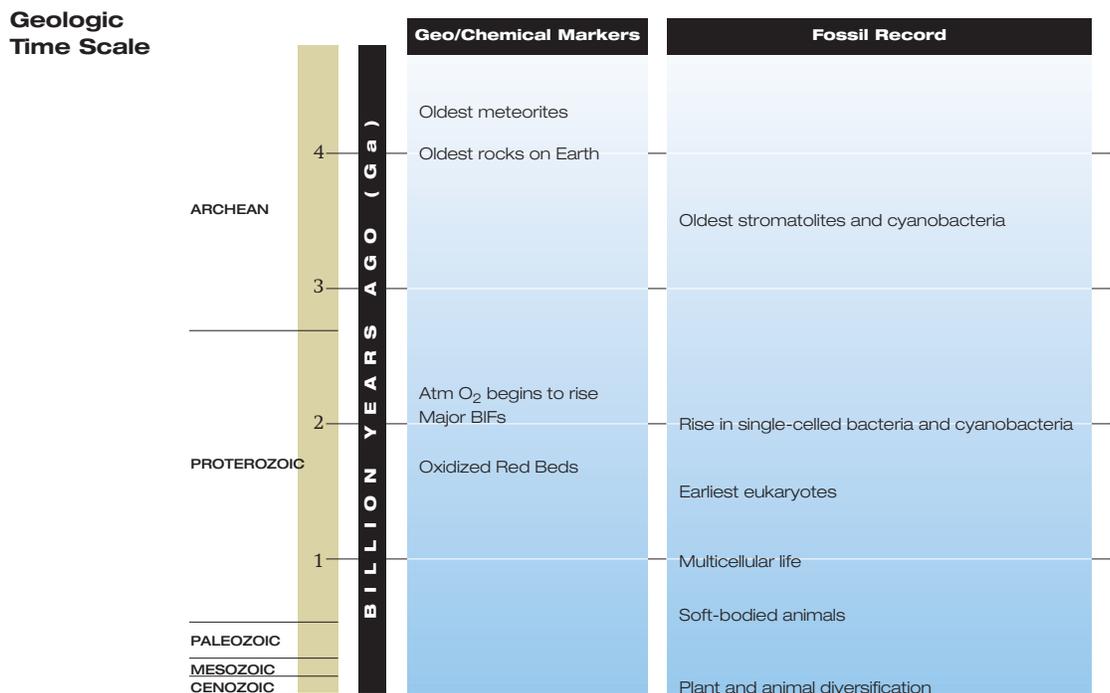
organisms that are not necessarily preserved morphologically (Knoll and Canfield, 1998). Organic geochemists use the term biomarker to describe certain distinctive compounds isolable from living systems that have related molecular fossil derivatives (Eglinton and Calvin, 1967). The salient features of biomarkers are structural distinctiveness, taxonomic specificity and preservation potential. Hence, the majority of biomarkers are lipids or pigments such as porphyrins; metabolites such as sugars, organic acids, amino acids and nucleosides do not make good bio-markers, as they generally are not specific and not preserved in the rock record.

Bacteriohopane-polyols are the most important class of biomarker compounds for bacteria, and acyclic isoprenoids for archaea. Cyclized and highly branched structures, such as found in the polyisoprenoid lipids, tend to be most resistant to biodegradation and to retain their structural integrity even after extended periods of burial in sediments (Summons and Walter, 1990). Recently, a significant step forward in Archean geobiology was made by the discovery of 2-methylhopane fossil analogues in ancient organic sediments (Summons et al., 1999; Brocks et al., 1999). This presents the most compelling evidence yet for the presence of

cyanobacteria and oxygenic photosynthesis back to at least 2700 Ma.

On the inorganic end, the use of emerging technologies to measure novel bioindicators holds promise for interrogating ancient samples. Work underway at the Jet Propulsion Laboratory in Pasadena, CA has shown that the distribution of the inorganic products of metal-precipitating microbes tends to be distinct and differentiable from the inorganic constituents of sediment or host rock, although their spatial distribution may alter over time (Douglas, 1998). The three dimensional distribution of elemental abundances, fractionation patterns of stable isotopes, and charge state distribution of cations challenge the traditional definition of biomarkers. Measurements of these parameters that are out of context with expected values for an abiological geological environment have potential as non-Earth-centric bio-markers. Synchrotron based x-ray

spectroscopes are presently being employed to probe biominerals with exciting results. Of special note is the ability to measure cation oxidation states at very small spatial dimensions with x-ray photo-electron emission spectroscopy (Conrad et al. 2000, DeStasio, et al. in press). This technology is currently being used to explore the distribution of differing charged states in both iron and manganese in biogenic rocks, and is likely to expand our ability to recognize bio-markers. Electron energy loss spectroscopy also holds great promise in this regard. No less than half a dozen high-energy analytical probes are presently being exploited in pursuit of inorganic biomarkers at high spatial and spectral resolutions. X-ray photons, ultraviolet and visible wavelength lasers, electrons, protons and gallium ions are being used for both imaging and spectroscopy.



M MORPHOLOGICAL FOSSILS

*A*ppreciation of molecular fossils (be they organic or inorganic) does not diminish the importance of being able to recognize morphological fossils (macro or micro) when they occur in the rock record. Major questions about whether certain features in ancient rocks are biogenic remain to be answered. To illustrate this, we highlight work currently being done on stromatolites and magnetotactic bacteria.

Stromatolites are attached, lithified sedimentary growth structures, accretionary away from a point or limited surface of initiation. Fossilized stromatolites are often cited as examples of early life. This is because the accretion process is commonly thought to result from the sediment-trapping or precipitation-inducing activities of microbial mats. However, little evidence of this process is preserved in most fossilized Precambrian stromatolites. It is not clear that the laminated structures seen in Archaean and Proterozoic stromatolites are biogenic, and criteria to discriminate abiotic processes from ones that are microbially-mediated must be developed. In detail, stromatolite growth is dependent on many processes that are interrelated in a complex fashion. A list of factors that might influence stromatolite development include light intensity, salinity,

nutrient supply, current velocity, sediment grain size distribution, mat community diversity, and carbonate saturation, to name a few. Although it would be difficult to test all of these experimentally, one potentially informative line of investigation would be to simulate stromatolite morphogenesis by studying accretionary processes in simple microbial communities, varying only a select number of environmental parameters.



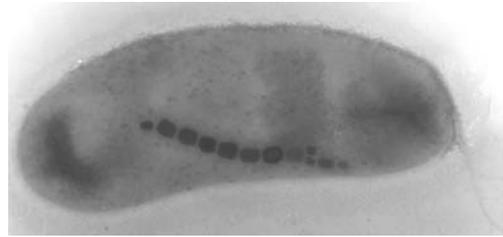
Growth model based on diffusion-limited aggregation and episodic sedimentation.



Branching columnar stromatolites of the Paleoproterozoic Talthelei Formation, north-west Canada, showing strong similarity to model results.

The successful study and interpretation of stromatolites requires a process-based approach, oriented toward deconvolving the replacement textures of ancient stromatolites. The effects of diagenetic recrystallization first must be accounted for, followed by analysis of the processes that create lamination and deduction of possible accretion mechanisms (both biological and abiotic). It seems likely that certain morphologic classes of stromatolites, such as those with coniform geometry, may prove to be largely or even uniquely biogenic. That said, morphology alone cannot always be used as an accurate measure of stromatolite biogenicity, as shown by recent numerical simulations that test abiotic accretion hypotheses (Grotzinger and Rothman, 1996). Application of this approach suggests that stromatolites were originally formed through *in situ* precipitation of laminae during Archean and older Proterozoic times, but that younger Proterozoic stromatolites grew through the accretion of carbonate sediments, most likely by microbial trapping and binding. This trend is thought to reflect the long-term evolution of the earth's environment rather than microbial communities (Grotzinger and Knoll, 1999).

Another example of where morphology has been used to infer biogenicity is the case of the Martian meteorite ALH84001 (McKay et al., 1996). Although still controversial, the most compelling arguments for relic biogenic activity in this sample spring from the similarity between some of the fine-grained magnetite (Fe_3O_4) in the meteorite and that found in magnetotactic bacteria. In a typical magnetotactic bacterium, individual



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

magnetite crystals (~50 nm) are enclosed in a lipid bilayer membrane and aligned in linear chains so as to maximize the net magnetic moment of the cells. Natural selection has perfected the size, shape, composition, crystallographic orientation, and ultrastructure of the cells in order to enhance their magnetic properties. Recent high resolution transmission electron microscopy of nearly 600 magnetite particles from the ALH84001 meteorite has shown that approximately 27% have an elongated, chemically-pure hexagonal prismatic morphology that is identical to the magnetite produced by *Magnetospirillum magnetotacticum* strain MV-1 (Thomas-Keprta et al., 2000). To date, these particles have not been found anywhere outside of the biosphere, nor have they been synthesized by materials scientists. In addition to providing possible evidence for life on Mars, it has been suggested that the magnetite biomineralization system might be the ancestral process responsible for biomineralization in later animal phyla (Kirschvink and Hagadorn, 2000). Understanding how the magnetosome forms and operates, therefore, may provide clues for unraveling the genetics of biomineralization in higher organisms.

SOTOPIC SIGNATURES

*F*or many years stable isotopic fractionations have been used to reconstruct past climates and recognize the products of specific metabolisms in the geologic record (Epstein et al., 1953). For example, the currently oldest known sedimentary rocks (in the range of 3.7-3.9 billion years) have been shown to contain graphite with a strong enrichment in isotopically light carbon, suggesting that this material comprises the fossil remains of the earliest organisms (Schidlowski et al., 1979; Mojzsis et al., 1996). This enrichment is the result of enzymatic fractionation in the first step of uptake of inorganic carbon by autotrophic organisms, and it testifies to the biochemically advanced state of microbial life already 600-700 million years after the formation of the planet. Until recently, most isotopic studies have focused principally on C, H, O, N and S isotopes (Chambers and Trudinger, 1979; Schidlowski et al., 1983; Des Marais, 1997; Knoll and Canfield, 1998). Recent advances in the ability to measure Fe isotopes now add this element to the isotopic arsenal and will likely prove to be important in learning about life on the early Earth (Beard and Johnson, 1999). Despite insights that have been gained from isotopic studies into the Archean and Proterozoic ecosystems (Canfield et al., 2000),

much remains to be learned using isotopic geochemistry. As an example, we discuss how carbon isotopic patterns may inform us about the evolution of the atmosphere.

Most geologists believe that atmospheric O₂ concentrations increased dramatically between about 2400 and 2000 Ma (Cloud, 1972; Walker et al., 1983; Kasting, 1993; Holland, 1994). The reasons for this increase, however, are not well understood.

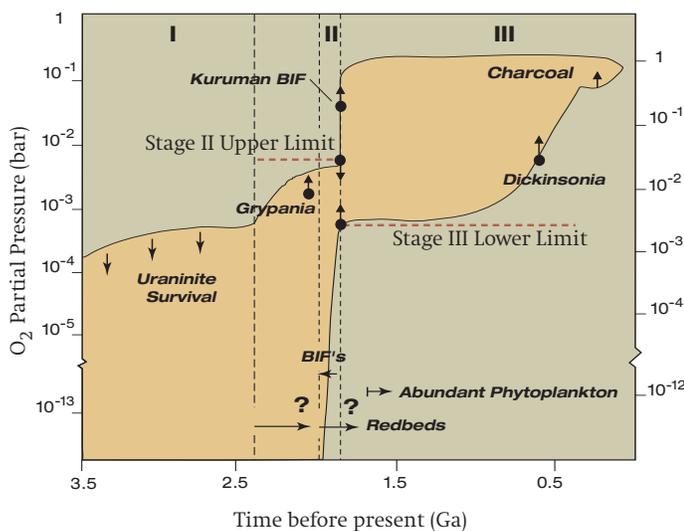
Conventionally, the rise in atmospheric oxygen has been attributed to the evolution of oxygenic photosynthesis by cyanobacteria. This argument assumes that changes in the production of O₂ controlled its atmospheric concentration. However, recent isotopic evidence suggests this is not necessarily the case. Differences in the isotopic composition of sedimentary organic C ($\delta^{13}\text{C}_{\text{org}}$) and carbonate C ($\delta^{13}\text{C}_{\text{carb}}$) reflect the aggregate effects of isotopic discrimination by biota and subsequent alteration in sedimentary rocks. Values of $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ in sediments, relative to the crustal average ($\delta^{13}\text{C}_{\text{avg}}$), indicate the relative rates of sedimentation and burial of organic and carbonate C. Because a greater relative rate of burial of organic C leads to a greater rate of accumulation of O₂ and/or other oxidation products (e.g., SO₄²⁻ and Fe³⁺), C isotopic

patterns convey implications for the cycles of other redox-sensitive elements, including oxygen. Mean $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{carb}}$ values have been remarkably stable for >3800 Ma, although significant excursions in both $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{org}}$ have occurred for relatively short (~ 200 Ma) intervals. This, in turn, implies that throughout that time, the net source for O_2 has been within a factor of two as large as today.

This leads us to conclude that oxygenic photosynthesis evolved well before the ~ 2300 Ma rise in atmospheric O_2 (biomarker studies have recently presented convincing evidence for 2700 Ma cyanobacteria), prompting us to ask the question why O_2 levels rose when they did. While many theories have been presented to resolve this paradox, one that is relevant to geobiology involves a role for microorganisms in controlling the chemistry of reduced gases. It is generally accepted that

the percent of outgassed CO_2 that is buried as organic C reflects a redox balance between the atmosphere and crust (Holland, 1978). Given this, it may follow that reduced gases (such as hydrogen and methane) were not escaping from the atmosphere at an appreciable rate during the Archean. It seems possible that hydrogen escape may have been suppressed due to the activities of methanogens and/or sulfate reducers, while methanotrophic bacteria suppressed atmospheric methane. If stratospheric levels of these gases were kept relatively low, then this would imply the escape rate was also low, and the C isotopic constraint could be satisfied. This hypothesis could be tested by culturing a microbial mat under anaerobic conditions and observing the gases given off by it and the amounts of C isotopic fraction produced within its various layers. An alternative, non-biological explanation for

Rise in Atmosphere Oxygen



*Putative stages in the rise of atmospheric O_2 over time. The shaded area represents the range of O_2 concentrations that are consistent with both geological and biological constraints. The transition from a reducing to an oxidizing atmosphere (Stage I to Stage II) appears to have been contemporaneous with the Huronian glaciation, which has been dated at ~ 2.3 Ga (billion years ago). Reprinted with permission from Kasting, J.F., *Earth's Early Atmosphere*. Science 259:920–926. Copyright 1993 American Association for the Advancement of Science.*

the suppression of hydrogen escape could be the existence of low exospheric temperatures at this point in Earth history; this could be tested with a quantitative model of upper atmospheric structure.

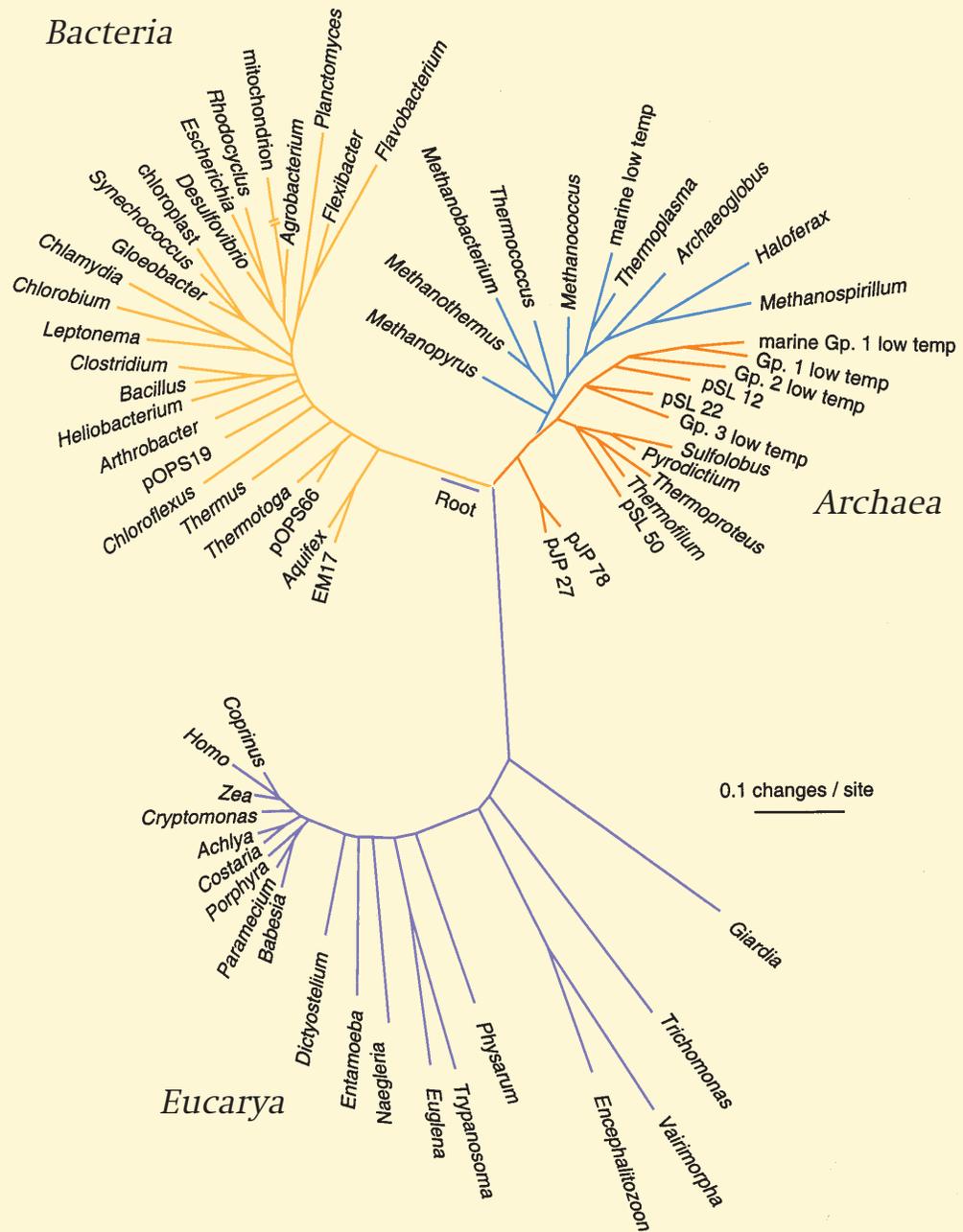
One current limitation to using isotope geochemistry in geobiology is that biological fractionations of stable isotopes commonly are not understood in sufficient detail to distinguish between the products of different metabolic pathways. Where such specificity is possible, it is a result of detailed experimental study of laboratory cultures and/or model enzymatic reactions. For example, there is a readily recognized difference between the stable isotope compositions of carbon fixed by C₃ and C₄ plants, resulting from differences in their photosynthetic pathways that are highly conserved among different species and therefore recognizable in the geological record. The simplicity and success of this 'fingerprint' offers a challenge to the next generation of geobiologists to resolve the isotopic fractionation pathways of major bioenergetic processes that may have played an important role on the early earth (e.g. chemoautotrophy and anoxygenic photosynthesis). Only once such fingerprints are known can isotopic data be used to test hypotheses about how biology has modulated atmospheric chemistry throughout geologic time.

Questions of Who and How: Deciphering the Mechanisms of Evolution

G ENETIC DIVERSITY

Understanding evolutionary diversification and how it has interfaced with the environment is one of the greatest challenges in geobiology. Beginning with Darwin's theory of natural selection, it has long been appreciated that life evolves in response to environmental stimuli (Darwin, 1859). The converse notion, that the environment itself is shaped by the workings of the biosphere, is relatively new (Lovelock, 1987; Margulis, 2000). Much of what we know today about biodiversity springs from the universal phylogenetic tree developed by Woese and others. Not only does the tree provide a molecular framework in which to evaluate evolutionary processes, but its revelation that the overwhelming diversity of life is microbial guides our approach to studying life's impact on earth history.

For years, microbiologists have recognized that the activities of the microbial world control and sustain life on this planet (Beijerinck, 1888; Winogradsky, 1889; Kluyver, 1924; Van Niel, 1967). Only recently, however, has the importance and extent of microbial diversity captured the attention of the rest of the scientific community, largely thanks to the creative application of molecular biological techniques to environmental samples (for review see Pace, 1997). These



Universal phylogenetic tree based on small subunit rRNA sequences. Reprinted with permission from Pace, N.R., *A Molecular View of Microbial Diversity and the Biosphere*. Science 276: 734-740. Copyright 1997 American Association for the Advancement of Science.

techniques utilize ribosomal RNA gene cloning and sequencing, single-cell hybridization probes, and applications of the polymerase chain reaction (PCR). Comparative interpretation of sequence data reveals the phylogenetic type of organisms in the environment. rRNA-based phylogenetic results go beyond simple taxonomy because some properties of otherwise unknown organisms can be inferred from the properties of their cultivated relatives, and the sequences provide tools, for instance hybridization probes, with which to analyze various aspects of particular organisms in their natural settings (see for example, Edwards et al., 2000). rRNA-based technology is being used to survey the types of genomes that occur in environmental ecosystems, including zones of hydrothermal circulation, endolithic communities, bioremediating aquifers, anaerobic sediments, unusual symbionts of animals, and human disease tissues. DNA is purified from environmental samples, then used for PCR amplification with primers that anneal to universally-conserved or phylogenetic group-specific sequences in 16S rRNA genes. PCR products are cloned and sequenced, and the results are analyzed phylogenetically. This strategy is revealing a spectacular wealth of unknown organisms that go well beyond the extent of known diversity. For example, what appear from rDNA sequence to be eight new eukaryotic kingdoms have recently been discovered by molecular probing of anoxic sediments (Dawson and Pace, in prep). How these uncultivated microbes affect the chemistry of the earth's environment is currently unknown.

MICROBIAL PHYSIOLOGY

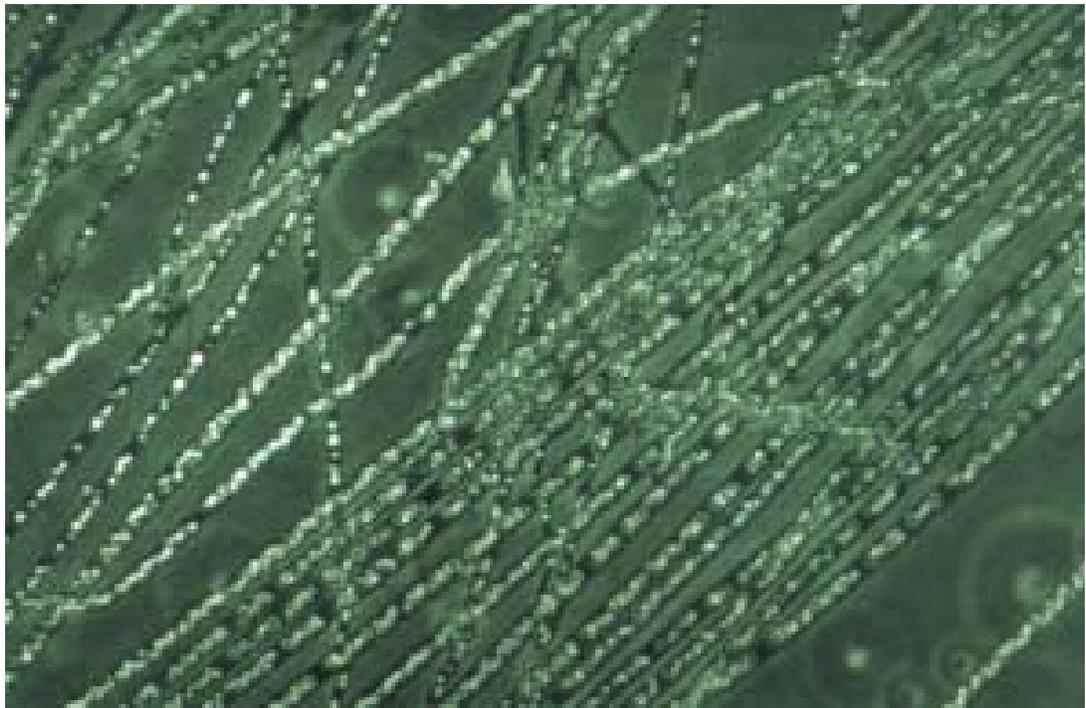
With the recognition that microbes have dominated the world's ecosystems throughout geologic time, a key question becomes: what have they been doing? While the interactions between life and the Earth are highly complex, it is by now axiomatic that the emergence of critical forms of life profoundly and irreversibly changed the chemistry of the planet. Despite this recognition, little is understood about biologically-mediated chemical changes that occurred on the early Earth.

Determining the connection between the activity of particular microbes (both present and past) and the chemistry of their environment lies at the heart of geomicrobiology. Virtually every month new discoveries are made about the occurrence of life in extreme environments, ranging from the freezing to the boiling point (Prisco et al., 1998; Stetter, 1996). The diversity of metabolisms being encountered span those that derive power from the sun to those that are driven by geochemical reactions occurring in the deep subsurface of the earth (Widdel et al., 1996; Ghiorse, 1997). Indeed, the diversity of chemical reactions catalyzed by microbes is remarkable and plays a fundamental role in the cycling of the elements between the earth's atmosphere, hydrosphere, and

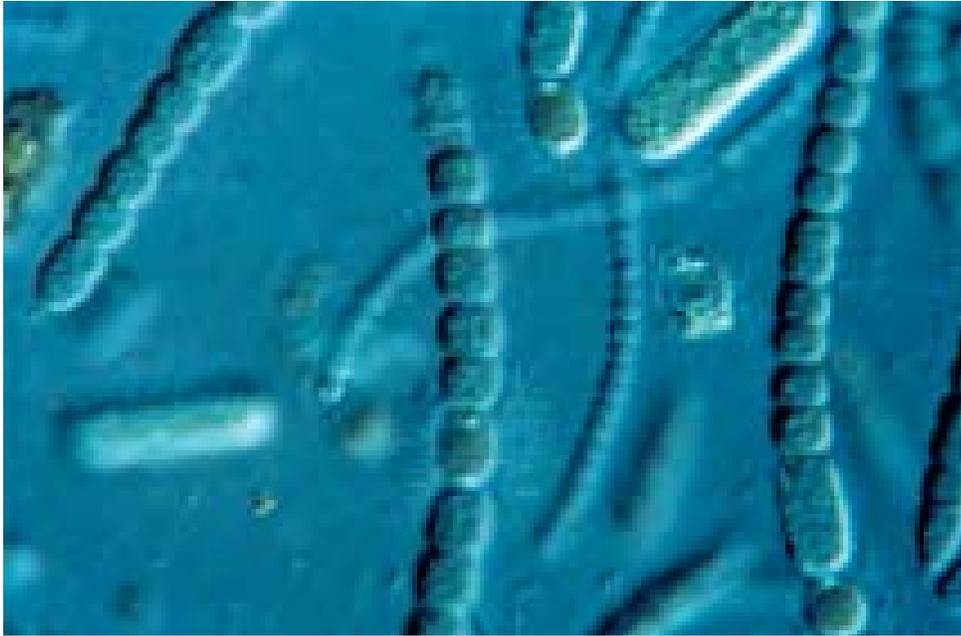
lithosphere (Ehrlich, 1990). One promising approach to understanding how the evolution of metabolism has shaped the chemical evolution of the earth is to investigate modern bacteria for clues into the lifestyles and products of ancient organisms. This can be thought of as exploring the genetics of geochemistry. A key assumption in this approach is that DNA is a fossil, i.e., by determining which genes in modern bacteria control geochemical reactions, we can gain insights into ancient metabolic processes and their mineral products. With the cost of DNA sequence analysis plummeting, more and more environmentally-interesting microbial genomes are becoming available. Sequence data from these genome projects will help to assess the relative importance of environ-

mental and biological processes on the co-evolution of life and the earth's surface (Banfield and Marshall, 2000). In concert with chemical, isotopic and mineral analyses, genetic and biochemical analyses may allow us to link the evolution of particular types of metabolism with events preserved in the rock record.

Microbe/mineral interactions have stimulated much research in recent years (Beveridge et al., 1997; Banfield and Nealson, 1998). This subfield of geomicrobiology is particularly amenable to interdisciplinary work, as exemplified by approaches to studying microbial metabolisms that are based on iron, such as Fe(II)-photosynthesis and Fe(III) respiration. Studying these processes from a variety of angles (e.g.,



*Microbes are members of biogeochemical cycles. Filamentous sulfide-oxidizing *Beggiatoa* sp. are important members of the sulfur cycle. They oxidize hydrogen sulfide and temporarily store elemental sulfur intracellularly. The white cellular inclusions are sulfur globules,*



a



b

Filamentous heterocystous cyanobacteria. Cyanobacteria invented oxygenic photosynthesis. The heterocysts are specially equipped cells able to fix atmospheric nitrogen and supply the organically bound forms to neighboring cells. a: Nomarsky image. b: Same frame in epifluorescence microscopy. The light harvesting and reaction center pigments show their red autofluorescence.

physiological, genetic, biochemical) is relatively straightforward, as model organisms that can be genetically manipulated are known (e.g. *Rhodobacter capsulatus*, *Shewanella putrefaciens*, *Geobacter sulfureducens*) and genome sequencing projects for these organisms are nearly complete. Furthermore, both of these metabolisms leave chemical signatures that may be subject to isotopic and mineralogic analyses: Fe(II)-photosynthesis generates oxidized iron minerals and Fe(III)-respiration generates reduced iron minerals that are found in an ancient class of sedimentary deposits known as banded iron formations (BIFs). It has been hypothesized that bacteria with these metabolisms may have played an important role in BIF deposition (Widdel et al., 1996), which, if true, would provide a solution to a major geological puzzle. Fe(III)-reduction and Fe(II) oxidation are also thought to be geochemically important in modern environments, as they are likely to contribute significantly to the mobility of toxic compounds and radionuclides (Lovley, 1991). Very little is understood about the mechanisms of Fe(III)-respiration, and even less is known about Fe(II)-photosynthesis. Genetic approaches to exploring microbe/mineral interactions at the molecular level are just beginning (Beliaev & Saffarini, 1998; Burnes et al., 1998; Newman and Kolter, 2000), and new results will undoubtedly change how we read microbial genomes for clues into the evolution of these important metabolic pathways.

EUKARYOTIC SYSTEMS

When considering microbes, it is important that this term not become a synonym for prokaryotes (e.g. bacteria and archaea). The majority of eukaryotic genetic diversity and evolutionary history is microbial, and the ecological significance of microbial eukaryotes is only beginning to be appreciated (Dawson and Pace, in prep). While molecular techniques have already taught us much about the roots of the Eucarya (reviewed in Knoll, 1992), the origin of the eukaryotic cell remains a fascinating puzzle, and work on unmasking the cell biology of deeply branching eukaryotes awaits the next generation of geobiologists. Identifying signature biomarkers from these organisms would greatly improve our ability to interpret the paleobiological record. Furthermore, recent applications of molecular techniques to diverse environmental samples has revealed that eukaryotic phyla often dominate the genetic pool at any given site, yet currently very little is known about their biogeochemical activity and therefore many opportunities for further research exist.

Comparatively more work has been done on the genetic structure of particular eukaryotic organisms. Research relevant to geobiology has focused on two separate problems: how the genome readies itself for

evolution, and how particular genes control development. Until recently, most researchers assumed that genomes evolve to minimize mutation rates and prevent random genetic drift. Barbara McClintock was a notable exception, recognizing early on that the genome in times of stress could initiate its own restructuring and evolution (McClintock, 1983). Studies currently being performed on the process of gene unscrambling in hypotrichous ciliates demonstrate how extensive this restructuring can be (Pennisi, 1998; Prescott, 1999). Work to date has focused on the scrambled germline (micronuclear) genes encoding DNA polymerase alpha and alpha telomere binding protein in *Oxtricha nova* and *Stylonychia lemnae*. For example, the intact somatic (macronuclear) gene encoding DNA polymerase alpha is assembled from 48 highly dispersed pieces in the micronuclear genome, present on two main loci with no apparent strand-asymmetry (Landweber et al., 2000). The mechanisms controlling this complex reassembly process in ciliates may provide insights into how genes first began to assemble themselves on the early Earth.

After their proper assembly, the ordered transcription of genes within eukaryotes governs major developmental processes. The field of developmental genetics, together with phylogeny and paleontology, is shedding light upon the evolution of multicellular life. For example, ever since the discovery of the Burgess Shale an important problem in evolutionary science has been to understand what drove the rapid diversification of animal phyla during the Cambrian explosion. The discovery of conserved families of



A soft-bodied animal from the late Paleozoic (middle Cambrian) Burgess Shale near Field, British Columbia.

transcription factors (such as the *hox* genes) as well as other gene families that control development in phylogenetically disparate animals (such as those involved in inter-cellular signaling), has enabled developmental biologists to speculate on the nature of primitive developmental pathways in Proterozoic animals. It now seems likely that major genetic innovations were already in place by the time of the Cambrian explosion, and it was the combination of favorable environmental and ecological factors that triggered the rapid diversification of animal phyla during this time (Knoll and Carroll, 1999). While the evolutionary diversification that is preserved in the Burgess Shale is a classic example, many other examples of radical change also can be found in the fossil record and are good targets for geobiological efforts.

EXPERIMENTAL PALEOGENETICS

Whether we are interested in understanding the genes involved in signal transduction in the first multicellular metazoan or the genes involved in the first iron respiring bacterium, all hypotheses regarding ancient genes and their function(s) remain pure speculation until we are able to test them. An unfolding area of research that holds great promise for geobiology employs techniques of recombinant DNA technology to reconstruct and study putatively ancient proteins in the laboratory. This line of investigation is called “experimental paleogenetics” and has been pioneered by Steven Benner’s group at the University of Florida. Experimental paleogenetics is based upon the premise that episodes of rapid sequence evolution in a particular protein family often correlate with the emergence of new physiological functions known from the fossil record. Likely episodes of rapid sequence evolution are identified by bioinformatics tools that enable a set of sequences to be compared to one another and grouped according to the changes in their genetic sequence. From such data sets, genes encoding proteins of interest can be reconstructed and expressed, and the behavior of the resulting proteins can be studied in the laboratory (Stackhouse et al., 1990).

For example, paleontological and sequence data suggested that the emergence of ruminant digestion in mammals (approximately 40 Ma) coincided with a time of rapid evolution of a family of proteins known by the name “ribonuclease A” (RNase A). Ruminants became the principal large herbivores in the period that followed, almost certainly because of climate changes associated with the closure of the Drake passage between South America and Antarctica. This plate tectonic event was associated with a dramatic cooling and drying of large parts of the continental land mass, and the biology responded. Tough grasses came to dominate savannahs. Ruminants, whose digestive systems use bacteria in a forestomach to ferment cellulose, were able to extract the energy in the energy-poor grasses more efficiently.

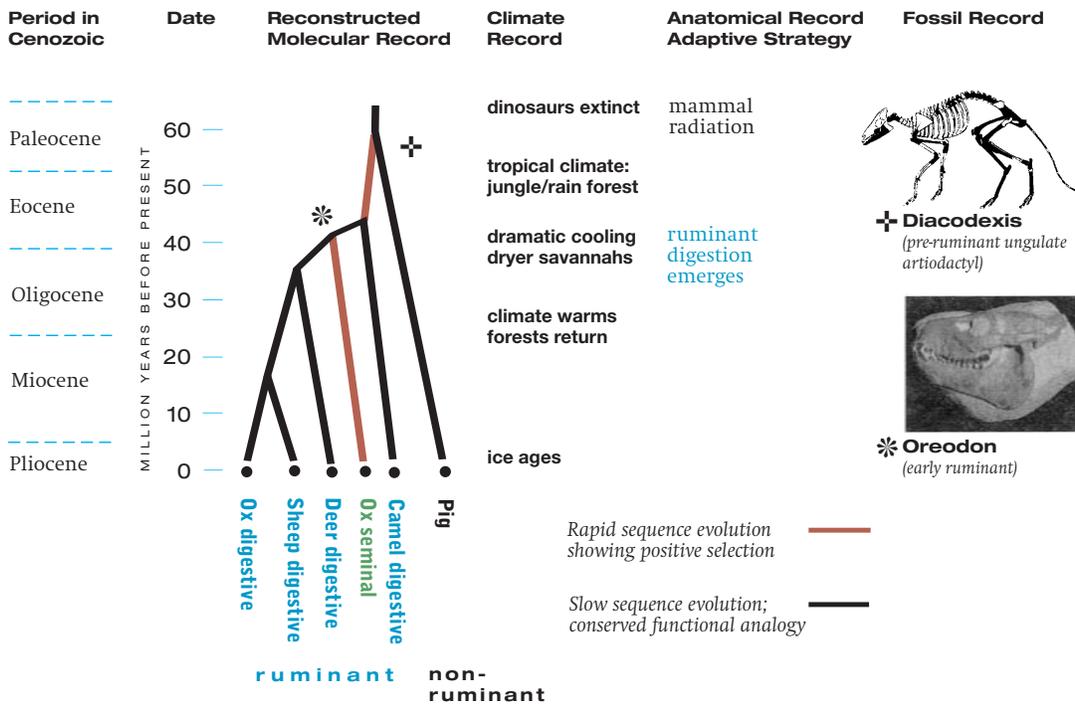
This model predicts that versions of RNase A found in ruminants (since 40 Ma) should be better suited for digestion than those found in pre-ruminants that lived earlier. To test this, genes predicted to encode ancient RNases both before and after ruminant digestion arose were synthesized, expressed in *E. coli*, and studied. RNases that existed before ruminant digestion showed properties not well suited for function in a

digestive tract. RNase reconstructions that post-dated the emergence of ruminant digestion had the *in vitro* properties expected for a digestive enzyme (Jermann et al, 1995). Thus, paleogenetic experiments confirmed the hypothesis that the episode of rapid evolution seen in the reconstructed sequence record of RNases reflected the acquisition of chemical properties that enabled an ancestral non-digestive enzyme to evolve into one suitable for the digestive tract, exactly at the time that the reconstructed molecular record suggested that the protein family had experienced an episode of rapid sequence evolution. This correlation between physiology, plate tectonics, paleoclimatology, and paleontology

(disciplines from natural history) and the molecular record (including structural biology) has defined a paradigm that is answering many questions having the form: "What is the function of this protein?"

Experimental paleogenetics provides a conceptual link between paleontology and prebiotic chemistry. Stimulated by the fossil record, genome sequences can be scanned for candidate genes responsible for major evolutionary events. The molecular underpinnings of evolutionary events can then be determined through laboratory experiments as described above. Pushing back the evolutionary clock from the time of ruminant fossils by several orders of magnitude to the

Paleobiochemistry



Models that combine the geological, paleontological, and molecular histories of life on Earth define "function" in a comprehensive and fundamental way.

time of early life on earth, molecular techniques can be used to probe the nature of the first biological systems (self-replicating molecular assemblies). It has been suggested that during the early history of life on earth RNA played a dual role as both genetic material and chief agent of catalytic function (Cech and Bass, 1986). This era, usually referred to as the “RNA world”, is thought to have preceded the emergence of DNA genomes and instructed protein synthesis.

While the details of how life emerged from geochemical systems are unclear, the laws of nature place restrictions on possible pathways of emergence (Mojzsis et al., 1999), and these are being intensely explored by model experiments and extraterrestrial observations. A number of groundbreaking experimental studies (Inouye and Orgel, 1983; Ferris and Ertem, 1993; Pitsch et al., 1995) support the early ideas of Goldschmidt (1947), Bernal (1951), Cairns-Smith (1982), and Wächtershäuser (1988) that interaction with and participation by solid mineral surfaces are likely to have played important geobiological roles in synthesis, concentration, and catalytic oligomerization of such source molecules that are required for the construction of macromolecular systems, capable of information transfer and metabolism in an early RNA world.

In the RNA world, catalytic RNA would have been responsible for replicating and maintaining an RNA genome and, ultimately, inventing protein synthesis. Over the past two decades a variety of RNA enzymes have been described, including several that were found in nature and a much larger number that were generated in the laboratory

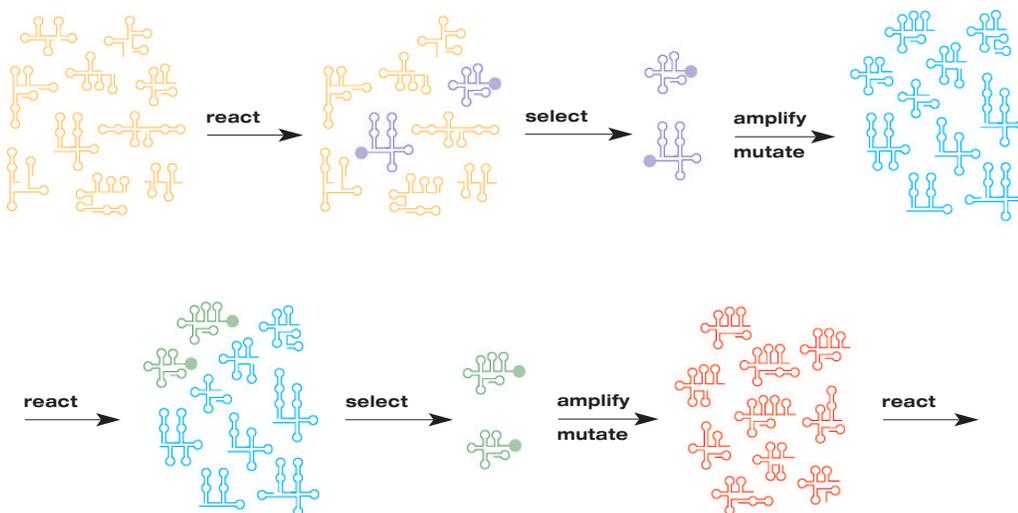
through test-tube (*in vitro*) evolution. The known catalytic activities of RNA include polymerization of nucleotides, aminoacylation of RNA, carbon-carbon bond formation, peptide bond formation, and glycosidic bond formation. Despite these observations, in considering the catalytic potential of RNA (especially in relation to proteins) the limited range of functional groups that exist among the four nucleotides is striking.

While the structure of RNA and DNA is something we take for granted, experimental prebiotic chemistry allows us to question natural selection (Joyce, 1988). Why did the major macromolecular building blocks develop the way they did? Could a different set of information storage molecules have emerged had the conditions on the early earth been different? If so, what would the conditions needed to have been, and what would these molecules have looked like? *In vitro* evolution of RNA and DNA molecules is beginning to provide some answers. For example, the compositional shortcomings of RNA may be offset by its ability to bind divalent metal cations and other small-molecule cofactors that can assist in catalysis. In addition, RNA may become covalently modified to acquire additional functionality. Yet another means of achieving functional diversification of RNA involves the replacement of one or more of the component nucleotides by a nucleotide analogue. In a prebiotic context, it is difficult to envision the availability of the four standard nucleotides without the coexistence of a variety of nucleotide analogues. The incorporation of nucleotide analogues, either at specific locations or throughout an RNA

molecule, may have conferred some functional advantage and therefore been favored by natural selection. DNA, lacking a 2' hydroxyl group, would seem to be even more functionally impoverished than RNA. Terrestrial biology apparently never had the

opportunity or incentive to invent DNA enzymes, although this has been accomplished in the laboratory (Li and Breaker, 1999). In this way, *in vitro* evolution allows us to explore hypotheses about nucleic acid chemistry in the prebiotic era.

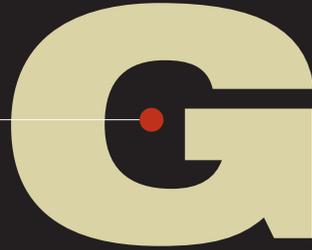
In vitro Darwinian evolution of *ribozymes*.



Top left, the molecules are challenged to catalyze a chemical reaction, thus becoming tagged in a way that distinguishes them from unreacted molecules (dark circles). Top right, tagged molecules are segregated, then amplified and mutated. Bottom, progeny molecules are treated similarly, and the process is repeated until molecules with the desired properties are obtained. Reprinted by permission from *American Biology Teacher* 60(1): 10–12. Copyright 1998 National Association of Biology Teachers.

Geobiology

Resource Needs



Given the interdisciplinary nature of geobiology, progress in this field will come from training and supporting a new generation of scientists to work at the interface between the biological and earth sciences. Particularly useful would be the establishment of an intensive summer course to introduce advanced researchers (graduate students, postdocs, professors) to the field, as well as career development grants for junior level scientists. In addition to investing in education, long-term progress in geobiology could be greatly enhanced by support for well-chosen core drilling projects, field expeditions and genome sequence projects.

An Intensive Training Course in Geobiology

Solving problems in geobiology requires an in-depth understanding of both biology and earth history. Currently, very few programs exist that support such interdisciplinary education and it is difficult for scientists from these disparate fields to communicate with one another. The establishment of an intensive summer course in geobiology would be an effective way to train a new generation of scientists to bridge this divide. We propose to develop such a course.

Currently, the Marine Biological Laboratory (Woods Hole, MA) runs a series of summer courses that serve as good models for a new course in geobiology. These courses typically run from 6-8 weeks and combine lectures with laboratory exercises. They are limited to 20 students per class. An appropriate site for a new geobiology course would provide access to interesting geological sites as well as state-of-the-art laboratory facilities. The goal of the course will be to expose researchers at the graduate, post-graduate, and professorial levels to current research and methods in geobiology. As the field of geobiology is inherently broad, the course will be organized around four themes that we believe are most central to the discipline: Microbial Diversity, Microbial Physiology, Earth Systems, and Earth History.

A team of core instructors (including biologists and geologists) will be assembled to lecture on these topics. As we recognize that this is a general and not a comprehensive list, the course will also sponsor symposia to bring in outside experts to discuss specific topics (eg. biogeochemistry, biomarkers, genomics, the RNA world). An important component of the course will be excursions to sites of interest to introduce students to field geology. Laboratory analysis of samples brought back from the field will be stressed.

In addition to providing a base for interdisciplinary training of a new generation of scientists, our expectation is that the gathering of biologists and geologists in this environment will promote the development of collaborations between established investigators to solve important problems in geobiology. The development of a common “geobiological language” that this course will facilitate is essential for progress in this field at all levels.

RECOMMENDATION

We recommend that an intensive summer course be established to support interdisciplinary training in geobiology.



Postdoctoral Fellowship Program in Geobiology

*T*he development of new research in geobiology logically requires that work be initiated in partnership between biology and geology departments. To the extent that this necessitates well defined projects most appropriately carried out by postdoctoral fellows, progress in this field could be advanced by a career development program that provides salary funding and a contribution towards research expenses for such fellows. One of the main goals of the program should be to encourage postdoctoral fellows to develop research projects under the joint guidance of biology and geology mentors. This will enable fellows with expertise in either biology or geology to use their postdoctoral training to acquire new skills while at the same time building upon their base in one of these disciplines. By providing a flexible framework that will allow postdoctoral fellows to travel between labs (both intra and inter-university), creative interdisciplinary research projects may be initiated.

RECOMMENDATION

We recommend that a public or private agency or a coalition of agencies establish a postdoctoral fellowship program that will allow creative young geobiologists to take on interdisciplinary problems.

S

upport for Specific Research Projects

Scientific Drilling Projects and Sample Archiving

Virtually all of our present knowledge about the geological history of Earth's biosphere is obtained through the study of ancient sedimentary rocks. However, many processes associated with surface weathering act to alter and obscure the physical and chemical properties of this rock record. Furthermore, the complete thickness of sedimentary rocks are rarely, if ever, exposed at the surface, making it difficult to obtain high-resolution time series of critical events in earth history. Although relatively easy to perform, the shallow drilling techniques that are often used for commercial mining and ore exploitation studies are usually considered too expensive for typical scientific investigations. If carefully archived and preserved, samples collected from such drilling projects can serve as a resource for generations of investigators.

Support for Expeditions

In order to learn about earth history, geologists typically must invest a significant amount of time in searching for field sites. Once found, the best sites are often in remote locales that are difficult to access. Requests for funding to support expeditions to these sites typically are one of the first line items cut from grant budgets, as they are perceived to be unessential. Yet there is no substitute for going to a site where the best samples can be found. Being able to observe the natural environment and collect samples to bring back to the lab for further study is an essential part of interrogating earth history. A large return for a relatively small investment could be made if public and private funding agencies were to support such expeditions.

New Genome Sequencing Projects

Due to innovations in high-throughput technologies, it is now possible to sequence entire microbial genomes in less than a week. Until recently, the majority of DNA sequence projects targeted human pathogens, and the evolutionary interest of the genome was not a high priority when selecting which organisms to sequence. From an evolutionary point of view, one of the great opportunities in genomics is to try to understand the old “tempo/mode” problem, or, to put it another way, to define “macroevolution” in molecular evolutionary terms (C.R. Woese, personal communication). How the evolutionary dynamic is driven by the interplay of mutation rate, the propensity to pick up foreign genes, the closeness of fit between an organism and its environment, and changes triggered by environmental shock, may be critically evaluated by comparative genomics. Given the wealth of evolutionary information preserved in genomes and the relative ease of sequence analysis today, support for DNA sequence projects of organisms of evolutionary and/or environmental interest (eg. representative microbes from different branches of the evolutionary tree, microbes from rapidly vs. slowly evolving lines, microbes that engage in biomineralization, microbes that dominate the oceans, microbes that live in extreme environments) would greatly stimulate the emerging field of geobiology.

RECOMMENDATION

We recommend that public and private funding agencies interested in geobiology support: 1) shallow drilling projects and subsequent study and archiving of samples and 2) expeditions (both reconnaissance and sampling) to field sites around the world.

We also recommend that both public and private DNA sequence centers place greater priority on DNA sequence analysis of the genomes of organisms of evolutionary/environmental interest.

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