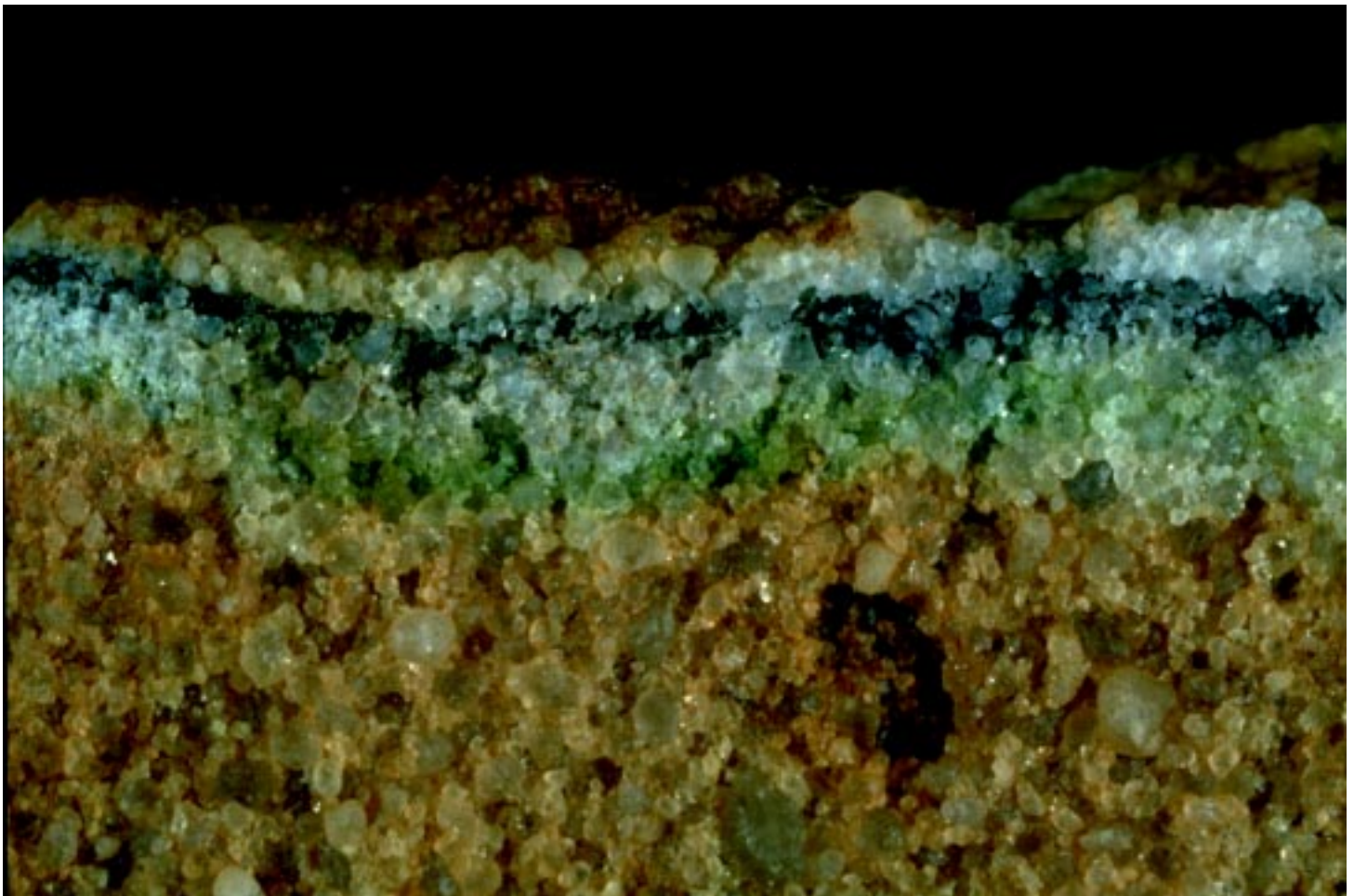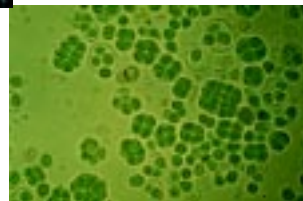In the high, dry valleys of Antarctica, one of the driest places on Earth, liquid water comes but once a year, and for only a few days. The clever algae, right, (*Hemichloris antarctica*) that make their home in the local rocks manufacture a polysaccharide to keep the water inside to nourish the microbial community, visible in the magnified layers below.

The Search for Extraterrestrial Life

by Kenneth H. Nealson

If I could do it all over again and relive my vision of the 21st century, I would be a microbial ecologist. Ten billion bacteria live in a gram of ordinary soil, a mere pinch held between the thumb and the forefinger. They represent thousands of species, almost none of which are known to science. Into that world I would go with the aid of modern microscopy and molecular analysis.

E. O. Wilson

E. O. Wilson, one of the prominent biologists of our time, has made immense contributions to our understanding of macroscopic life on Earth, and in his book, *The Naturalist*, he expressed the opinion that it is now time to move such thinking to the microbial level. Such thoughts resonate well with my own thinking, since I'm a microbial chauvinist whose career has centered on the definition of life in extreme environments on Earth.

Last year I came to Caltech and the Jet Propulsion Laboratory to set up an astrobiology group, to search for signs of life *off* Earth. NASA has defined astrobiology as everything from the Big Bang to human ecology (and even beyond), and at JPL we have staked our claim in this immense topic in the following way: initially to investigate the earliest stages of metabolic life on our planet and to relate this to the early evolution of Earth. Metabolic evolution, one of the keys that enabled life to become a global phenomenon, was already in full swing more than 500 million years ago. Most of Earth's geology, and many of its atmospheric properties that we still see today, were in place by that time. So, if we want to search for life elsewhere, we must keep in mind that there is no guarantee that a particular planet will have evolved to the same advanced stages we have on Earth. A historical perspective is thus key to developing a strategy for life detection. To put it another way, we must know the early history of a planet in order to frame the search for life properly. One clue we follow in this search is the development of what we call biosignatures—the traces that organisms leave behind. Most of the organisms I will discuss are bacteria, extremely small creatures whose biosignatures can be very subtle. To be a Sherlock Holmes at the bacterial level, one must develop appropriately sensitive and definitive techniques.

Since Earth is the only place where we are certain that life exists, it will serve as our laboratory for the development of the search strategy. The overall strategy is still in its early stage of definition, but a general idea consists of three parts:

1. the development of non-Earth-centric biosignatures for life detection;

2. the testing of these biosignatures on earthly samples to see just how good they are;

3. the eventual use of these biosignatures and tests for the analyses of extraterrestrial samples.

From my perspective as a biologist, this entire process is not only a new endeavor, but also involves asking fundamentally new questions. I don't recall in my entire career anyone handing me a rock and asking: "Is it alive?" or "From this sample, can you prove whether there was ever life on Earth?" Rather, I was given a frog and asked, "How does it work?" "What is it made of?" These days the questions have changed to "What genes are there?" and "How do they function?" But the general problem remains: biologists are trained to study life, not to detect it. Yet detection is what we will be faced with in a few years when samples are returned from Mars. If another planet were, like Earth, teeming with life, this would not be a difficult task. It would be relatively easy to tell that Earth was (and is) alive from almost any distance, and especially so if samples were available for detailed physical and chemical analysis. You could be a very bad chemist and still figure out that there was life on Earth. If the signs of life are subtle or unfamiliar, however, then the task becomes much more difficult. This difficulty is

If, from space, you had been looking for complex life on Earth, you would have thought it dead until the last few hundred million years; and if you were looking for signs of intelligent life, you wouldn't have found any until 70 years ago when the radio was invented.

demonstrated by the present controversy surrounding the now famous Mars meteorite, ALH 84001. Two years ago, this 4.5 billion-year-old rock was reported to contain evidence for life on Mars. But even now, after extensive research, the jury is still out as to whether the evidence is convincing. The problems stem from many fronts, including the age of the sample, the difficulties in separating indigenous signals from those due to Earth contamination, and the very definition of life and how to prove that it is (or was) present. What this meteorite really has taught us is that we have a lot to learn about how to distinguish life from nonlife.

You would think that, as a group, biologists would be extremely well suited to detect life. Because we understand biochemistry so well, it should be easy to detect life. Indeed, there are molecules that can be detected at very high sensitivity, allowing us to find a single bacterium in a liter of water. If these key indicator molecules are not there, however, it may not be so easy, and we certainly can't depend on the likelihood that life elsewhere would contain the same key molecules that we recognize. The problem then takes on a different aspect: if we rely solely on Earth-centric indicators of life, we may unwittingly fail to

detect life that differs in its chemical makeup from our earthly standards.

To this end, our astrobiology group is focusing on what we consider the two fundamental properties of all life: structure and chemical composition, both of which can be detected and measured. Historically, structures are the paleontologists' keys to recognition of past life on Earth. It is structures that characterize life as we know it, and we should expect structures to characterize any new forms of life we encounter. We don't know in advance the nature of the structures or the size scales over which to search, but we do expect structural elements to be associated with any life forms.

In addition, we should be able to recognize these structures by a characteristic chemistry that is easily distinguished from the background chemistry of the planet. On Earth, life is carbon-based with a peculiar and remarkably constant elemental composition (hydrogen, nitrogen, phosphorous, oxygen, carbon, etc.), which is remarkably out of equilibrium with the crustal abundance of our planet. In other words, there is more or less of some elements than would be present if there were no life on Earth. While there are other properties of life that may be measurable (such as replication, evolution, and energy exchange with the environment), and that may leave traces in the geological record, we believe that if life does or did exist, then it will best be detected by the existence of structures and their distinctive chemistries.

In the past few years, a number of new findings in the biological community have greatly changed our appreciation of life on Earth. These new developments, which must be considered in the search for extraterrestrial life, can roughly be grouped into three areas:
1. the early emergence of life on Earth;
2. its nature and diversity;
3. its toughness and tenacity.

From recent studies of ancient rocks of the Issua formation in Greenland, traces of metabolic activity (carbon metabolism) indicate that life existed on Earth as early as 3.8 billion years ago. This suggests that the invention of life took place rather rapidly, roughly within 200 million years of

Boulder fields in Hawaii (left) and on the moon (right) look equally barren. But don't judge from appearances: in general, earthly rocks, even those in inhospitable environments, are teeming with microbial life.

when the planet cooled and became hospitable for carbon-based life. These results have triggered speculation about life in general (particularly the problems associated with the invention of such complex systems), as well as about the possibility that similar living systems might have evolved on other planets. For example, it is generally agreed that in the early period of planetary development, and up until about 3.5 billion years ago, Mars and Earth may have shared similar planetary conditions. This has led many to posit that life might have had adequate time and the proper conditions to develop on early Mars as well. The subsequent loss of the Martian atmosphere and hydrosphere suggest that extant surface life on Mars would be very unlikely, but, based on our knowledge of the history of the planet, the possibility that it may have once existed cannot be excluded.

While there are few truly ancient fossils from which to judge ancient Earth life, it seems clear that simple, unicellular life dominated the early Earth; multicellular eukaryotes did not appear until about 2 billion years ago. Complex multicellular eukaryotes— the big organisms like ourselves—were not present until approximately 500 million years ago, when oxygen reached current levels and the Cambrian explosion of life and species (often called the Big Bang of evolution) occurred. From that point onward, Earth began to take on what we would consider a familiar appearance: occupied by plants, animals, and fungi. Before that time, even though it was teeming with microscopic life, by most biological measuring sticks it might have been scored as a rather dead place. (If, from space, you had been looking for complex life on Earth, you would have thought it dead until the last few hundred million years; and if you were looking for signs of intelligent life, you wouldn't have found any until 70 years ago when the radio was invented.) This perspective must be kept in mind when searching for life on other planets of unknown evolutionary age.

In the past two decades we have moved from a peculiarly eukaryotic-centric view of life to one that openly admits that the small, single-celled creatures that were once ignored play a vitally important role in the metabolism of our planet. The classification of life that most of us learned from our biology teachers contained five kingdoms. It was derived through the work of Linnaeus and others in the mid-1700s, and relied upon observation of the visible features of organisms to give each a name (for example, *Homo sapiens*

Mars (shown at right in a three-dimensional map created recently with data from the Mars Global Surveyor) shared similar conditions with Earth during its early development—up until about 3.5 billion years ago. Might it have also developed primitive life before its water and atmosphere vanished? The Viking Lander, which sampled the Martian surface in 1976 (below, right), found no evidence that life exists now. But was it looking for the right signs that might indicate that life had once existed?
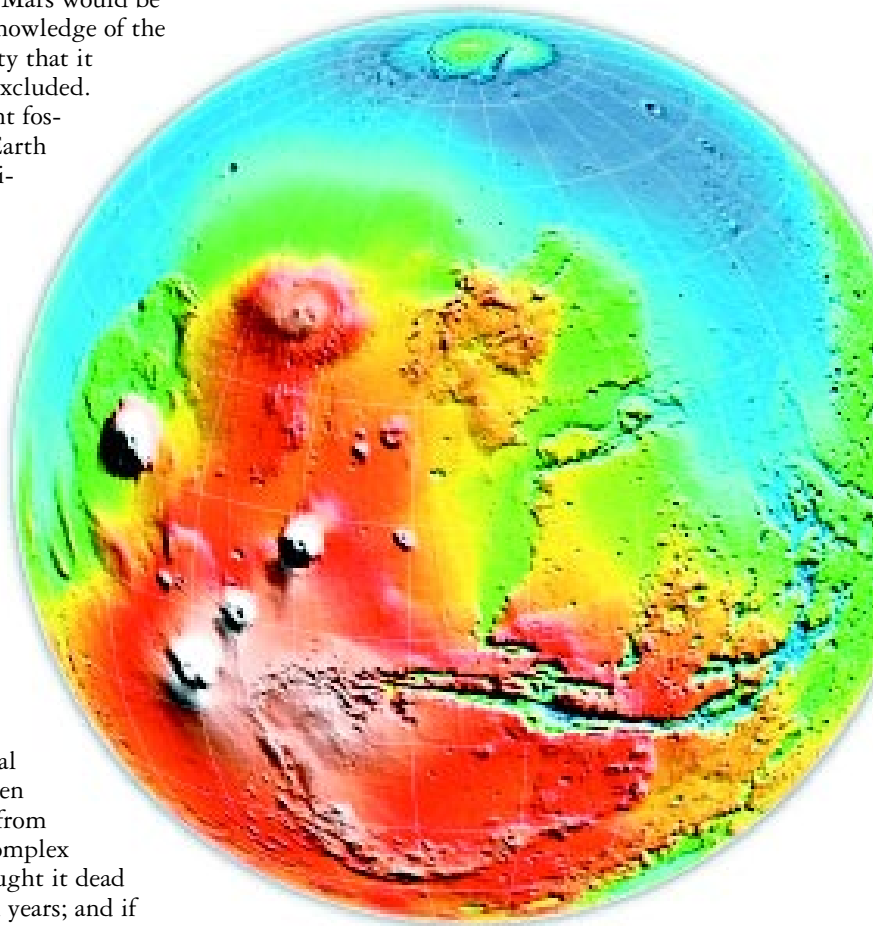
The Linnaean taxonomic system (below) classified forms of life according to features that could be observed: legs, antennae, seed pods, etc. Because they exhibited more visible diversity, the plant, animal, and fungi kingdoms are at the top of the tree. Below them are the protists—the amoebas, parameciums, and the like, and bringing up the bottom are the single-celled bacteria, or monera, thought relatively unimportant in the days before powerful microscopes.
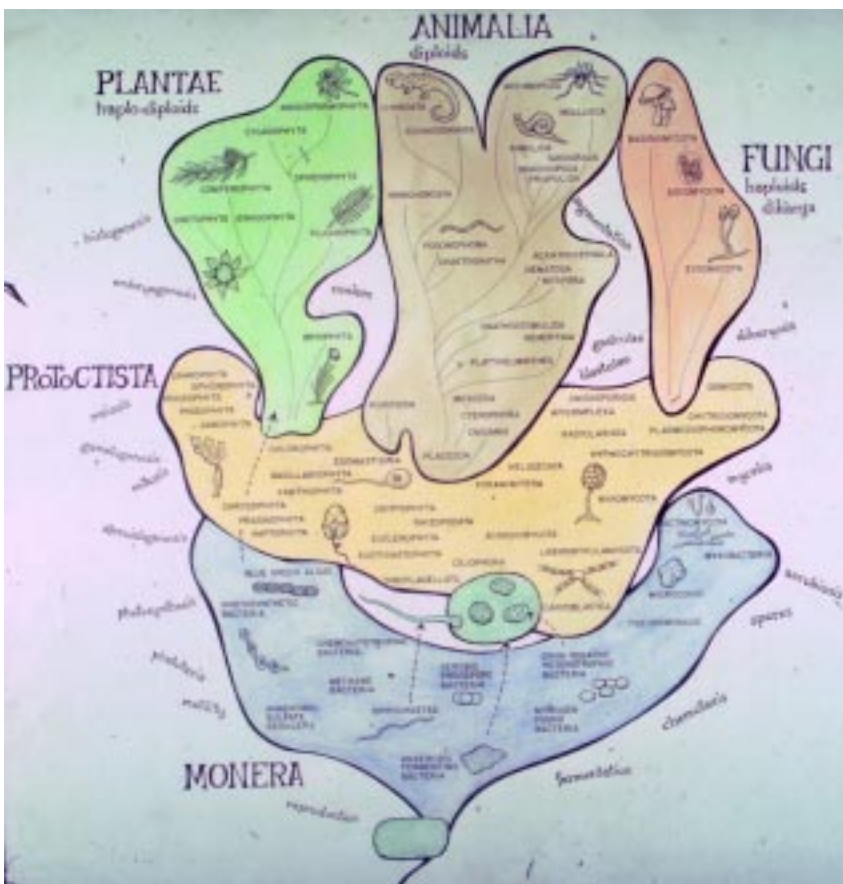
I refer to these organisms [prokaryotes] as the Timex watches of the living world—they're simple; they're rugged; they don't break; you can drive cars over them; it's hard to get rid of them.

for humans), and to group organisms of similar appearance together. The diagram shown at left is referred to as a phylogenetic tree, which illustrates the presumed evolutionary progression—which groups preceded which in time.

Largely because of the nature of the tools available (human eye, hand lens, and later, simple microscopes), it is not surprising that such trees were dominated by the macroscopic, many-celled eukaryotes such as the fungi, plants, and animals. The tiny eukaryotic protists (amoebae, paramecia, etc.), being visible but not understood, were relegated to the next-to-the-last rung of the ladder, while the prokaryotes (bacteria) were handily put at the bottom where they could be acknowledged but not seriously so. This entire approach was reasonable at the time, in the sense that structural diversity was driving classification, and the single-celled, anucleate prokaryotes have little that is comparable to the structurally and behaviorally diverse larger organisms.

This view of the biosphere changed dramatically in the last decade with the advent of molecular taxonomy and phylogeny. The basic idea behind this approach is that there are some molecules common to all earthly life (16 S ribosomal RNA, for example), and that, if one could sequence such molecules and compare the sequences, it might be possible to use this chemical information to compare all life, even that which can be seen only with a microscope.

We now had a way of putting numbers on the evolutionary tree of bacteria. The germ of this idea is actually decades old, but it has become feasible only recently with the development of new techniques in sequencing nucleic acids and the use of this information for comparison of organisms. This approach, called molecular phylogeny and pioneered by Karl Woese of the University of Illinois, has completely overturned the way we look at life on Earth. Instead of five kingdoms, four of which are eukaryotic, we now recognize three kingdoms—and two of them are *prokaryotic.* Even more dramatic, however, is the realization that the three formerly dominant kingdoms (plants, animals, and fungi) are actually clustered at the end of the eukaryotic assemblage, and display only a modicum of genetic diversity. Based on the distances along these phylogentic branches, the genetic distance between a methanogenic bacterium and *E. coli* (the common colon symbiont of humans) is far greater than that between man and a slime mold. Apparently, it is possible to achieve structural and behavioral diversity (traits that have appeared only in the last 500 million years) while remaining genetically quite homogeneous. This idea frightened those who were used to the classical view, but it shouldn't be so astonishing. Given that multicellular eukaryotes evolved only recently, and that for nearly 3 billion years the prokaryotes dominated the surface of the Earth, we should not be sur-

"CLASSICAL" PHYLOGENY

MOLECULAR PHYLOGENY
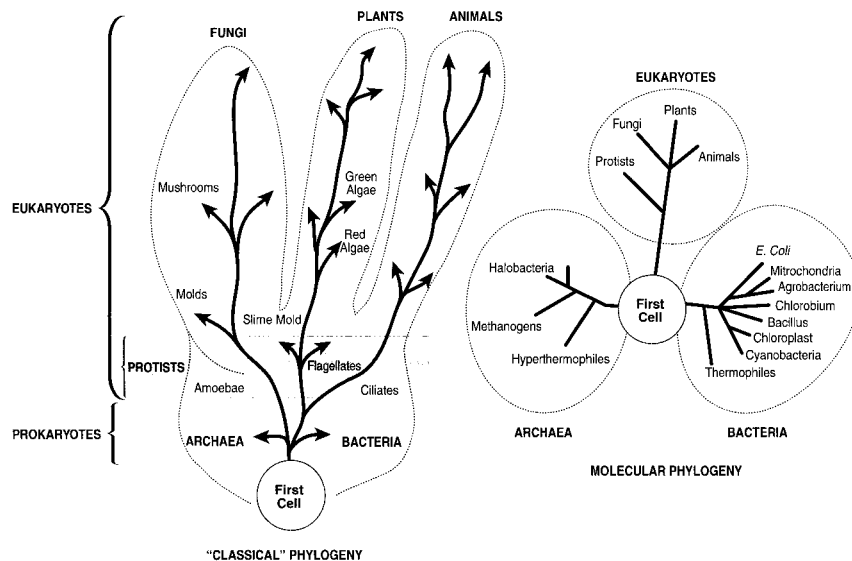
prised that the bulk of the apparent genetic diversity on the planet resides in the latter.

The third critical feature of life on Earth deals with the toughness and tenacity of life. In the illustration below, I have delineated some of the key properties that distinguish the prokaryotes from their more complex eukaryotic cohorts. The eukaryotes are defined by the presence of a nucleus and nuclear membrane in their cells (eu = true; karyon = nucleus), and in general are characterized by complex structures, complex behavioral features, and simple metabolism. Their metabolism is oxygen-based respiration of organic carbon, and the sizable energy yields from this process are used to support their complex structural and behavioral investments. Basically, plants make the organic carbon via photosynthesis, and animals eat the plants (and other animals), leading to the kind of complex communities we easily recognize under the general heading of predator-prey cycles. The very existence of complex structures (both intracellular organelles, and multicellular tissues and organs) renders the eukaryotes sensitive to environmental extremes often easily tolerated by their structurally simple prokaryotic relatives. Above 50° C, it is almost impossible to find a functional eukaryotic cell, for example. Eukaryotes are not tough; put them in boiling water and they soften up right away and you can eat them.

On the other hand, the prokaryotes are the environmental "tough guys"—tolerant to many environmental extremes of pH, temperature, salinity, radiation, and dryness. I refer to these organisms as the Timex watches of the living world—they're simple; they're rugged; they don't break; you can drive cars over them; it's hard to get rid of them. A number of fundamental properties distinguish them from the eukaryotes. First, they are small—they have optimized their surface-to-volume ratio for the most efficient chemistry. On average, for the same amount of biomass, a prokaryote may have 10 to 100 times

more surface area. Thus, in your own body, whose mass may be composed of a few percent bacterial biomass (as gut symbionts), the bacteria make up somewhere between 24 and 76 percent of your effective surface area. (This means that most of the chemistry being done inside you is not being done by you but by your bacteria.) For many environments, such as lakes and oceans, where bacterial biomass is thought to be approximately 50 percent of the total, the bacteria make up 91 to 99 percent of the active surface area, while for anaerobic environments, where the biomass is primarily prokaryotic, the active surface areas are virtually entirely prokaryotic. In essence, if you want to know about environmental chemistry, you must ask the prokaryotes.

Prokaryotes have rigid cell walls, which preclude life as predators. They are restricted to life as chemists and do their metabolism via transport and chemistry. This is in marked contrast to the eukaryotes, whose cells are capable of engulfing other cells. Because prokaryotes have a rigid cell wall, they can't engulf other organisms. So they're put into an evolutionary state in which there is no advantage to getting bigger. The engulfing eurkaryotes, however, have a tendency to get

Small size (1-2 μm) (high S/V ratio) favors chemistry

Rigid Cell Wall requires transport extracellular enzymes favors chemistry
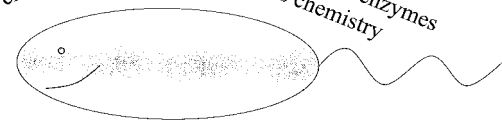
Metabolic Diversity
Alternate energy sources -- organics, inorganics
Alternate oxidants -- O_2, metals, CO_2, etc.

The word extremophile has crept into our vocabulary in the past decade, coined to accommodate organisms that are resistant to, and even thrive in, extreme conditions. These extremophiles can be resistant to chemical (pH, salinity), physical (temperature, dryness), or metabolic extremes.

Some bacteria love extreme salinity. The red halobacteria trapped in the salt crystal below are responsible for the color of the salt ponds near San Francisco (below, near right) and the Dead Sea mounds (far right), where a different species has been bleached by the sun.

bigger and bigger; obviously, if you can get larger than the organism next to you, you can eat it. If it were an advantage for the prokaryotes, they would be big. They've had 3.5 billion years to do it, and, by God, they'd be BIG. They would have figured it out.

The full effect of such evolution is seen in the genomic analyses of prokaryotes, where it is common to see 25 percent or more of the total genome involved with uptake, transport, or other membrane- or cell-wall-related processes. Eukaryotes, on the other hand, devote much of their DNA to development, regulation, differentiation, or even duplication. If the prokaryotes are the chemists, the eukaryotes take on the role of the biologists.

Prokaryotes are metabolically very diverse, while the eukaryotes are quite restricted in their metabolic abilities. The prokaryotes have developed a metabolic repertoire that allows them to utilize almost any energetically useful chemical abundant on Earth. Being opportunists, these ingenious chemists have simply harvested every worthwhile

corner of the chemical market, learning to utilize both organic and inorganic energy sources. Among the major sources of energy available on Earth today, eukaryotes exploit only light and organic carbon, mainly in the form of glucose. These eukaryotes were smart; they developed a very good fuel. And they use the best oxidant, molecular oxygen, to "burn" that fuel. In marked contrast, prokaryotes have figured out how to derive energy from all sorts of combinations of inorganic fuels (such as hydrogen sulfide, nitrogen, or iron) and anaerobic oxidants—even carbon dioxide, which is the worst oxidant of all. If there is energy in any such fuel/oxidant combination, some microorganism will find it. While eukaryotes have sacrificed metabolic diversity for high energy yield, the prokaryotes occupy the diverse, lower-energy habitats. It's not easy growing on hydrogen sulfide, but this metabolic diversity has served them well.

But what about their toughness? In the past, most people interested in bacteria were trained in medical schools. There you study *E. coli* and the pathogenic bacteria that live in the wonderfully rich environment of our bodies. Of course, these bacteria are not very tough and versatile because we give them everything they want. But the word extremophile has crept into our vocabulary in the past decade, coined to accommodate organisms that are resistant to, and even thrive in, extreme conditions. These extremophiles can be resistant to chemical (pH, salinity), physical (temperature, dryness), or metabolic extremes. And it is seldom in nature that an organism encounters just one extreme. For example, under high temperatures, it is common to find anoxic conditions, because oxygen is not very soluble in hot water. Furthermore, due to high evaporation rates, warm systems are often associated with high salinity. Desert ponds often exhibit high pH and salinity, since evaporating water and the minerals trapped there interact to produce extreme conditions.

The most notorious extremophiles are perhaps those associated with high-temperature environments—bacteria capable of growth at 100° C and above. The maximum temperature of any hyper-

Alkaline Mono Lake (below) combines high salinity with a pH of 10 or higher—like lye. A peek under the edges of the carbonate tufa towers reveals a green layer of cyanobacteria (left) hiding from the sunlight.

Too-hot-to-handle rocks from recently erupted Mount St. Helens were home to vast communities of thermophiles growing happily on volcanic sulfur.

thermophile is about 115° C, well above the boiling point of water; these organisms can be grown only under pressure where the water is stable and will freeze to death at temperatures as high as 80° C, temperatures that would result in severe burns to humans. Not too long after Mount St. Helens erupted, a group of us was allowed into the area wearing asbestos shoes and protective clothing. We picked up rocks that were 65 to 85° C—too hot to handle with our bare hands. But when we looked at them in the scanning electron microscope, there were wall-to-wall bacteria growing on hydrogen sulfide and sulfur coming up out of the volcano.

We know of bacteria that live in saturated salt brines (the red halobacteria, for example, which are trapped in mounds in the Dead Sea), and at pH values as low as minus 3 and as high as 11. The eukaryotes, on the other hand, are in general much more restricted in their ranges of tolerance.

One of the strategies of life that often emerges when things get tough is an endolithic lifestyle— the ability to associate with rocks, either on or just under the surface. In California's alkaline Mono Lake, for example, we can see that the tufa mounds that dominate the alkaline lake, and which appear to be dead, are teeming with life. A few millimeters under the rock surface are populations of cyanobacteria that hide from the intense sunlight, positioning themselves for optimum growth in the now-filtered light. A similar situation occurs in many desert soils, where the photosynthetic microbes are found under the surfaces of rock layers.

Bacterial communities have also been found in the high, dry valleys of Antarctica, where liquid water can be found on only a few days a year. About 20 to 30 percent of the rock surfaces have a considerable amount of color in them, and when you crack these rocks open, inside is a well-developed microbial community that manufactures a polysaccharide, which forms a layer in the rock to keep the water inside. These microbes can survive all year-round just waiting for the first thaw. On the few days when there's liquid water, these bacteria have some of the highest metabolic rates that we know about; during the rest of the year their metabolic rate is effectively zero.

In pursuit of other extremophiles, let us return to the issue of metabolic diversity. Given that eukaryotes are almost entirely limited to growth on organic carbon with oxygen as the oxidant, any set of conditions in which organic carbon or oxygen are absent constitutes an extreme environment and is a potential life-threatening situation. For the prokaryotes, however, such environments are simply opportunities to exploit the environment via a different nutrition. This might be called metabolic extremophily. The very existence of such diversity forces those of us hunting for life to include such extreme habitats in the search, and to broaden the definition of life to include metabolic abilities that a few years ago might have been summarily dismissed. The ability to grow on energy sources such as carbon monoxide, ferrous iron, hydrogen sulfide, or hydrogen gas (my personal favorites are the bacteria that "breathe" iron and manganese) implies that bacteria could

The core at right drilled from the Siberian permafrost (below) has been frozen for perhaps a million years or more. Yet enormous colonies of ordinary bacteria that aren't even particularly fond of the cold have managed to thrive.

Bacteria entombed in the stomachs of insects petrified in amber (right) for 10 million years can be revived.

inhabit worlds not heretofore considered as candidates by most scientists seeking extraterrestrial life, and must now be included in any search strategy that is designed.

A final point regarding the prokaryotes relates to their tenacity and ability to survive for long periods of time. There are many examples of bacteria being revived after long-term storage, but perhaps none more dramatic than those from the Siberian and Antarctic permafrost, where soils that have been permanently frozen for 3 million years or more have yielded copious numbers of living bacteria. We have obtained samples from David Gilichinsky and his colleagues from Puschino, Russia, who have been drilling in such sites in Siberia and Antarctica for many years now. It is not unusual to find 10^6 to 10^7 viable bacteria from each gram of permafrost. These are not cold-loving (psychrophilic) bacteria that have adapted to these freezing conditions, but simply meso-philic organisms that usually thrive in moderate temperatures, which have been trapped within this

icy storage facility for millions of years.

Ambers found in the Dominican Republic, which can be dated at 10 to 40 million years old, contain perfectly preserved insects with symbiotic bacteria in their stomachs. Some of these bacteria that have been entombed in the amber for more than 10 million years have been successfully cultured. The fact that viable bacteria can be isolated from samples preserved for millions of years has changed the way many of us feel about the interplanetary transport of life. It is not so easy to discount it as it once was.

So, if we are to proceed to another celestial body in search of life, our definition of habitability must be different from what we would have relied on just a few years ago. We must consider that the physical and chemical conditions tolerant to life are broader than we once thought. We must examine the potential energy sources available (Jupiter's moon Europa, for example, has a huge tidal energy probably equal to its solar energy) and look carefully for life forms utilizing any such energy. We must be prepared for subtle, single-celled life that may not be obvious at first glance, and we should look in places where life might have been preserved in dormant form.

But what precisely will we look for when we go to another planet or when we are fortunate enough to bring samples back to Earth? This is the question of the day for the new astrobiology group, which currently consists of, besides myself, a physicist, a high-energy physicist, a physical chemist, an inorganic chemist, an organic chemist, and a geologist. As I noted earlier, we're looking for properties of life that are universal and measurable, and the two features that we feel are of some obvious value: structure and chemistry.

Structures, as mentioned above, are the standard fare of the paleontologist, and when the structures of life are already known, they serve us very well. But when we are hunting in a new spot, dependence on known structures has a number of

Realistically, Mars will have to be our next laboratory. We need to practice

studying Mars and get good enough to convince ourselves that we can detect

life in other places without bringing a sample back.

potential traps, including the fact that we might discard structures simply because they are unfamiliar. It will be important to remain open-minded about the types and sizes of structures found in samples from new sites.

We cannot, however, rely simply on structural information alone. While we believe that life will be linked to some structural elements, these alone will not prove the existence of life. Coupling structural analysis with the determination of chemical content may well provide a tool for strongly inferring the presence of life. Life is,

almost by definition, a source of negative entropy: a structure composed of groups of chemical monomers and polymers whose existence would not be predicted on thermodynamic grounds, given the abundance of chemicals in the atmosphere and crust of the planet. The exact nature of these chemicals is not so important as the fact that they are grossly out of equilibrium with their surrounding geological environment. If methods were available for analysis of the chemistry of structures at the proper size scales, then the possibility of detecting extant (or even extinct) life would be greatly increased. Ultimately, we would like to have samples from many places in our solar system and beyond, but Europa is a 10-year round-trip and a journey to Saturn's moon Titan and back would take 20 years. Realistically, Mars will have to be our next laboratory. We need to practice studying Mars and get good enough to convince ourselves that we can detect life in other places without bringing a sample back.

As our ability to measure structures and chemistry improves (and we have already developed the capacity for remote identification of elements by x-ray photoemission spectroscopy), the possibility of answering the question of whether life does or does not exist off Earth will improve as well. We will need a strategy for exploration, sample collection and return, and finally, sample analysis. Given the number of other solar systems already known to exist, and the emerging numbers of planets around far-away stars, it seems unlikely that life will *not* be found elsewhere. Development of the proper strategy, and definition of those conditions that do and do not support life will be key to the ultimate discovery of extraterrestrial life. With the proper strategy and approach, the question seems to be not one of whether there is life, but when we will find it. □