

The possibility of alternative microbial life on Earth

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Abstract: Despite its amazing morphological diversity, life as we know it on Earth today is remarkably similar in its basic molecular architecture and biochemistry. The assumption that all life on Earth today shares these molecular and biochemical features is part of the paradigm of modern biology. This paper examines the possibility that this assumption is false, more specifically, that the contemporary Earth contains as yet unrecognized alternative forms of microbial life. The possibility that more than one form of life arose on Earth is consistent with our current understanding of conditions on the early Earth and the biochemical and molecular possibilities for life. Arguments that microbial descendants of an alternative origin of life could not co-exist with familiar life are belied by what we know of the complexity and diversity of microbial communities. Furthermore, the tools that are currently used to explore the microbial world – microscopy (with the aid of techniques such as DAPI staining and fluorescence *in situ* hybridization), cultivation and PCR amplification of rRNA genes – could not detect such organisms if they existed. Thus, the fact that we have not discovered any alternative life forms cannot be taken as evidence that they do not exist.

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Introduction

Finding a form of life that differs in its molecular architecture and biochemistry from life as we know it would be profoundly important both from a scientific and a philosophical perspective. There is compelling evidence that life as we know it on Earth today shares a last universal common ancestor (LUCA; Woese 1998, 2004). It is unlikely that LUCA was the earliest form of life on Earth since it was already quite sophisticated, having nucleic acids and proteins, as well as complex metabolic processes. In short, life as we know it represents a single example of a fairly advanced stage of life. One cannot safely generalize from a single example to all life, wherever or whenever it may be found. Indeed, in the absence of additional examples of life we are in a position analogous to that of a zoologist trying to formulate a theory of mammals based only upon their experience with zebras. It is unlikely that she will focus on their mammary glands since they are characteristic only of the females. Yet the mammary glands tell us more about what it means to be a mammal than the ubiquitous stripes seen in both male and female zebras. Finding a form of life having a different molecular architecture and biochemistry would help us to understand the nature of life in general – the processes that led to its emergence and the various forms it may take, whether on the early Earth or elsewhere in the Universe. Furthermore,

it would have profound philosophical implications for our understanding of our place in the Universe.

When scientists speak of searching for ‘life as we *don't* know it’ they typically have in mind extraterrestrial life. Considerable attention has been given to the question of what life might look like in other places in the Universe and how we might detect its presence with the aid of remote and *in situ* robotic devices. There is, however, another possibility that is rarely considered, and that is that the contemporary Earth itself might host forms of life differing at the molecular level in fundamental ways from life as we currently know it.

Discussions of the origin of life on Earth have appeared in the literature over a period of many decades (for an excellent overview, see Fry 2000). While most researchers have assumed that life originated only once on Earth, a few pioneers have considered the idea of multiple origins of life: Shapiro (1986) has suggested that familiar life may have originated more than once on Earth. Sleep *et al.* (1989) have suggested that familiar life may have emerged and been extinguished several times on the early Earth during the period of ‘heavy bombardment’. Wächtershäuser (1992) has suggested that primitive surface metabolists preceded cellular life and might even persist in habitats that cannot be occupied by heterotrophs. Here we discuss in detail a possibility that has received little attention. We suggest that if life originated

more than once on Earth, it may have produced proto-organisms differing at the molecular level in fundamental ways from the forerunners of our form of life and moreover, that microbial descendants of some of these proto-organisms may still be with us on Earth today, as yet unrecognized for what they represent. We argue that this idea, which is contrary to the paradigm that all life on Earth today descends from a common ancestor, should not be dismissed. Davies & Lineweaver have discussed this idea, although with little biological and chemical detail, in a recent paper attempting to quantify the likelihood of the emergence of multiple forms of life on Earth (Davies & Lineweaver 2005) (*vide infra*).

What modestly different life might look like?

Life as we know it on Earth today shares a number of fundamental characteristics at the molecular level. It contains catalytic and structural macromolecules made of protein, and genetic material made of nucleic acids. It is clear that proteins and DNA are remarkably well suited for their particular functions, and many alternative structures that have been considered fall short in terms of providing suitable structures for these functions. However, it is also clear that some of the molecular building blocks of proteins and nucleic acids could have been different. Indeed, it is an open question as to whether all life (wherever it may be found) is constructed of proteins and nucleic acids. This question is difficult to answer outside the context of a general theory of living systems, something that we currently lack. We do not explore the possibility here of forms of life that differ radically at the molecular level because, as discussed below, detection of even modestly different life forms poses a tremendous challenge.

Familiar life utilizes nucleic acids to store its hereditary information. DNA is well suited for this function for a number of reasons. First, it is double-stranded, and the resulting redundancy provides the correct sequence information in case of damage to one strand of DNA that must be repaired. The poly-anionic backbone causes DNA to adopt an extended structure that facilitates replication. Importantly, this extended structure is quite insensitive to the exact sequence of bases in the DNA (Benner & Hutter 2002). Finally, the interaction between the two complementary strands that is mediated by hydrogen bonding interactions between the Watson–Crick faces of the bases is strong enough to provide molecular recognition and structural integrity, but not so strong that the strands cannot be easily separated to allow replication. Much effort has been invested into the exploration of alternative structures for a genetic polymer. The possibility of alternative backbone structures or alternative sugars has been explored, but with limited success in terms of reproducing the ability of DNA to form an extended double-stranded structure regardless of the identity of the bases in the polymer (Miller *et al.* 1981; Huang *et al.* 1993; Richert *et al.* 1996; Eschenmoser 1999; Benner & Hutter 2002; Reddy & Bruice 2003). However, the identity of the bases used in DNA is a characteristic that might have been substantially different. Benner and

Table 1. Number of distinct codons available for various combinations of base pairs and codon sizes

Number of base pairs	Number of positions in codon			
	2	3	4	5
1	4	8	16	32
2	16	64	256	1024
3	36	216	1296	7776
4	64	512	4096	32 768

co-workers (Piccirilli *et al.* 1990; Benner 1994, 2004; Benner & Switzer 1999; Geyer *et al.* 2003) have explored the possibility of different base pairs, and have shown that a number of alternative base pairs can be accommodated in duplex DNA. In addition, life as we know it employs a triplet genetic code, although the code is not universal – there are some variations in codon assignments, particularly in mitochondria and ciliates. The possibility of codes that utilize a different number of bases or different sizes of codons can be considered (see Table 1). However, if we assume that approximately 20 amino acids are required to create good protein structures, then most of the possible codes listed in Table 1 either have too little coding capacity or far too much (a situation that would probably introduce too much complexity into the process of translation). Only a triplet code using four bases and a doublet code using six bases have coding capacities in the right range.

Extant life on Earth uses proteins for the majority of structural and catalytic functions. Proteins are particularly suited for these functions because of the structural properties of polymers of amino acids. The polyamide backbone of proteins is neutral, unlike that of nucleic acids, and thus the polymer is able to fold into globular structures. The planarity of the amide functionalities in the backbone restricts rotation around the C–N bond, thus providing some restrictions on the number of conformers that can be adopted. Furthermore, the repeating pattern of hydrogen bond donors and acceptors in the backbone allows interactions along the strand that promote the formation of stable secondary structures, such as alpha helices and beta sheets. The linkage between amino acids is quite stable, but not infinitely so, and it can be relatively easily hydrolysed by enzymes to allow the turnover of proteins within cells. This propitious combination of properties is conferred by the amide bonds linking the amino acids in the polymer; polymers linked by ester, thioester, ether or C–C bonds would lack one or more these properties.

Life as we know it builds its proteins primarily from the same 20 amino acids¹. Yet there are many other amino acids

¹ Seleno-cysteine is found in a small number of enzymes, it is incorporated during protein synthesis at the ribosome using a tRNA that recognizes what would normally be a stop codon. Seleno-methionine is incorporated into proteins randomly in place of methionine. Post-translational modifications of some amino acids in proteins occur in specific cases; examples include the formation of dehydroalanine from serine and γ -carboxy-glutamate from glutamate.

that could have been utilized. While it is important that the collection of amino acids used in proteins includes a sufficient number of small, large, hydrophilic, hydrophobic and charged amino acids, the exact identities of the amino acids in each of these classes may not be critical. Moreover, the amino acids utilized for protein synthesis by familiar life are all L-amino acids, and there is no reason to think that D-amino acids could not have been utilized instead. Indeed, proteins that have been chemically synthesized from D-amino acids fold correctly and are functional (Milton *et al.* 1992; Zawadzke & Berg 1992; Fitzgerald *et al.* 1995; Canne *et al.* 1997).

Given that alternative combinations of bases in DNA and amino acids in proteins might have been chosen, why does the form of life, with which we are familiar, construct its proteins and nucleic acids out of the particular combination of molecular building blocks that it does? Given our current understanding of chemistry and molecular biology, the best explanation is that these building blocks resulted from chemical and physical contingencies present on the early Earth. Had circumstances been relevantly different, so would life on Earth. This suggests an intriguing possibility. Perhaps a number of different locations on the early Earth were conducive to the formation of life. Locations that have been proposed as important sites for the pre-biotic chemical reactions that provided the building blocks of life include hydrothermal vents (Holm & Andersson 1998; Martin & Russell 2003), mineral surfaces (Wächtershäuser 1988; Cairns-Smith *et al.* 1992; Cody 2004) and organic aerosol particles (Dobson *et al.* 2000). In addition, a variety of amino acids were deposited on Earth from meteorites derived from both asteroids (Oró *et al.* 1971; Anders 1989; Glavin *et al.* 1999; Botta & Bada 2002) and comets (Chyba *et al.* 1990). Racemic mixtures of 70 amino acids, only eight of which are utilized by life on Earth today, have been identified in meteorites (Anderson & Haack 2005). A novel, and as yet untested, theory proposes that the synthesis of amino acids might have been catalysed on the primitive Earth by dinucleotides, once conditions favourable for their production arose (Copley *et al.* 2005). It is unlikely that the chemical conditions in these incipient 'cradles of life' were identical. Thus, the building blocks available for life, as well as the stability of critical intermediates and the types of reactions that might have been catalysed, would certainly have differed in different locations. If conditions conducive to the emergence of life were present at a number of different locations, then corresponding differences in biomolecules might have arisen in the earliest Terran life forms, perhaps communities of self-replicating RNA molecules (Gilbert 1986; Joyce 2002) or vesicles containing self-reproducing populations of molecules (Oparin 1957; Dobson *et al.* 2000). The hypothesis that the early Earth hosted multiple, alternative origins of life is thus compatible with our current chemical and biological understanding of the nature of familiar life. As we discuss below, some of these alternative types of life might still exist on Earth.

It is, of course, possible that the origin of life is an exceedingly improbable affair, and that life either originated only once on Earth or originated elsewhere and was brought to Earth in a meteorite. But it is important to bear in mind that ignorance concerning how life actually originated on Earth does not provide support for the claim that the origin of life is a cosmic coincidence of some sort; ignorance cannot support a knowledge claim of any sort except perhaps for the trivial claim that we simply do not know. Besides, to the extent that science operates under the guiding principle that natural phenomena are explicable in terms of natural processes, appeal to unnatural occurrences, whether cosmic coincidences or supernatural creation, is self-defeating. If, like other natural phenomena, life is the product of natural processes operating under certain kinds of chemical and physical constraints, then it seems more likely than not that the early Earth hosted more than one origin of life. Some of these separate origins might have produced primitive organisms differing in their basic molecular building blocks in some of the ways discussed above.

Could alternative life co-exist with familiar life on Earth?

If there were alternative origins of life on Earth, it seems clear that they did not give rise to proto-organisms that evolved into large organisms such as higher plants and animals. However, there is little reason to suppose that the processes of evolution inevitably produce large organisms. Microbes are the most abundant form of life on Earth. In most cases, they multiply more rapidly than large organisms, allowing them to evolve more rapidly in response to changing environmental conditions. Microbes exploit more energy resources than multicellular organisms. Some of them photosynthesize, others metabolize organic material and others metabolize inorganic material such as ammonia, hydrogen sulphide and iron. They prosper under an astonishingly wide range of environmental conditions, being found in highly acidic streams, boiling hot springs, several kilometres beneath the Earth's crust and in the coldest regions of Antarctica (Rothschild & Mancinelli 2001). In other words, the biological diversity of the microbes is much greater than that of large multicellular organisms. Indeed, organisms such as higher plants and animals seem to be the exception rather than the rule on Earth. This may also be true of the Universe as a whole; Ward & Brownlee (2000) argue that microbial forms of life are probably very common in the Universe, but that large complex organisms are not. The point is the absence of large complex descendants of alternative forms of early life does not count as evidence that alternative life forms did not exist early in Earth's history, or that they could not persist today.

It might be argued that our form of life is so aggressive and evolutionarily robust that any form of alternative life would have been eliminated long ago. This argument does not bear up under consideration of the structure and dynamics of microbial communities. Although small in

number, rare microbes successfully compete in environments swarming with common microbes (McCaig *et al.* 1999; Spear *et al.* 2005; Walker *et al.* 2005). Indeed, they typically participate with other organisms in an interdependent biological system, producing or utilizing material that is utilized, produced or ignored by other microbes. There is little reason to suppose that the microbial descendants of an alternative origin of life could not participate in such a system. For example, a microbe that used only D-amino acids for protein synthesis could survive quite well in a milieu containing L-amino acids simply by having a suite of racemases to convert the abundant L-amino acids to D-amino acids. Bacteria typically have such racemases to generate the D-amino acids used for peptidoglycan synthesis (Yoshimura & Esaki 2003).

There are also at least two plausible alternative evolutionary scenarios. Those forms of novel life that differed the most from familiar life (e.g. where the proteins utilized a very different suite of amino acids) might have had an evolutionary edge. Familiar life would have found them the most difficult to metabolize, and hence the poorest source of nutrition. Such micro-organisms might not only have survived, but gone on to evolve their own independent, interlocking ecological system of predator–prey relations. Another way in which novel forms of early Earth life might have survived is by becoming adapted to environments that are less hospitable to familiar microbial life. In short, rather than being eliminated, novel forms of early life might have evolved in such a way as to remove themselves from competition with familiar life.

Another hypothetical objection that might be raised against the possibility of novel forms of life, descended from an alternative origin of life, has to do with lateral gene transfer. Lateral gene transfer contrasts with vertical ('normal') transfer, which is what happens when genes are transferred from parent to offspring. Lateral gene transfer involves the transfer of genetic material from one organism to another without replication or reproduction. This material can be incorporated into the recipient's genome and passed on to its offspring. Lateral gene transfer is known to have played a significant role in the evolution of microbes. Indeed, many microbiologists believe that the earliest life consisted of a community of proto-organisms that shared genetic material (Woese 1998). If this were the case then (the argument goes) primitive microbes deriving from different origins of life would have been amalgamated into this homogeneous pool of primitive proto-organisms, which subsequently evolved into familiar life.

However, this scenario glosses over some serious problems. Lateral gene transfer as we know it today presupposes significant similarities in the genomes of the microbes involved. Familiar microbes could not incorporate pieces of a genome utilizing alternative base pairs, not to mention different numbers of bases, into their genomes or *vice versa*. If microbes deriving from alternative origins of life exchanged biomolecules they must have done it before the complex cooperative arrangement between proteins and

nucleic acids that characterize familiar life was worked out. Indeed, exchanges among proto-organisms may have been indiscriminate, involving precursor biomolecules of all kinds. In other words, it is not at all clear that a community of diverse proto-organisms deriving from alternative origins of life could have hybridized into a single form of life that evolved into life as we know it. Given our limited understanding of the origin and early development of life, we cannot dismiss the possibility that familiar life arose from a fortuitous mixture of chemicals and that fortuitous mixtures of different chemicals produced alternative forms of microbial life.

Of course none of this proves that such organisms ever existed, let alone still exist. The point is only that many of the arguments that are commonly advanced against their possibility do not hold up well under close scrutiny. How likely is it that they exist? Answering this question is difficult because we know of only one form of life, and we do not yet understand the mechanism by which it emerged. Indeed, Davies & Lineweaver (2005) have recently argued that an alternative origin of life on Earth is not only possible, but also highly probable; they calculate the probability to be 90%. We believe their calculation is based upon questionable assumptions. Their argument is based upon the assignment of a 50% probability for the emergence of life over a 100 million year period. We simply cannot assign such a probability based upon the single data point we have. However, the possibility of alternative life forms cannot be ruled out on the basis of our current knowledge of chemistry and biology.

Limitations to current technologies

The possibility that an early alternative life form could have evolved into microbes that either co-exist with familiar life as part of a single, unified biosphere or exist in an independent, parallel biosphere should not be discounted out of hand for a very simple reason: our current technology would not allow us to detect an alternative form of life². We have three major tools with which to explore the microbial world. The first is microscopy. Unfortunately, the morphology of most non-eukaryotic microbes provides little insight into their phylogenetic classification or metabolic capabilities, and we are unlikely to be able to distinguish between normal life and alternative life just by looking. Moreover, molecular biology has taught us that superficial similarities in morphology can hide important differences in molecular architecture and biochemistry. The Archaea provide a particularly salient example. Most Archaea look pretty much like bacteria under a microscope. However, the Archaea are genetically and biochemically more different from bacteria than they are from eukaryotes. Indeed, the discovery that the Archaea are so different from bacteria revolutionized biological taxonomy, with the five kingdoms of familiar life (animals,

² Davies & Lineweaver mention this point, but do not discuss it in detail.



Fig. 1. DAPI-stained microbes in a water sample taken from a flooded area in New Orleans after Hurricane Katrina (1000× magnification). Photo courtesy of Mari Rodriguez and Mark Hernandez.

plants, fungi, protists and monera (bacteria)) being replaced by three domains of life (Archaea, Eubacteria and Eukarya; Woese *et al.* 1990). The moral is that morphology does not allow one to eliminate the possibility of *shadow microbes*, i.e. microbes that resemble Archaea and Eubacteria in their gross morphology, but differ from them in fundamental ways at the molecular level. This possibility is underscored by the fact that evolutionary pressures can produce similar adaptations from different biological building blocks. It is probable that conditions on the early Earth favoured the development of a morphology along the lines of the Archaea and the Eubacteria, just as conditions on Earth later favoured the independent development of wings in insects, birds and bats. It would thus be a mistake to conclude that every microbe that resembles a bacterium under a microscope is an Archaeon or a Eubacterium.

The power of microscopy has been expanded by the use of stains for specific cellular components such as nucleic acids (see Fig. 1) and lipids, and particularly by fluorescence *in situ* hybridization (FISH) techniques, in which oligonucleotide probes targeting specific genes are used to identify specific organisms in an environmental sample. However, these approaches do not allow us to conclude that all cells visible under a microscope are representatives of familiar life. Stains such as 4',6-diamidino-2-phenylindole (DAPI) intercalate into double-stranded nucleic acids, and would probably stain nucleic acids in a different life form that contained different bases or a different backbone in its genetic material. Stains for lipids cannot distinguish between familiar and alternative life forms, since the presence of a lipid membrane reveals nothing about the components of the genetic material and proteins enclosed within. Finally, FISH as it is usually performed is not at all useful, as it can only be used to identify cells containing a gene complementary to the probe being used. If an alternative form of life uses a different type of

genetic material, oligonucleotide probes will not hybridize. Thus, microscopy, even combined with standard molecular tools such as FISH, cannot eliminate the possibility that alternative life forms, even those that are not very different from known life, are present in natural microbial populations.

A second tool that has provided us with most of our information about the genetic composition and physiological properties of microbes is cultivation. By growing large quantities of a single microbe, we can determine the chemical components of its genetic and structural materials, the composition of its membrane, the types of metabolic processes it uses to obtain nutrients and energy, and, assuming that its genetic material is DNA, the sequence of its genome. However, we can currently culture less than 1% of the microbes that can be visualized by microscopy (Pace 1997). Efforts to improve cultivation techniques are bearing fruit (Leadbetter 2003), and this situation may improve in the next few years. However, difficulties in cultivation certainly limit our ability to detect alternative forms of microbial life, particularly since they would be more likely than familiar life to require growth conditions that we might not expect. Without being able to culture a shadow microbe, it would be difficult to determine, for example, that it utilized different bases in its DNA or different amino acids in its proteins.

Finally, PCR amplification of 16S rRNA from environmental samples has provided an extraordinarily powerful tool for identifying non-culturable components of microbial communities (Pace 1997). Unfortunately, however, DNA amplification is not useful for detecting novel forms of microbial life. The process requires 'universal primers' capable of supporting amplification of 16S rRNA from one of the three domains of familiar life. Their effectiveness in amplifying microbial DNA depends upon whether it contains coding regions that are sufficiently similar to those on the DNA of familiar life. This poses a serious problem for the prospect of identifying shadow microbes by means of DNA amplification. Even if an alternative life form had DNA as we know it, its ribosomal RNA (assuming that it has ribosomes) might be so different from those of familiar life that it could not be amplified by PCR. Moreover, if the backbone, sugars, or bases of the genetic material were different in an alternative form of life, its genetic material could not be amplified using PCR.

Questioning the paradigm of life on Earth

The paradigm for modern biology includes the assumption that life as we know it is the only form of life on Earth. We owe our understanding of the role played by paradigms in science to the work of Kuhn (1970). As Kuhn argued, scientific research is conducted within the confines of a paradigm. In addition to theories, paradigms include methods, instrumentation and subsidiary assumptions concerning a particular subject matter. Even though our theoretical understanding of life does not preclude alternative forms of microbial Terran life, the supposition that they do not

exist is tacitly incorporated into the paradigm of modern biology.

Paradigms are invaluable tools for scientific research. They facilitate the construction of hypotheses, the design of experiments and the interpretation of results. However, as Kuhn discussed, paradigms sometimes act as blinkers, hindering the exploration of nature by discouraging certain avenues of exploration and biasing the way in which results are interpreted. As a result, important scientific discoveries, and the theoretical advances that wait upon them, may be delayed for many years. Kuhn (1970, pp. 115–117) illustrated this point with several examples from astronomy. William Herschel's discovery of the planet Uranus is particularly salient for our purposes. Between 1690 and 1781 some of Europe's most eminent astronomers reported seeing a star in positions that we now know were occupied by Uranus. Twelve years later Herschel observed the same object with a newly developed, more powerful telescope, and what he saw stumped him. Under higher magnification, it appeared disc shaped, which was highly unusual for a star. Further investigation revealed that the mysterious object moved among (rather than with) the stars. Herschel concluded that he had discovered a new comet. However, as subsequent investigations revealed, the orbit of the object did not conform to that of a comet. After several more months of investigation, another astronomer ventured that the orbit was planetary. Thus, what had been taken to be a star was discovered to be something quite different, namely, a planet. The discovery of Uranus was rapidly followed by the discovery of numerous smaller objects having planetary orbits. Kuhn speculates that the minor paradigm change imposed upon astronomers by the discovery of Uranus prepared them to see objects (namely, asteroids) that they had not seen before but that had nonetheless been there all along. In this light, it is instructive to consider some analogous examples from the biological sciences. These cases resemble the cases discussed by Kuhn insofar as they involve discoveries that were astonishing at the time but nevertheless represented phenomena that had been present all along, unrecognized because they conflicted with a reigning paradigm.

In the Middle Ages infectious diseases were attributed to such things as bad air, supernatural influences and humoral imbalances, in conjunction with the constitution of the body. The foundations for the modern paradigm for infectious disease were laid by Louis Pasteur, Robert Koch and others towards the end of the 19th Century (Reid 1974; Madigan & Martink 2006). Koch, who first identified a bacterium (*Bacillus anthracis*) as the cause of anthrax, developed techniques for culturing and propagating bacteria, and for determining that a particular bacterium is the cause of a particular disease. Koch's new paradigm (the germ theory of disease) was powerful but it was unable to establish that bacteria caused all infectious diseases. In particular, his techniques were unable to identify viruses, which were far too small to be seen using the technology available at the time and which could not be cultured in isolation. Yet experimental work strongly suggested that infectious agents

of some sort were involved in the transmission of the diseases concerned. The mystery was finally solved in the early 20th Century by a combination of two new technologies. Electron microscopy allowed the visualization of the extremely tiny viral particles, and cultivation in the presence of cells (in particular, in eggs) allowed the propagation of viruses. In more recent times, our understanding of the causes of infectious disease has been shaken again by the discovery of prions, proteinaceous infection particles that cause diseases such as scrapie and bovine spongiform encephalopathy (Prusiner 1998). Prions are simply proteins, and the idea that a protein could transmit an infectious disease was so revolutionary that a Nobel Prize was awarded to Stanley Prusiner for the discovery of prions in 1997.

The discovery of the Archaea provides a particularly salient example since it involved the discovery of a previously unsuspected form of microbial life, which resulted in the overthrow of a dominant biological paradigm (Woese 2004). Prior to 1977, scientists believed that living organisms fell into two categories – bacteria and eukaryotes. This way of thinking originated from microscopic studies, which revealed that bacteria were small and contained no membrane-bound organelles, while eukaryotic cells were larger and contained several membrane-bound organelles, including the nucleus, mitochondria and endoplasmic reticulum. The demise of this paradigm started modestly in the 1960s, when Carl Woese began to sequence ribosomal RNA in order to generate phylogenetic trees based on molecular characteristics. As a large database of rRNA sequences became available, it became evident that the rRNA sequences of some microbes clustered together to the exclusion of bacteria and eukaryotes. This group was initially called the Archaeobacteria, but the name was later changed to Archaea, as it was realized that these organisms are fundamentally different from bacteria and, in fact, constitute the third domain of life. The Archaea resemble bacteria in terms of morphology, transcribe RNA using machinery that is more similar to that of eukaryotes than that of bacteria, and have a cell wall structure that is markedly different from both bacteria and eukaryotes. The discovery of Archaea required the development of molecular techniques because Archaea and bacteria look similar under a light microscope. Indeed, what are now understood to be telling chemical differences in the cell membranes of the Archaea and bacteria were originally interpreted as mere adaptations to what was perceived to be extreme environments. In the words of Brock (1978): 'The fact that *Sulfolobus* and *Thermoplasma* have similar lipids is of interest, but almost certainly this can be explained by convergent evolution. This hypothesis is strengthened by the fact that *Halobacterium*, another quite different organism, also has lipids similar to the two acidophilic thermophiles'. Consequently, the presence of a third domain of life was completely unexpected. Biologists had stumbled across a new form of microbial life without recognizing that they had done so.

A final example is the discovery of catalytic RNA, which upset what is fondly known as the 'Central Dogma'. The

Central Dogma posits that DNA is transcribed into mRNA, which is then translated into proteins. Proteins were believed to carry out the interesting catalytic, structural and regulatory functions required for life. Although Carl Woese had speculated, as early as 1967, that nucleotides might catalyse chemical reactions (Woese 1967), this idea was not given serious consideration. RNA was seen as simply the intermediary between DNA and protein. This paradigm was upset by the unexpected and independent discoveries by Tom Cech (Kruger *et al.* 1982) and Sidney Altman (Guerrier-Takada *et al.* 1983) that RNA could catalyse chemical reactions. Cech and Altman shared the Nobel Prize in 1989 for this discovery. More recently, important roles of RNA in regulating gene expression have been discovered, requiring yet another remodelling of the Central Dogma paradigm. In summary, some of the most lauded work in scientific history has upset the paradigm prevailing at the time. Yet, we continue to operate in the framework of paradigms because they are so useful. Paradigms can be upset by the emergence of new technology that allows exploration in a new way, or by recognition that the results of an experiment do not fit the paradigm and are so compelling that revision of the paradigm is necessary. As discussed above, exploration of the microbial world has continued to yield new and unexpected discoveries. It is not unreasonable to think that this process will continue as we develop more sophisticated methods and tools for probing the invisible world of microbial life.

Conclusions

The possibility of microbial descendents of alternative origins of life on Earth cannot be dismissed based on current knowledge. The fact that we have not discovered any does not mean they do not exist, since the tools that we currently use to explore the microbial world could not detect them if they existed. Furthermore, arguments to the effect that alternative microbes could not co-exist with familiar life are belied by what we know of the complexity and diversity of microbial communities. If such microbes exist, there is little doubt that they cast heretofore unrecognized physical and chemical shadows (so to speak) upon our familiar biosphere, and hence could be detected with the right tools. The challenge, of course, is to develop methods for recognizing these elusive chemical and physical traces. However, even if shadow microbes do not exist on Earth today, the development of such tools would be an invaluable contribution to the search for unfamiliar forms of microbial life on other planets and moons.

The discovery of a shadow Terran biosphere would have profound scientific and philosophical ramifications. It is clear that life as we know it on Earth has a common origin, which means that we are currently limited to a single sample of life. One cannot generalize on the basis of a single sample. In order to formulate a truly general theory of living systems we need examples of unfamiliar forms of life. Although we have good theoretical reasons for believing that life on Earth

could have been at least modestly different in its biochemistry and molecular architecture, we do not know how different it could have been. It is important that we do not artificially constrain our thinking about the origin of life on Earth and the possibilities for extraterrestrial life on the basis of a limited and possibly very misleading example of life. Indeed, a dedicated search for shadow microbes might produce surprising results, providing us with unexpectedly novel forms of microscopic life. Given that the possibility of alternative forms of life on Earth cannot be discounted and the profound importance such a discovery would represent, we believe that a dedicated search for them ought to be seriously considered.

Finding an alternative form of life on Earth poses an enormous technical challenge. First, we cannot predict the most likely place for finding an alternative life form on Earth. With no knowledge of the biology of such a life form, we cannot predict whether it would more probably be found in rich ecosystems with much microbial diversity, or in extreme conditions, where only a few types of familiar microbes thrive. Indeed, we have often been surprised by finding familiar microbes in unexpected places; for example, the discovery of abundant *Mycobacterium* species in an endolithic community from a highly acidic silica rock sample from Yellowstone's Norris Geyser Basin (Walker *et al.* 2005) was unexpected, as *Mycobacteria* are generally found at only very low levels in environmental samples. Second, the problem resembles finding the proverbial needle in a haystack. The extent of microbial diversity is staggering. A recent study estimates that soil carrying 2×10^9 cells g^{-1} can contain nearly 10^7 species, with 99.9% of the species present at levels of less than 10^5 cells g^{-1} (Gans *et al.* 2005). Even identifying the presence of a rare eubacterium is challenging under these circumstances. Finally, as described above, our current methods are inadequate for detecting forms of life that do not have DNA containing the four canonical bases.

It is worthwhile considering what new methods would allow us to identify and characterize an alternative life form. Studies of the unique biology of an alternative life form would require a sample large enough for the chemical analysis of its constituent macromolecules. Growth in a pure culture would be the optimal way to accomplish this. However, we do not have the methods for high-throughput screening of cultivation conditions that could ensure the growth of a pure culture of an alternative microbe from an environmental sample. Thus, initial efforts might best be directed toward detecting potential alternative life forms *in situ*. The most expedient way to detect an alternative form of life might be to develop reagents that can distinguish typical DNA from other genetic materials differing either in the backbone or the nature of the bases. Such reagents could be used to stain environmental samples to look for cells that do not bind the reagent, always keeping in mind the possibility that an unusual cell wall structure might lead to an inadequate permeabilization of the cell and a consequent lack of staining. For example, antibodies against DNA should be able to

discriminate between typical DNA and alternative nucleic acids containing a different type of backbone; this could easily be tested using synthetic analogues of DNA. An alternative approach would be to develop reagents that would recognize alternative backbone structures. Although the creation of such reagents is certainly feasible, this approach would be a fishing expedition limited by our ability to predict what backbone structures might be found in alternative life forms. However, an advantage to this approach would be that a fluorescent probe that does not stain normal cells could be used to collect stained cells using fluorescence-activated cell sorting (FACS).

A different methodology would be required to detect alternative life forms utilizing a different suite of bases in DNA. Antibodies that recognize the bases of DNA as well as the backbone could be used. Antibodies that recognize alternative bases in a DNA context could also be developed and used to stain environmental samples to look for cells that utilize different bases in DNA. Again, fluorescent probes might be used to sort out cells binding the probe for additional analyses. Alternatively, antibodies against DNA containing non-standard bases could be immobilized and used to capture DNA containing unusual bases from bulk DNA isolated from environmental samples, possibly in amounts sufficient for chemical characterization. Each of these proposed methods poses technical challenges, particularly the daunting signal-to-noise problems inherent in trying to detect a rare microbe in a large and diverse population. However, a search for shadow microbes on Earth should be considered because finding an alternative form of life would be one of the greatest scientific discoveries of all time.

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