Hypothesis Article

Signatures of a Shadow Biosphere

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Abstract

Astrobiologists are aware that extraterrestrial life might differ from known life, and considerable thought has been given to possible signatures associated with weird forms of life on other planets. So far, however, very little attention has been paid to the possibility that our own planet might also host communities of weird life. If life arises readily in Earth-like conditions, as many astrobiologists contend, then it may well have formed many times on Earth itself, which raises the question whether one or more shadow biospheres have existed in the past or still exist today. In this paper, we discuss possible signatures of weird life and outline some simple strategies for seeking evidence of a shadow biosphere. Key Words: Weird life—Multiple origins of life—Biogenesis—Biomarkers—Extremophiles—Alternative biochemistry. Astrobiology 9, 241–249.

1. Background

THE HISTORY OF OUR DEVELOPING UNDERSTANDING of life on Earth has been characterized by repeated discovery, driven largely by improvements in techniques to explore the Earth's biosphere. The age of enlightenment brought exploration technologies that led to the discovery of new biota in the Americas, Australia, and Africa. The invention of the microscope uncovered an unexpected microbial world. RNA sequencing in the 1960s and 1970s revealed that the prokaryotic biosphere itself consists of two domains that are as different from each other as they are from eukaryotes. Together, these discoveries revolutionized our understanding of the history of life on Earth over the past three billion years.

Today, it is believed that microbes constitute the vast majority of terrestrial species. Nevertheless, the microbial realm remains poorly explored and characterized. Less than 1% of microbes has been cultured and described (Amann *et al.*, 1995; Pace, 1997; Hugenholtz *et al.*, 2006). Because microbial morphology is very limited, it is in most cases difficult, if not impossible, to deduce much about the nature of microbial life by simply looking at it. Gene sequencing has so far proven to be the only reliable method to determine the relationship of a given microbial species to other known life. This extensive ignorance raises the intriguing issue of how sure we can be that all microbial types have been identified. Might it be the case that the exploration of the biosphere is not complete, and deep additional branches of the tree of life have so far been overlooked? Is it even possible that microbial life exists that does not share a common descent with familiar organisms and, therefore, constitutes a different tree altogether, deriving from an independent genesis?

It is relatively uncontroversial that at least one very different kind of life existed on early Earth. It had no encoded proteins but rather used RNA as the sole genetically encoded component of biocatalysts. This conjecture is supported by the catalytic properties of RNA and the detailed structure of the ribosome, a complex structure built from both protein and RNA, but where the RNA is clearly responsible for the protein synthesis (Moore and Steitz, 2002). It is not clear that existing life-detection strategies, which mainly target the ribosomal machinery, would register any surviving RNA or-

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ganisms that diverged from the life we know prior to the invention of the ribosome. Still less likely would they be to work with any of the radically different forms of life that may have arisen from multiple independent episodes of terrestrial biogenesis.

In this paper, we discuss the working hypothesis that there might exist an alternative microbial realm on Earth that constitutes a type of "shadow biosphere." We leave open the possibility that a shadow biosphere may have become extinct in the remote past yet left identifiable traces in the form of inexplicable mineral alterations or unusual biomarkers. Our work elaborates the preliminary ideas contained in several earlier papers (Benner, 1999; Benner and Switzer, 1999; Cleland and Copley 2005; Davies and Lineweaver, 2005). We outline strategies whereby evidence of past or extant alternative forms of microbial life—dubbed "weird life" in a recent National Research Council report (Baross, 2007)—might be identified, presumably in the form of a biological anomaly that merits further investigation. We use the term "terran life" to refer to all life on Earth, regardless of its biochemistry (Baross, 2007) and "standard life" to refer to life that uses the general biochemical architecture for life on Earth as it is taught in standard textbooks. This article concerns the possibility of nonstandard terran life, especially terran life whose biochemistry is so nonstandard that it would not be detected by life-detection tools targeted at standard terran biochemistry.

2. Life on Earth as We Do Not Know It

A fundamental obstacle in searching for life as we do *not* know it is that we cannot be sure what to look for. It is therefore useful to construct a hierarchy of weirdness that progressively departs in its biochemistry from standard life. It starts with branches on the tree that may have diverged before standard life split into Eubacteria, Archaea, and Eucarya, and progresses to life that might exploit standard biochemistry but does not share a common ancestry with known terran life. Finally, we speculate about life that differs in various degrees in its choice of chemical pathways that support its genetics and metabolism. A plausible example of the latter is life that employs the same elements as standard life but combines them differently, such as by using the same protein backbone with different sets of amino acid side chains or by using the same DNA phosphate backbone with different sets of nucleotides. The possibility of such forms is suggested by laboratory studies in synthetic biology (Benner and Sismour, 2005). A more radical example would be carbon-based biochemistry with one of the additional elements crucial to standard life substituted with a chemical surrogate; for example, with P replaced by As (see for example, Wolfe-Simon *et al.*, 2009). In this paper, we leave aside still more speculative departures from known biochemistry, such as forms of life that use solvents other than water or use silicon in place of carbon as the key scaffolding element.

The hierarchy of increasing weirdness is paralleled by the increasing difficulty in our ability to detect that life, even if it is located in our immediate vicinity. For example, current life-detection strategies focus on ribosomal RNA, a technique that has successfully found life with unexpectedly different metabolic pathways and replicative mechanisms. A similar strategy might be able to detect weird life that used a different set of encoded amino acids, but it would be less likely to detect remnant RNA-world organisms and would not work at all with life that employs a different set of encoding nucleotides or with any of the forms of life farther up in the weirdness hierarchy.

Any search for a shadow biosphere must consider the role of ecological niches and address the issue of why standard life could not/did not invade and conquer the locales harboring weird life. Standard life has, after all, adapted to nearly every ecological niche that possesses liquid water and a source of free energy (one possible exception being ocean vent locales above about 130°C). Standard proteins have proven able to catalyze steps in the wide variety of metabolisms found in those niches. Given the demonstrated adaptability of standard biochemistry, it might seem reasonable to expect that standard life would have displaced whatever weird life-forms might have previously occupied such niches, on the assumption of greater Darwinian fitness. But this line of reasoning is a little too pat. First, there may be niches in which conditions are simply too extreme for standard life, though not for weird life (*e.g.*, at high pressure with temperatures above 130°C). Second, there is the possibility of "peaceful co-existence." One of the lessons from the last half century of microbiology has been that metabolic similarity is a poor indicator of evolutionary affinity. Two organisms might perform similar functions with very different biochemical machinery, perhaps due to evolutionary convergence. If so, then there would be little incentive for organisms of one class to "eat" those of the other. Archaea and Eubacteria, two microbial domains that diverged perhaps three billion years ago, coexist in the same environmental space (for example, in the rumen of a buffalo). Each contributes a class of metabolism that has not evolved in the other. A classic example is provided by methanogenesis, which is a widespread form of metabolism among Archaea. It is a surprising fact that methanogenesis has not been co-opted by Eubacteria via lateral gene transfer, despite the apparent ability of Eubacteria to out-compete Archaea in virtually every other niche. As a result, methanogenic Archaea can happily follow their distinctive metabolic path alongside Eubacteria without being out-competed. Conversely, Archaea have not acquired from Eubacteria whatever genes may be needed to allow it to conquer the classical Eubacterial niches. In the same vein, a radically different organism, for which lateral gene transfer with standard life is even less likely, might also peacefully coexist by exploiting a metabolic pathway that standard life ignores.

Another reason why weird life might fend off conquest by standard life is if it enjoyed a biochemical advantage of some sort. For example, eukaryotes devote about 70 percent of their physical volume to translation (Benner, 1999). By contrast, RNA organisms, by not having translation, can be much smaller and thus dominate the "small size" niche. RNA organisms might survive on modern Earth in environments where small size is advantageous. It is conceivable that some of the reports of nanobacteria, or nanobes, might be observations of such organisms (Folk, 1993; Kajander and Çiftçioglu, 1998; Uwins *et al.*, 1998). To take another example, RNA-world organisms would use RNA (containing phosphorus but not sulfur) as catalysts instead of proteins (which contain sulfur but not phosphorus). Thus, they may have an advantage over standard life in environments that
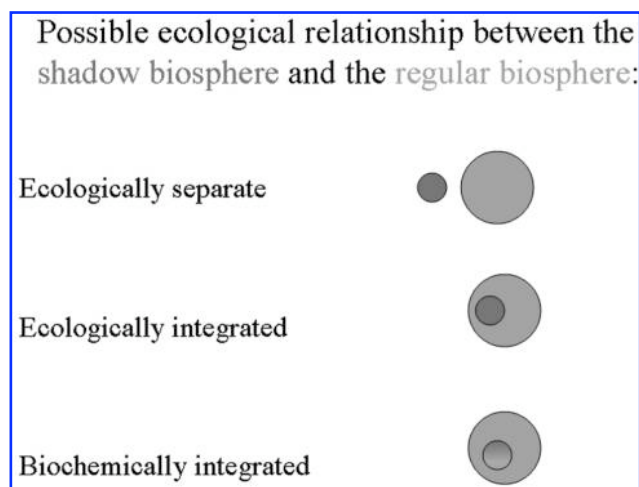
FIG. 1. Schematic representation of various possible relationships between known and weird life.

possess appreciable phosphorus yet are poor in sulfur, for example, during the Archean (Canfield, 1998).

It is convenient to define three distinct relationships between weird life and standard life: ecologically separate, ecologically integrated, and biochemically integrated (Fig. 1). In terms of identification, ecological isolation offers the easiest relationship to contemplate. It isn't hard to imagine stumbling across a radically different form of life in an extreme terrestrial environment hostile to all forms of standard life. We discuss this case in Section 3. In Section 4, we consider the problem of detecting weird life if it overlaps in both physical and parameter space with known life, especially if weird microbial life is present at low levels and intermingled with abundant Eubacteria and Archaea. In Section 5, we offer a few thoughts about the problem of establishing a second genesis for weird life if it should turn out to be not only ecologically but biochemically integrated with known life.

3. Ecologically Separate Shadow Biosphere

The easiest form of shadow biosphere to consider is one that is ecologically separate from known life. There may be some terrestrial environments in which conditions are too harsh for standard life to survive but are nevertheless acceptable to a more resilient form of weird life. The exploration of life in extreme environments is now a major research project. Our interest focuses on environments devoid of standard life yet which nevertheless show evidence of some sort of biological activity. The question which then arises is how putative weird life might betray its presence in the harsh environment. This issue was confronted many years ago in the context of the Viking missions to search for an unknown form of life on Mars.

Signatures of life divide into three broad categories: structure, function, and molecular composition. Structural evidence for life can take many forms—for example, geological alterations (mineral deposits, fossils, stromatolites). Viking sought evidence of extant life by looking for carbon cycling. Similar techniques could be used to detect weird terrestrial life. If weird life is metabolizing in an ecologically isolated environment, there should be a distinctive chemical dis-

equilibrium as well as a throughput of matter and energy where none might otherwise be expected, on the basis that standard life is absent there. It would therefore make sense to plan a "mission to planet Earth," involving a "super-Viking" suite of experiments. Notwithstanding their post-Viking sophistication, these experiments would be far cheaper than their Viking counterparts because they would not have to be transported to another planet, nor would they need to be sterilized, since by assumption super-Viking would be sampling environments that are either lethal to known life or have already been exposed to known life over an extended duration.

Information processing and replication imply a thermodynamic cost for reasons of basic physics. One way in which generic biogenic chemical disequilibrium might be manifested is through patterns in organic molecules, *i.e.*, a distinctive molecular composition. This point is based on what McKay terms the "Lego Principle" (McKay, 2004). In contrast to abiotic mechanisms, biological processes build from a small subset of the complete range of possible organic molecules. In organic material of nonbiological origin, molecules with similar chemical properties are present at roughly similar concentrations. In life, by contrast, there can be a selection between such chemically similar molecules. Examples include the use of L- not D-amino acids in proteins and the use of D-sugars in polysaccharides. The selectivity of biological processes is shown schematically in Fig. 2 by the distribution of spikes, in contrast to a smooth, nonbiological distribution. Weird life may display unfamiliar spikes; it may not even use amino acids. The advantage of this analysis is that the specific spikes are less important than the overall spikiness. [A complicating factor that first needs to be addressed, however, is that nonbiological processes can also sometimes generate a non-uniform distribution of species in small molecule structure space, in a manner that may depend on available mineral catalysis and other physical factors (Ricardo *et al.*, 2004).] A crucial proviso for identifying weird life this way is that it would need to produce patterns distinctively different from those of standard life and its breakdown products and from abiotic processes; otherwise, one could not rule out contamination by standard life. Even
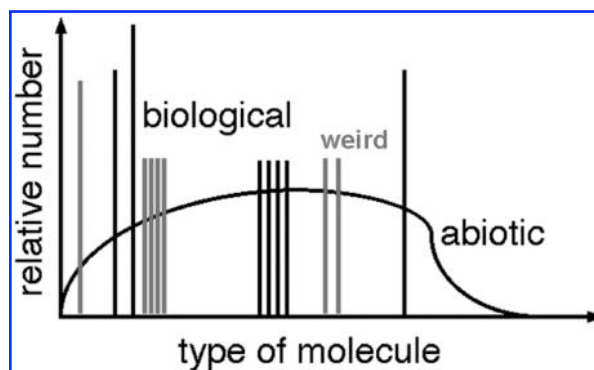

FIG. 2. Comparison of biogenic with nonbiogenic distributions of organic material. Nonbiological processes produce smooth distributions of organic material, illustrated here by the curve. Biology, in contrast, selects and uses only a few distinct molecules, shown here as spikes (*e.g.*, the 20 L-amino acids used by familiar life).

if shadow life occupies a separate ecological niche from standard life, the organic detritus from the latter could still invade the former as a result of water or wind transportation. If weird life is present at very low levels, its organic signature could be swamped by the breakdown products of much more abundant standard life, which would create a major signal-to-noise problem. Conversely, we might detect trace products of ecologically separate weird life that had diffused or been transported into the regular biosphere.

Interestingly, there are known examples of profoundly isolated microbial ecosystems that could, in principle, be populated by weird life, though all three of them play host to standard life. These systems provide examples of how a community of shadow life could thrive even if unconnected to the regular biosphere. Two of the isolated microbial ecosystems are based on methanogens that utilize H_2 released by rock-water reactions, and one derives ultimately from radioactive decay.

Stevens and McKinley (1995) discovered an example of a microbial community that is completely independent of surface photosynthesis and grows deep beneath the Columbia River. This subsurface ecosystem includes hydrogen-consuming methanogens that use the basic chemical reaction $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$. The H_2 in this reaction is produced by the serpentinization of olivine, in which H_2O oxidizes iron in basaltic rock. Chapelle et al. (2002) found a similar system in the massive basalts in the Twin Falls area of Idaho. Lin et al. (2005, 2006) reported another anaerobic chemoautotrophic microbial ecosystem in the deep subsurface that derives its energy from a redox reaction produced by radioactive decay. Primary production in the latter system is based on sulfur-reducing bacteria, such as *Desulfoprofundis tokoloshe*. The basic chemical reaction in this case is $4H_2 + H^+ + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$, where the $4H_2$ is produced by radioactive dissociation of H_2O. The sulfate is produced by the reaction of FeS_2 in the host rock with the oxidants O_2 and H_2O_2, which are also produced by the radiolysis of H_2O.

Standard life depends on a "habitable window" in several parameters, the most notable being temperature, salinity, pH, concentration of metals and other potentially toxic substances, and background radiation. There is thus a multi-dimensional parameter space, the boundary of which delineates the outer reaches of known life. A systematic search strategy should consider variations in two or more parameters simultaneously. We might, for instance, seek life at extremes of both acidity and temperature, or metallicity and radiation (e.g., in the tailings of uranium mines). A clear signature of ecologically isolated shadow life would be an occurrence where standard life reaches a limit, as a particular parameter, or set of parameters, is varied; then, after a gap, some additional evidence for life is found. For example, as the temperature is raised, it seems likely that standard life would cease at about 130°C. If no life is found in the range 130–150°C, but there is evidence for life at, say, 150–180°C, this would constitute strong evidence for hyperthermophilic shadow life.

Environments contiguous with the known biosphere that nevertheless seem to have exceeded the outer limits for standard life include ultra-dry deserts (such as the core of the Atacama), ice sheet plateaus at high latitudes, highly elevated mountaintops, the mid to upper atmosphere, hot ocean vents above a maximum temperature, and the subsurface of the Earth's crust below a maximum depth (these possibilities are summarized in Table 1). If carbon-based shadow life has taken up residence in one of these locations, we might at the very least expect to find carbon cycling from extant representatives or carbon concentrations from extinct representatives, or both. Navarro-Gonzalez and coauthors (2003) reported on studies of the ultra-dry core of the Atacama Desert. They found only low, and often undetectable, levels of normal life, but no evidence of any carbon (or nitrogen) cycling by processes that might indicate the presence of weird life. Similarly, there is no evidence for unexpected carbon cycling on the surface of polar ice without the presence of liquid melt (e.g., McKay et al., 1992). However, no systematic study has been undertaken at this time of all the suggested extreme environments. A limitation of this line of investigation is that life may persist in extreme nutrient-poor environments at very low metabolic rates, which would make carbon cycling very hard to detect. For example, in the deep subsurface, standard life is known to be present, but in an almost dormant state (Jorgensen and Boetius, 2007; Lipp et al., 2008; Roussel et al., 2008).

As a complement to direct exploration, one might create a controlled artificial environment, designed such that standard life cannot survive, introduce material from candidate shadow ecosystems into that environment, and then test for residual biological activity. This was, in fact, how the highly radiation-resistant organism *Deinococcus radiodurans* was discovered, growing in a supposedly radiation "sterilized" environment.

The most promising signature of ecologically isolated weird life would be the identification of distinctive biomolecular structures adapted to the extreme environment (as in the case of lipid membranes that may permit Archaea to thrive at high temperatures). We can hazard a guess as to which biomolecular structures might confer survival value in various extreme conditions. For example, helices are important folding elements in proteins. Protein chains made from 2-methylamino acids (which replace a hydrogen atom in a standard amino acid by a CH_3 group) fold to give helices that are more stable than those built from standard

TABLE 1. SEARCH LOCATIONS FOR SHADOW LIFE

A. Natural environments with little, or no, standard life
  Dry deserts
  Ice sheets
  Hot vents and deep subsurface
  High-altitude mountaintops and plateaus
  Airborne particles in the upper atmosphere
  High-radiation environments
  Highly saline environments
  Extreme high or low pH
  Sites heavily contaminated with metals and other
    poisons
  Environments strongly depleted in a key element
    (e.g., P, S)
B. Artificial environments
  Introduce toxins specific to DNA life
  Remove all P or another vital element
  High temperature (> 150°C)
  Water replaced by an alternative solvent such as
    ammonia

amino acids. Thus, proteins made from 2-methylamino acids may be able to maintain their folded, active form at higher temperature than proteins built from standard amino acids. In this way, 2-methylamino acids may allow a weird form of life to survive in an environment too hot for standard life.

Another example is provided by consideration of pH. Strongly acidic environments (with pH as low as 1) are not habitable by standard organisms because of the intrinsic basicity of adenine and cytosine. Conversely, the intrinsic acidity of thymine and guanine makes them implausible genetic elements at high alkalinity (Geyer *et al.*, 2003). Standard life extends its range of pH toleration by pumping protons across cell membranes to maintain near-neutral conditions within the cell. But weird life might avoid the need for proton pumping by using genetic materials that are less acidic in high pH conditions or less basic in low pH conditions. While structures of nucleobases that have those specifications are easily proposed (Benner, 2004), a wide range of alternative genetic structures is conceivable. Any of these alternative structures would escape current methods for detecting standard life.

If we knew what we were looking for, such weird biochemistry would be easy to identify. For example, a homochiral collection of alpha-methyl amino acids in a biological sample would be a strong indicator of weird life. Hydrazinolysis of a sample with a chiral hydrazine derivative could easily detect these. Unfortunately, it would not determine whether they were translated (and hence the product of a different genetic code). A weird genetic system would, however, be suggested by the detection of a limited collection of heterocycles. Both discoveries would immediately justify deeper investigation. The fundamental difficulty is that these proposals for weird biochemistry are just three of many. It is always more difficult to detect the unexpected.

4. Ecologically Integrated Shadow Biosphere

Microbial communities are highly organized, well-integrated systems that modify their environments, both chemically and physically, in significant ways. They then maintain these modifications, which creates an extensive collection of fairly stable ecological niches that would not otherwise exist. The phylogenetic diversity of microbial communities is staggering; they do not at all resemble pure laboratory cultures. Some varieties of microbe are represented in huge numbers, whereas others are represented in very small numbers. The number of microbes that occupy a given niche reflects its "size," *i.e.*, the pertinent physical and chemical resources made available by the community. Being a rare microbe is not necessarily an evolutionary disadvantage in that they occupy different ecological niches than common microbes occupy and produce or utilize material that is ignored by other varieties. Indeed, recent studies suggest that rare microbes supply the bulk of the phylogenetic diversity of many microbial communities (Baker *et al.*, 2006; Sogin *et al.*, 2006). It would thus be a mistake to limit a search for shadow microbes to environments hostile to familiar life.

If a fraction of microbial life "under our noses" (or beneath our microscopes) is life as we don't know it, how can we identify any weird component? As microbial morphology is very limited, it may be impossible to tell, just by looking, whether a given microbe is known or weird life. Thus, there is a major challenge to find the weird life signal in the face of the noise of the regular biosphere, especially if weird life is present in low concentrations.

Again, a search could target either structure or function. An alternative biochemistry will use at least some distinctively different organic molecules. One strategy would be to identify potentially biologically useful molecules that are not incorporated in standard life or produced in its decay products and seek evidence for them in the biosphere. For example, bacteria and archaea make use of several different cell wall structures, but other possibilities can be conceived. Another example concerns amino acids, for which standard life uses only a restricted subset. Yet a much broader class of amino acids forms readily in nature: the Murchison meteorite contains several dozen, for example. As remarked in Section 3, alpha-methyl amino acids are not used by any known organism, but, on the face of it, could fulfill a biological role. If they were found in conjunction with biological activity such as carbon cycling, they would constitute a strong indicator of alternative biochemistry. A fairly simple laboratory test would be to examine the amino acid inventory of organisms, as that could be an indicator of a departure from the universal genetic code. A potentially promising location for weird life of this sort would be a geological setting, such as a hydrothermal system, where copious abiotic production of a wide selection of amino acids is taking place.

An indirect sign of an ecologically integrated shadow biosphere might come from the discovery of viral parasites in the general environment that are clearly maladapted to known life. Because viruses are easily transported, they might be indicative of either ecologically separated or integrated shadow biospheres. Seawater is teeming with vast numbers of different viruses (Breitbart and Rohwer, 2005); a shotgun analysis along the lines pioneered by Venter *et al.* (2004) would seem to be an efficient way to proceed.

Another universal signature of life is homochirality. The detection of concentrations of chiral organic molecules of the same stereo configuration would be strong evidence for biological activity of some sort and has long been considered in the context of extraterrestrial life (Klein *et al.*, 1976). In the case of extinct weird life, racemization would eventually destroy the chirality signature and indeed degrade any distinctive pattern in the distribution of organics. However, in low-temperature environments such as permafrost, the Antarctic dry valleys, and high mountaintops (if protected from solar ultraviolet radiation), racemization is extremely slow, and a chiral signature could be preserved for hundreds of millions of years.

Although there is no agreement on its origin, the specific chiral signature of L-amino acids and D-sugars may very well be a frozen accident. If life were to start over again, there would be a chance that the opposite chirality—"mirror life"—would emerge next time. A search for D-amino acids and L-sugars in the environment could provide clues to the presence of weird life, if it could be demonstrated that these molecules were neither racemization products of standard life nor abiotic in origin. A more convincing signature of weird life could be provided by mirror steranes; unlike amino acids, these organic molecules have no known abiotic origin (Mackenzie *et al.*, 1982).

A complicating factor is the widespread use by standard

life of racemases, which can convert L- into D-amino acids and D- into L-sugars. Sugars are designated D or L based on the placement in space of the oxygen attached to the next-to-last carbon in the sugar's carbon chain (see Fig. 3). This means it is possible to convert D-sugars into L-sugars without epimerization, which is generally a slow process. Instead, some D-sugars can be converted into certain L-sugars by oxidation of one end and reduction of the other (Fig. 3). This process is used in animals to make vitamin C from D-glucose via conversion into L-gulcose (where the name "gulcose" was suggested by Fischer because it turned around two letters in the name of the sugar "glucose"). Likewise, L-xylulose rather than its D counterpart is the principal sugar in plants.

The situation with amino acid chirality is less clear cut, because an epimerization is always required to interconvert the D and L forms. However, we note that 2-ketoacids are universal precursors for L-amino acids, and these molecules are achiral. Moreover, D-amino acids are used in certain roles even by non-exotic organisms (including *E. coli*), while epimerases and D-amino acid oxidases are frequently encountered in the biosphere. This suggests that the chiral characterization of life isn't a straightforward matter of one-handedness versus the other. The bottom line is that chirality remains a promising tool for identifying weird life, but its application requires a high degree of sophistication.

More speculative is the possibility of weird life exploiting novel sources of energy (see, for example, Schulze-Makuch and Irwin, 2004). One possibility is ultraviolet radiation, which is lethal to all standard life. Solar ultraviolet light is abundant at high latitudes and altitudes, and especially the largely unexplored upper atmosphere, and would be a good source of energy for photosynthesis if its damaging bond-breaking effects could be mitigated (Wolstencroft and Raven, 2002). One way that the radiation sensitivity of the genetic matter might be reduced is through the use of alternative acid components. For example, a thymidine analogue that does not so easily form thymine dimers would extend the range of organisms into environments with exposure to harder ultraviolet light. Another speculative idea is organ-

isms that use gravitational energy via pressure gradients, for example, by being anchored at a fixed depth in a tidal zone and using the pressure variations to drive a thermodynamic cycle coupled to a chemical cycle. Variations in ambient electric field gradients could also be, in principle, harnessed as an energy source.

An even more radical departure from known life would be organisms that employ a different set of elements from the familiar C, H, N, O, P, S set. One example that has received some attention (Wolfe-Simon *et al.*, 2009) is arsenic (As), which is chemically similar to phosphorus (P) and could substitute for it in some biochemical roles. The kinetic lability of the arsenic-oxygen bond makes it unlikely that arsenic could entirely replace the structure and function of phosphorus in nucleic acids, lipid membranes, and ATP; but it might serve a transient kinetic role in weird metabolism. On the other hand, it also offers a number of dissimilatory metabolic pathways either via the reduction of arsenate to arsenite (the analogous reduction of phosphate to phosphite has a stronger redox demand) or the photosynthetic oxidation of arsenite to arsenate (Kulp *et al.*, 2008). Although its lower abundance makes arsenate less favorable than phosphate for modern biochemistry, there remain niches that are arsenic rich and phosphorus poor. Known examples are deep ocean volcanic vents, shallow hydrothermal systems, Mono Lake in California, and desert varnish (Oremland *et al.*, 2002). It is possible that a weird biochemical system lived, or now lives, in one or more of these niches. A targeted search could look for organisms with As being systematically incorporated into key organic molecules. Another approach would be to try to culture organisms from high-As environments and measure their metabolic or reproductive rate as a function of As concentration. If the rate declined as the As concentration was reduced to zero, it would be a strong indicator of As having a crucial biochemical function.

An ecologically integrated weird life-form would likely differ less in its basic genetic structures than a weird life-form adapted for an environment where standard life cannot survive. It is therefore more difficult to find reasons why it would not have been conquered by standard life. As re-
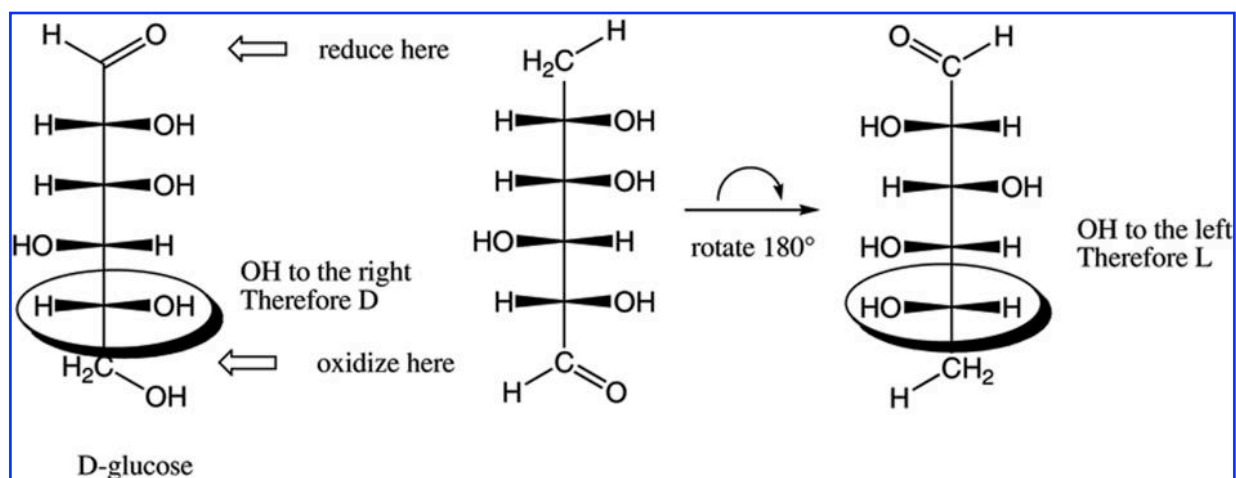

FIG. 3. Chirality switch. Reduction and oxidation of opposite ends of a glucose molecule can flip the chirality without the need for epimerization.

marked in Section 2, a possible survival strategy would be a symbiotic relationship between weird life and sympatric standard life, analogous to the history of Archaea and Eubacteria. Prior to genomic analysis, Archaea were not even recognized as distinctive classes of microorganisms on a phylogenetic tree but rather as examples of a kingdom of Monera (Margulis, 1998). Presumably, any symbiotic relationship between weird life and standard life would also involve the exchange of some biochemical structures such as nutrients, enzymes, and possibly even genetic material—perhaps analogous to the lateral gene transfer between Archaea and Eubacteria. One consequence of this biochemical integration would be to make weird life that much harder to discriminate from standard life.

5. Biological Anomalies: Has Shadow Life Already Been Discovered?

It is conceivable that the presence of alternative microbial life has already been noted, just as Archaea had long been noticed before their distinctiveness was appreciated. A gram of soil typically contains a million different species of microbes, of which only a tiny fraction have been sequenced or even characterized (Amann et al., 1995; Pace, 1997; Hugenholtz et al., 2006). If a shadow biosphere is ecologically integrated with the known biosphere, the respective member organisms would be intermingled, and it is unlikely that morphological differences alone would constitute a distinctive signature. Shadow organisms would presumably resist attempts at standard culturing and gene sequencing, and for this reason may have been shrugged aside as "uncooperative." But any microbial species that fails to respond to standard biochemical techniques is a candidate for alternative life and should be scrutinized for novel chemical content.

Recognizing shadow microbes would not be easy. It is very likely that all life so far studied descended from a common origin. Known organisms share a similar biochemistry and use an almost identical genetic code, which is why microbiologists can sequence their genes and position them on a single tree of life. But there is an obvious circularity here. Organisms are analyzed via chemical probes that are carefully customized to respond to life as we know it. These techniques may well fail to respond appropriately to a different biochemistry. If shadow life is confined to the microbial realm, it is entirely possible that it has been overlooked.

In light of these considerations, the best strategy to adopt in searching for shadow microbes is to cast a wide net and search for biological or biochemical anomalies (Cleland and Copley, 2005; Cleland, 2007). If shadow microbes exist or have existed, they have undoubtedly left traces in their environments. The fact that we haven't recognized them yet is hardly surprising. First, they would be missed by standard molecular biology techniques (polymerase chain reaction amplification of rRNA genes and metagenomic methods). Second, the default assumption when biologists are faced with a perplexing, seemingly biological, trace is that either it was produced by familiar life or it has an abiotic origin. This assumption makes it unlikely that biologists would immediately appreciate the significance of traces of weird life even supposing that they encountered them.

To illustrate the notion of a biological anomaly, consider the case of desert varnish, a hard, dark coating found on rock in arid regions (Perry et al., 2006; Cleland, 2007) that has been a source of puzzlement since at least the time of Darwin (Darwin, 1871). There is no scientific consensus on how it is produced despite the fact that it has been extensively studied by geologists. Consisting of extremely thin chemical and mineralogical layers, it bears a morphological resemblance to stromatolites. Even more provocatively, varnish coatings are enriched in manganese, arsenic, and iron despite the fact that the rocks on which they are found are not. To many geologists, this intriguing combination of microstructural and chemical features suggests a microbial origin, particularly since bacteria and algae commonly produce manganese or iron as by-products of metabolism. Yet microbes are infrequently found on varnish surfaces, as they are on living stromatolites. Polymerase chain reaction analysis of 16S rRNA genes extracted from coatings and surrounding rocks and soils vary from region to region, which suggests that no one group of bacteria, let alone a single variety, is responsible for the coatings. Furthermore, attempts to produce varnishlike coatings in laboratory settings with bacteria and algae have been unsuccessful. As a result, the question as to whether desert varnish has a biological origin is still hotly debated. The most recent contribution to this debate (Perry et al., 2006) postulates a very complex series of inorganic chemical reactions, though it still cannot account for the anomalously high concentration of iron and manganese.

6. When Is a Tree Really a Branch? Defining Biogenesis

In the event that a new form of life is discovered, a major challenge will be to determine whether it is derived from a genuinely independent biogenesis, i.e., it represents a second tree of life, or whether it is simply a hitherto undiscovered side branch on the known tree of life. This issue is of crucial significance to astrobiology. If it can be established that life on Earth has originated more than once, it implies that life will emerge readily in Earth-like conditions, and it will therefore be very likely to arise on other Earth-like planets, too. On the other hand, if weird life is merely a highly divergent outlying branch on the same tree as familiar life, we could not draw the sweeping conclusion from its discovery that life is widespread in the universe. At best, the discovery of a shadow biosphere might merely extend the parameter range over which extraterrestrial life is expected to exist. It is, therefore, of crucial importance to develop criteria for distinguishing a single bushy tree of life from a forest of independent trees.

The more the new life differs biochemically from known life, the more plausible it is that we would be dealing with multiple genesis events. For example, it is very likely that the familiar triplet genetic code has evolved from a simpler and more primitive precursor, perhaps a doublet code (Patel, 2005). It is conceivable that some ancient microorganisms have survived by using the earlier doublet code. As before, our view of the survivability of such primitive microorganisms is colored by our view of whether a doublet code could confer Darwinian advantage, at least in some environments. These organisms would *not* be a genuinely new form of life; rather, they would be "living fossils" that occupy a new, deep branch on the known tree of life, having bifurcated from the main branch before the establishment of the mod-

ern code (presumably before the split into Eubacteria, Archaea, and Eucarya). Another example of a "living fossil" would be a little-modified surviving vestige of the RNA world—a hypothesized biochemical precursor of standard contemporary life, according to some. Even though relic RNA life would have different biochemistry at the level of biopolymer sequence and information flow, it would nevertheless betray its common origin with standard life by sharing many chemical similarities at lower levels (Benner *et al.*, 1989). By contrast, the discovery of mirror life might well indicate an independent origin, because it is hard to imagine an earlier achiral form of life that split into left- and right-handed versions, as achiral molecules arguably lack the complexity necessary to support life. Homochirality in biopolymers has distinct advantages over heterochirality (Siegel, 1998).

A complicating factor is the possibility that a shadow biosphere might have biomolecular structures and metabolisms similar to those found in the known biosphere. The power of evolutionary convergence might be strong enough for independently originating life-forms to "discover" the same biochemical solutions to some problems. Thus, we find in standard life functionally very similar enzymes that are nevertheless coded for by DNA sequences with little or no homology. It may thus be the case that evolutionary convergence would progressively obliterate the distinctive evolutionary pathways of two independently originating forms of life, making their separate provenance hard to discern.

We have deliberately avoided providing either a general definition of life or a specific scenario for biogenesis, as they are not necessary for the purposes of this paper. It is possible that life emerged from nonlife abruptly, after the fashion of a phase transition in physics, which would support a well-defined notion of an origin of life at a particular place and time. It is equally possible that the transition from nonlife to life was a complicated sequence of events extended over time. If so, the notion of multiple genesis events becomes blurred or even vacuous (Lineweaver, 2006). Certainly, it would be much harder to establish whether a shadow biosphere is descended from an independent second genesis if there is no clear demarcation between the living and nonliving realms. Indeed, the only criterion would seem to be absolute physical isolation, which is extremely unlikely if both genesis events occurred on Earth. There is, of course, the possibility of a shadow biosphere of extraterrestrial origin, though even a martian origin would probably not constitute a watertight case for physical isolation (Davies, 1998).

We do not attempt to resolve these mainly philosophical issues here. Our position is that we should first attempt to discover weird life on Earth and worry about its relationship, if any, to familiar life once we have had a chance to study it in detail. We do, however, note that, if the threshold of life is indeed ill defined, we may discover intermediate forms that fall short of possessing the complete autonomy of familiar cells but nevertheless are important players in the overall biosphere on account of being biochemically as well as ecologically integrated. It is conceivable that nanobacteria belong to the class of forms intermediate between the realms of chemical complexity and true biology.

This paper is based on the idea that the best strategy for discovering the existence of weird terrestrial life, assuming for the sake of argument that it exists, is to search for bio-logical anomalies. As Kuhn (1970) emphasized, anomalies are the driving force behind scientific revolutions; they stand out against the backdrop of accepted scientific belief, driving new conceptual schemes and paving the way for yet more discoveries. It is hard to imagine a discovery of greater significance to science than a shadow biosphere of weird life on Earth. There is, of course, no guarantee that such a biosphere exists or has ever existed. But a systematic search for one, even if unsuccessful, is a worthwhile strategy anyway, if only because it may very well uncover hitherto unknown highly exotic forms of standard life. Certainly, a shadow biosphere cannot be ruled out on the basis of our current scientific understanding (Cleland and Copley, 2005; Davies and Lineweaver, 2005); and, given the profound importance that such a discovery would represent, it is well worthy of scientific investigation. As discussed, identifying weird terrestrial life for what it represents against the "noisy" backdrop of familiar life would not be easy, but we have sketched some promising strategies for overcoming these difficulties. We have also identified some puzzling, seemingly biological, anomalies that just might represent traces (or shadows) of as-yet-unrecognized weird terrestrial life.

Acknowledgments

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