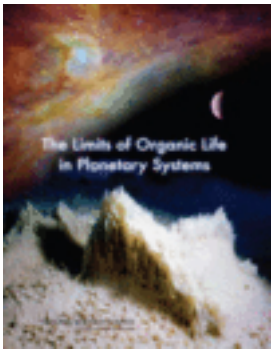
The Limits of Organic Life in Planetary Systems



Committee on the Limits of Organic Life in Planetary Systems, Committee on the Origins and Evolution of Life, National Research Council

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The Limits of Organic Life in Planetary Systems

Committee on the Limits of Organic Life in Planetary Systems
Committee on the Origins and Evolution of Life

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Board on Life Sciences
Division on Earth and Life Studies

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Dedicated to

Non-Human-Like Life Forms,
Wherever They Are

Preface

As the search for life in the solar system expands, it is important to know what exactly to search for. Previous life-detection experiments have been criticized for being too geocentric. This study aims to inform research program managers, policymakers, and mission designers about the possibilities for life on other solar system bodies. Further, during planetary protection exercises at the National Aeronautics and Space Administration (NASA), questions concerning the possibility of nonterran[a] life recur repeatedly. Remarkably little knowledge is organized that might shed light on the plausibility of bizarre life as a concern for planetary protection.

The search for signs of life, present or past, is an important goal of NASA's robotic solar system exploration programs and, ultimately, for its astronomical programs designed to probe the gross characteristics of extrasolar planetary systems. To date, that search has been governed by a model of life that is based on the life that we know on Earth—terran life. Several features of terran life have attracted particular focus:

- Terran life uses water as a solvent;
- It is built from cells and exploits a metabolism that focuses on the carbonyl group (C═O);
- It is thermodynamically dissipative, exploiting chemical-energy gradients; and
- It exploits a two-biopolymer architecture that uses nucleic acids to perform most genetic functions and proteins to perform most catalytic functions.

As a consequence, most of NASA's mission planning is focused on locations where liquid water is possible, and it emphasizes searches for structures that resemble cells of terran organisms, small molecules that might be the products of carbonyl metabolism, particular kinds of chemical-energy gradients, and tests for amino acids and nucleotides similar to those found in terrestrial proteins and DNA. This approach is defensible given the absence of a general understanding of how life might appear if it had an origin independent of Earth. Experiments in the laboratory, however, are suggesting that life might be based on molecular structures substantially different from

[a]The Committee on the Limits of Organic Life in Planetary Systems uses the term "terran" to denote a particular set of biological and chemical characteristics that are displayed by all life on Earth. Thus "Earth life" has the same meaning as "terran life" when the committee is discussing life on Earth, but if life were discovered on Mars or any other nonterrestrial body, it might be found to be terran or nonterran, depending on its characteristics.

those known in contemporary terran life. These results suggest that if life originated independently, even within our own solar system, it might have nonterran characteristics and, thus, not be detectable by NASA's in situ or remote-sensing missions designed explicitly to detect terran biomolecules or their products.

Further, if life is possible in solvents other than liquid water, it might exist in planetary environments other than the few that are currently targeted as potential hosts of nonterran life. Other than on Earth, liquid water is now considered possible only on subsurface Mars and in sub-ice environments of the Galilean moons of Jupiter (Europa, Ganymede, and Callisto), and perhaps on Saturn's moon, Enceladus. Nonaqueous solvents might, however, be present in other planetary environments. Because some of these spots (e.g., the surface of Titan) could be more accessible via spacecraft missions than either the deep subsurface of Mars or sub-ice Europa, evidence for life in solvents other than water might redirect missions to these other locales, and substantially improve the design of life-detection instrumentation generally. Similarly, nonterran life may change the gross characteristics of planetary environments in ways that differ from influences stemming from terran life, and these differences (e.g., the relative abundances of atmospheric species) may ultimately be observable over interstellar distances with astronomical facilities now on the drawing board.

This report explores a limited set of hypothetical alternative chemistries of life by following a hierarchy of possibilities that have been ranked through experimental, exploratory, and theoretical work done in the past. The study briefly reviews current knowledge concerning the following questions or hypotheses and provides suggestions for future research.

1. What environments on Earth that are extremes by terran standards harbor life? How must life-detection strategies be altered to discover this life on Earth? What extreme environments have not received attention? Are there synthetic environments that better represent conditions on alien worlds?
2. What environments on Earth are so extreme that life with standard terran biochemistry has been unable to occupy it?
3. What life forms are possible, still based on carbon and still functioning in water, but with a fundamental difference in the method of reproduction? Issues to be explored include the following:
   - What types of polymeric structures, other than proteins built from the standard 20 amino acids, might support catalysis in water? For example, can 2-amino-2-methyl-carboxylic acids, which have been found to be enantiomerically enriched in meteorites, be the basis for a catalytic system? In the absence of biopolymers, would selected monomers provide catalysis sufficient to sustain life?
   - What types of polymeric structures, other than nucleic acids built from the standard four nucleotides, might be replicatable and might support Darwinian evolution in water?
   - Can a functioning genetic system be established that is not based on a linear molecular structure? For example, can a compositional genome (a collection of monomers) sustain heredity?
   - Can a system capable of Darwinian evolution be demonstrated in the laboratory using nonstandard biopolymers or a compositional genome in water?
4. What life forms are possible, still based on carbon, but not functioning in water? Issues to be explored include these:
   - Can membranes be constructed in the laboratory that separate an organic solvent inside a cell from an organic solvent outside a cell?
   - What kinds of polymeric structures (or monomer collections) might support catalysis and genetics in nonaqueous environments, particularly in solvents found on solar system bodies other than Earth?
   - Can mineral systems be identified that interact in interesting ways with organic compounds in nonaqueous systems?
   - Can asymmetric induction, and spontaneous resolution that leads to the homochirality assumed to be necessary for life, be achieved in nonaqueous solvents, especially those found on solar system bodies other than Earth?
   - Can a system capable of Darwinian evolution be demonstrated in the laboratory using nonstandard monomers and/or biopolymers in nonaqueous environments?

The purpose of this study is twofold:

1. To evaluate the possibility that nonstandard biochemistry (i.e., biochemistry different from what we find as the universal biochemistry on Earth) might support life in known solar system environments and conceivable extrasolar environments; and
2. To define broad areas that might guide NASA and the National Science Foundation to fund efforts to expand knowledge in this area.

The results of this study are meant to aid in the development of a new generation of life-detection experiments that can be conducted in situ on planetary surfaces or conducted on samples returned from other solar system bodies.

Held on April 25, 2002, at the National Academies' Georgetown facility in Washington, D.C., the "weird life" planning session was chaired by John Baross (University of Washington) and included presentations from Chris Chyba (SETI Institute and Stanford University), Steven Benner (Foundation for Applied Molecular Evolution), Jack Szostak (Harvard University), George Cody (Carnegie Institution of Washington), and Robert Shapiro (New York University). A discussion session was led by Mitch Sogin (Woods Hole Marine Biological Laboratory). A planning session for the Workshop on the Limits of Organic Life in Planetary Systems was held at the Constitution Avenue building of the National Academies in Washington, D.C., on March 2-3, 2004, and chaired by John Baross with input from NASA staff members Michael Meyer, Marc Allen, and John Rummel.

The Workshop on the Limits of Organic Life in Planetary Systems was held on May 10-11, 2004, at the Constitution Avenue building of the National Academies, Washington, D.C. The co-chairs were Jack Szostak (Harvard University) and John Baross (University of Washington); panel moderators were Norman Pace (University of Colorado), James Kasting (Pennsylvania State University), Pascale Ehrenfreund (Leiden University), and Steven Benner (Foundation for Applied Molecular Evolution). Participants included Robert Blankenship (Arizona State University), Roger Summons (Massachusetts Institute of Technology), Ruth Blake (Yale University), Jonathan Eisen (Institute for Genomic Research), Eric Mathur (Diversa), Peter Ward (University of Washington), Christopher McKay (NASA Ames Research Center), David DesMarais (NASA Ames Research Center), James Ferry (Pennsylvania State University), Bruce Jakosky (University of Colorado), Robert Pappalardo (Jet Propulsion Laboratory), Jeffrey Kargel (U.S. Geological Survey), James Scott (Dartmouth College), Donald Button (University of Alaska at Fairbanks), Leslie Orgel (Salk Institute), Jonathan Lunine (University of Arizona), Dirk Schulze-Makuch (Washington State University), Douglas Clark (University of California, Berkeley), and George Cody (Carnegie Institution of Washington).

A writing meeting was held on March 14-16, 2005, at the National Academies' Arnold and Mabel Beckman Center, Irvine, California, and chaired by John Baross (University of Washington), with presentations from Steven Benner (Foundation for Applied Molecular Evolution), William Baines (Rufus Scientific), and Jonathan Lunine (University of Arizona).

Acknowledgments

The committee thanks Space Studies Board (SSB) interns Stephanie Bednarek, Matthew Broughton, and Brendan McFarland and Board on Life Sciences program officer Evonne Tang for their work on compiling the glossary and researching references. The committee also thanks SSB research assistant Victoria Swisher for assistance with the report review process.

This report has been reviewed by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's (NRC's) Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the authors and the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The contents of the review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their participation in the review of this report:

Robert H. Austin, Princeton University,
Paul Davies, Macquarie University, Australia,
Jack Farmer, Arizona State University,
Katherine H. Freeman, Pennsylvania State University,
James F. Kasting, Pennsylvania State University,
Anthony Keefe, Archemix Corporation,
Peter B. Moore, Yale University,
Kenneth H. Nealson, University of Southern California, and
Norman H. Sleep, Stanford University.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Leslie Orgel, Salk Institute for Biological Studies. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Executive Summary

Reflecting the near inevitability of human missions to Mars and other locales in the solar system where life might exist, and given the interest of the public in the question, Are we alone?, the National Aeronautics and Space Administration (NASA) commissioned the National Research Council, which formed the Committee on the Limits of Organic Life in Planetary Systems, to address the following questions:

- What can be authoritatively said today about limits of life in the cosmos?
- What Earth-based research must be done to explore those limits so that NASA missions would be able to recognize, conserve, and study alien life that is encountered?

Theory, data, and experiments suggest that life requires (in decreasing order of certainty):

- A thermodynamic disequilibrium;
- An environment capable of maintaining covalent bonds, especially between carbon, hydrogen, and other atoms;
- A liquid environment; and
- A molecular system that can support Darwinian evolution.

Earth abundantly displays life that uses solar, geothermal, and chemical energy to maintain thermodynamic disequilibria, covalent bonds between carbon, water as the liquid, and DNA as a molecular system to support Darwinian evolution. Life with those characteristics can be found wherever water and energy are available.

The natural tendency toward terracentricity[a] requires that we make a conscious effort to broaden our ideas of where life is possible and what forms it might take. The long history of terran chemistry tempts us to become fixated on carbon because terran life is based on carbon. But basic principles of chemistry warn us against terracentricity. It is easy to conceive of chemical reactions that might support life involving noncarbon compounds, occurring in solvents other than water, or involving oxidation-reduction reactions without dioxygen.

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The committee found no compelling reason to limit the environment for life to water as a solvent, even if life is constrained to use carbon as the scaffolding element for most of its biomolecules. In water, a wide array of molecular structures are conceivable that could (in principle) support life but be so different from those for life on Earth that they would be overlooked by unsophisticated life-detection tools. Evidence suggests that Darwinian processes require water, or a solvent like water, if they are supported by organic biopolymers (such as DNA). Although macromolecules that use silicon are known, few thoughts suggest how they might have emerged spontaneously to support a biosphere.

Many of the definitions of *life* include the phrase *undergoes Darwinian evolution*. The implication is that phenotypic changes and adaptation are necessary to exploit unstable environmental conditions, to function optimally in the environment, and to provide a mechanism to increase biological complexity. The canonical characteristics of life are inherent capacities to adapt to changing environmental conditions and to increase in complexity by multiple mechanisms, particularly by interactions with other living organisms.

One of the apparent generalizations that can be drawn from knowledge of Earth life is that lateral gene transfer is an ancient and efficient mechanism for rapidly creating diversity and complexity. The unity of biochemistry among all Earth's organisms emphasizes the ability of organisms to interact with other organisms to form coevolving communities, to acquire and transmit new genes, to use old genes in new ways, to exploit new habitats, and, most important, to evolve mechanisms to help to control their own evolution. Those characteristics are likely to be present in extraterrestrial life even if it has had a separate origin and a very different unified biochemistry from that of Earth life.

Because we have only one example of biomolecular structures that solve problems posed by life and because the human mind finds it difficult to create ideas truly different from what it already knows, it is difficult for us to imagine how life might look in environments very different from what we find on Earth. Recognizing the challenges in mitigating that difficulty, the committee chose instead to embrace it. In constructing its outlook, it exploited a strategy that began by characterizing the terran life that humankind has known well, first because of its macroscopic visibility and then through microscopic observation that began in earnest 4 centuries ago.

As the next step in its strategic process, the committee assembled a set of observations about life that is considered exotic when compared with human-like life. The committee asked, Can we identify environments on Earth where Darwinian processes exploiting human-like biochemistry cannot exploit available thermodynamic disequilibria? The answer to that question is an only slightly qualified no. It appears that wherever the thermodynamic minimum for life is met on Earth and water is present, life is found. Furthermore, the life that is found appears to be descendent from an ancestral life form that also served as the ancestor of humankind (we might not have recognized it if its ancestry were otherwise), and it exploits fundamentally human-like biochemistry. The committee reviewed evidence about abiotic processes that manipulate organic material in a planetary environment. It asked whether the molecules that we see in contemporary terran life might be understood as the inevitable consequences of abiotic reactivity.

The committee then surveyed the inventory of environments in the solar system and asked which ones might be suitable for life of the terran type. The survey made clear that most locales in the solar system are at thermodynamic disequilibrium, an absolute requirement for chemical life, and that many locales at thermodynamic disequilibrium also have solvents in liquid form and environments where the covalent bonds between carbon and other lighter elements are stable. Those are weaker requirements for life, but the three together appear, perhaps simplistically, to be sufficient for life. The committee asked whether it could conceive of a biochemistry adapted to those exotic environments, much as human-like biochemistry is adapted to terran environments. Because few detailed hypotheses are available, the committee reviewed what is known, or might be speculated, and considered research directions that might expand or constrain understanding about the possibility of life in such exotic environments. Finally, the committee considered more exotic solutions to problems that must be solved to create the emergent properties that we agree characterize life.

The committee found that using thermal and chemical energy to maintain thermodynamic disequilibria, covalent bonds between carbon atoms, water as the liquid, and DNA as a molecular system to support Darwinian evolution is not the *only* way to create phenomena that would be recognized as life. Indeed, the emerging field of synthetic biology has already provided laboratory examples of alternative chemical structures that support genetics, catalysis, and

Darwinian evolution. Organic chemistry offers many examples of useful chemical reactivity in nonwater liquids. Macromolecular structures reminiscent of those found in terran biology can be formed with silicon and other elements.

Accordingly, the committee identified high-priority Earth-based laboratory and field studies aimed at doing the following:

- Explore the limits of life on Earth, with an emphasis on detection of life in extreme environments that might have chemical structures and metabolisms different from those of terran life that has already been characterized.
- Pursue the origin of life, especially on the basis of information from NASA missions, the inventory of organic materials in the cosmos, and interactions between organic materials and minerals set in a planetary context.
- Contribute basic research to understand interactions of organic and inorganic species in exotic solvents, including water under extreme conditions (as found on Venus, Mars, Europa, Enceladus, and elsewhere), water-ammonia eutectics at low temperatures (as might be possible on Titan), and liquid cryosolvents (as found on Triton and elsewhere).
- Contribute to laboratory synthetic-biology research into molecular systems that are capable of Darwinian evolution but are different from standard DNA and RNA, especially those designed to improve understanding of the chemical possibilities of supporting Darwinian evolution.

The committee offers the following recommendations:

Recommendation 1. The National Aeronautics and Space Administration and the National Science Foundation should support these kinds of laboratory research:

- Origin-of-life studies, including prebiotic-chemistry and directed-evolution studies that address physiologies different from those of known organisms;
- Further studies of chirality, particularly studies focused on the hypothesis that specific environmental conditions can favor chiral selection, or on an alternative model that life with L-amino acids and D-sugars is better "fit," from an evolutionary perspective, to evolve into complex organisms; and
- Work to understand the environmental characteristics that can affect the ability of organisms to fractionate key elements, including not only carbon but also sulfur, nitrogen, iron, molybdenum, nickel, and tungsten.

Recommendation 2. The National Aeronautics and Space Administration and the National Science Foundation should support these kinds of field research:

- A search for remnants of an RNA world in extant extremophiles that are deeply rooted in the phylogenetic tree of life;
- A search for organisms with novel metabolic and bioenergetic pathways, particularly pathways involved in carbon dioxide and carbon monoxide reduction and methane oxidation coupled with electron acceptors other than oxygen;
- A search for organisms that derive some of their catalytic activity from minerals rather than protein enzymes;
- A search for organisms from environments that are limited in key nutrients, including phosphorus and iron, and determination of whether they can substitute other elements, such as arsenic, for phosphorus;
- A search for life that can extract essential nutrients—such as phosphorus, iron, and other metals—from rocks, such as pyrites and apatite;
- A search for anomalous gene sequences in conserved genes, particularly DNA- and RNA-modifying genes;
- Study of the resistance of microorganisms that form biofilms on minerals to the harsh conditions of interplanetary transport; and
- A search for life that stores its heredity in chemicals other than nucleic acids.

Recommendation 3. The National Aeronautics and Space Administration should support these kinds of space research:

- Programs that combine the exploration of potential metabolic cycles with the synthetic biology of unnatural nucleic acid analogues and their building blocks and that use the results to guide the design of instruments;
- Astrobiology measurements that can potentially distinguish between life on Mars (and possibly other bodies) that arrived via material ejected from Earth (or vice versa) and life that emerged on another body independently of life on Earth;
- Inclusion in missions planned for Mars of instruments that detect lighter atoms, simple organic functional groups, and organic carbon to help distinguish between "replicator-first" and "metabolism-first" theories of the origin of life; similar considerations should guide inclusion of small-organic-molecule detectors that could function on the surfaces of Europa, Enceladus, and Titan; and
- Consideration, in view of the discovery of evidence of liquid water-ammonia eutectics on Titan and active water geysers on Saturn's moon Enceladus, of whether the planned missions to the solar system should be reordered to permit returning to Titan or Enceladus earlier than is now scheduled.

1

Introduction

1.1 THE SEARCH FOR LIFE IN THE COSMOS

The National Aeronautics and Space Administration (NASA) has long given high priority to missions that ask whether extraterrestrial life might exist in the solar system and beyond. That priority reflects public interest, which was enhanced in the mid-1990s when fragments of Mars delivered to Earth as meteorites were shown to contain small structures reminiscent of microbial life.

The proper interpretation of those structures remains controversial, but it is certain that nothing would alter our view of humanity and our position in the cosmos more than the discovery of alien life. Nothing would contribute more to NASA's goal of exploring the cosmos, or to inspiring and educating the next generation of students in the hard sciences and engineering, than a search for alien life. Nothing would be more unfortunate than to expend considerable resources in the search for alien life and then not recognize it if it is encountered.

The search for life in the cosmos begins with our understanding of life on Earth. This understanding has grown enormously over the past century. It is now clear that although terran life is conveniently categorized into millions of species, studies of the molecular structure of the biosphere show that all organisms that have been examined have a common ancestry. There is no reason to believe, or even to suspect, that life arose on Earth more than once, or that it had biomolecular structures that differed greatly from those shared by the terran life that we know.

Our only example of life has been quite successful in dominating the planet. Earth itself presents a variety of environments, some extreme by human standards. One lesson learned from studies of terran biochemistry and its environmental range on Earth is that the life we know requires liquid water. Wherever a source of energy is found on Earth with liquid water, life of the standard variety is present.

That observation has already helped to guide NASA missions through the directive to "follow the water" in searching for life in the solar system. Environments where liquid water might be or might have been present are high on the list of locales planned for NASA missions. Excitement runs high when sites are found where the geology indicates with near certainty the past presence of liquid water in substantial amounts.

As pragmatic as the strategy is, scientists and laypeople alike have asked whether it might be parochial, or "terracentric." As Carl Sagan noted, it is not surprising that carbon-based organisms breathing oxygen and composed of 60 percent water would conclude that life must be based on carbon and water and metabolize free oxygen.[1]

The depth and breadth of our knowledge of terran chemistry tempts us to focus on carbon because terran life is based on carbon, and organic chemistry as we know it emerged from 19th-century natural-product chemistry

based on the isolation of compounds from nature. If terran life had provided silicon-based molecules, then our knowledge of silicon-based chemistry would now be advanced.

The natural tendency toward terracentricity requires that we make an effort to broaden our ideas of where life is possible and what forms it might take. Furthermore, basic principles of chemistry warn us against terracentricity. It is easy to conceive of chemical reactions that might support life involving noncarbon compounds, occurring in solvents other than water, or involving oxidation-reduction reactions without dioxygen. Furthermore, there are reactions that are not redox. For example, life could get energy from NaOH + HCl; the reaction goes fast abiotically, but an organism could send tendrils into the acid and the base and live off the gradient. An organism could get energy from supersaturated solution. It could get relative humidity from evaporating water. It is easy to conceive of alien life in environments quite different from the surface of a rocky planet. The public has become aware of those ideas through science fiction and nonfiction, such as Peter Ward's *Life as We Do Not Know It.*[2]

The public and the scientific community have become interested in authoritative perspectives on the possibility of life in environments in the solar system very much different from the ones that support life on Earth and life supported by "weird" chemistry in exotic solvents and exploiting exotic metabolisms. To NASA those ideas would help to guide missions throughout the solar system and permit them to recognize alien life if it is encountered, however it is structured. Given the inevitability of human missions to Mars and other locales potentially inhabited by alien life, an understanding of the scope of life will improve researchers' chance to study such life before a human presence contaminates it or, through ignorance or inaction, destroys it.

In broadest outline, this report shows that the committee found no compelling reason for life being limited to water as a solvent, even if it is constrained to use carbon as the scaffolding element for most of its biomolecules. In water, varied molecular structures are conceivable that could (in principle) support life, but it would be sufficiently different from life on Earth that it would be overlooked by unsophisticated life-detection tools. Evidence suggests that Darwinian processes require water, or a solvent like water, if they are supported by organic biopolymers (such as DNA). Furthermore, although macromolecules using silicon are known, there are few suggestions as to how they might have emerged spontaneously to support a biosphere.

1.2 DEFINING THE SCOPE OF THE PROBLEM

For generations the definition of life has eluded scientists and philosophers. (Many have come to recognize that the concept of "definition" itself is difficult to define.[3]) We can, however, list characteristics of the one example of life that we know—life on Earth:

- It is chemical in essence; terran living systems contain molecular species that undergo chemical transformations (metabolism) under the direction of molecules (enzyme catalysts) whose structures are inherited, and heritable information is itself carried by molecules.
- To have directed chemical transformations, terran living systems exploit a thermodynamic disequilibrium.
- The biomolecules that terran life uses to support metabolism, build structures, manage energy, and transfer information take advantage of the covalent bonding properties of carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur and the ability of heteroatoms, primarily oxygen and nitrogen, to modulate the reactivity of hydrocarbons.
- Terran biomolecules interact with water to be soluble (or not) or to react (or not) in a way that confers fitness on a host organism. The biomolecules found in terran life appear to have molecular structures that create properties specifically suited to the demands imposed by water.
- Living systems that have emerged on Earth have done so by a process of random variation in the structure of inherited biomolecules, on which was superimposed natural selection to achieve fitness. These are the central elements of the Darwinian paradigm.

Various published definitions of life understandably incorporate those features, given that we are the life form defining it. Indeed, because the chemical structures of terran biomolecular systems all appear to have arisen through Darwinian processes, it is hardly surprising that some of the more thoughtful definitions of life hold that it is a "chemical system capable of Darwinian evolution."[4]

1.3 IS EVOLUTION AN ESSENTIAL FEATURE OF LIFE?

Many of the definitions of life include phrases like *undergoes Darwinian evolution.* The implication is that phenotypic changes and adaptation are necessary to exploit unstable environmental conditions and to function optimally in the environment. Evolutionary changes have even been suggested for the hypothesized "clay crystal life" of Cairns-Smith,[5] referring to randomly occurring errors in crystal structure during crystal growth as analogous to mutations. Would a self-replicating chemical system capable of chemical transformations in the environment be considered life? If self-replicating chemical compounds are not life, replication by itself is not sufficient as a defining characteristic of life. Likewise, the ability to undergo Darwinian evolution, a process that results in heritable changes in a population, is also not sufficient to define life if we consider minerals that are capable of reproducing errors in their crystal structure to be equivalent to evolution. Although that property of clays may have been vital in the origin of life and particularly in the prebiotic synthesis of organic macromolecules and as catalysts for metabolic reactions, can the perpetuation of "mistakes" in crystal structure result in the selection of a "more fit" crystal structure? It is important to emphasize that evolution is not simply reproducing mutations (mistakes in clays), but also selecting variants that are functionally more fit.

The canonical characteristics of life are an inherent capacity to adapt to changing environmental conditions and to interact with other living organisms (and, at least on Earth, also with viruses).[6] Natural selection is the key to evolution and the main reason that Darwinian evolution persists as a characteristic of many definitions of life. The only alternative to evolution for producing diversity would be to have environmental conditions that continuously create different life forms or similar life forms with random and frequent "mistakes" in the synthesis of chemical templates used for replication or metabolism. Such mistakes would be equivalent to mutations and could lead to traits that gave some selective advantage in an existing community or in exploiting new habitats. That random process could lead to life forms that undergo a form of evolution without a master information macromolecule, such as DNA or RNA. It is difficult to imagine such life forms as able to "evolve" into complex structures unless other mechanisms, such as symbiosis or cell-cell fusion, are available.

Evolution is the key mechanism of heritable changes in a population. However, although mutation and natural selection are important processes, they are not the only mechanisms for acquiring new genes. It is understood that lateral gene transfer is one of the most important and one of the earliest mechanisms for creating diversity and possibly for building genomes with the requisite information to result in free-living cells.[7] Lateral gene transfer is also one of the mechanisms to align genes from different sources into complex functional activities, such as magnetotaxis and dissimilatory sulfate reduction.[8] It is possible that this mechanism was important in the evolution of metabolic and biosynthetic pathways and other physiological traits that may have evolved only once even though they are present in a wide variety of organisms. Coevolution of two or more species is also a hallmark of evolution manifested in many ways, from insect-plant interactions to the involvement of hundreds of species of bacteria in the nutrition of ruminant animals. Organisms and the environment also coevolve, depending on the dominant characteristics of the environment and the availability of carbon and energy sources.

If the ability to undergo Darwinian evolution is a canonical trait of life no matter how different a life form is from Earth life, are there properties of evolving extraterrestrial organisms that would be detectable as positive signs of life? Evolution provides organisms the opportunity to exploit new and changing environments, and one piece of evidence for the cosmic ubiquity of evolution is that on Earth life occupies all available habitats and even creates new ones as a consequence of metabolism. Another hallmark of evolution is the ability of organisms to coevolve with other organisms and to form permanent and obligatory associations. It is highly probable that an inevitable consequence of evolution is the elimination of radically different biochemical lineages of life that may have formed during the earliest period of the evolution of life. Extant Earth life is the result of either selection of the most fit lineage or homogenization of some or all of the different lineages into a common ancestral community that developed into the current three major lineages (domains). All have a common biochemistry based on presumably the most "fit" molecular information strategies and energy-yielding pathways among a potpourri of possibilities.

Thus, one of the apparent generalizations that can be drawn from extant Earth life, and the explanation for the development of a "unity of biochemistry" in all organisms, is that lateral gene transfer is an ancient and efficient mechanism for rapidly creating diversity and complexity. Lateral gene transfer is also an efficient mechanism for

selecting the genes that are most "fit" for specific proteins and transferring them into diverse groups of organisms. The results are the addition of genes and the replacement of less-fit genes that have similar functions. Natural selection based solely on mutation is probably not an adequate mechanism for evolving complexity. More important, lateral gene transfer and endosymbiosis are probably the most obvious mechanisms for creating complex genomes that could lead to free-living cells and complex cellular communities in the short geological interval between life's origin and the establishment of autotrophic CO_2 fixation about 3.8 billion years ago and microbial sulfate reduction 3.47 billion years ago on the basis of isotope data.[9] An important implication of the existence of viruses or virus-like entities during the early evolution of cellular organisms is that their genomes may have been the source of most genetic innovations because of their rapid replication, high rates of mutation due to replication errors, and gene insertions from diverse host cells.[10]

Is evolution an essential feature of life? Cells are more than the information encoded in their genomes; they are part of a highly integrated biological and geochemical system in whose creation and maintenance they have participated. The unity of biochemistry among all Earth's organisms emphasizes the ability of organisms to interact with other organisms to form coevolving communities, to acquire and transmit new genes, to use old genes in new ways, to exploit new habitats, and, most important, to evolve mechanisms to help to control their own evolution. Those characteristics would probably be present in extraterrestrial life even if it had a separate origin and a unified biochemistry different from that of Earth life.

1.4 BRIEF CONSIDERATIONS OF POSSIBLE LIFE FORMS OUTSIDE THE SCOPE OF THIS REPORT

As discussed in the literature,[11] chemical models of non-Earth-centric life reveal much about what the scientific community considers possible, particularly regarding ways in which systems organize matter and energy to generate life. Thus, truly "weird" life might utilize an element other than carbon for its scaffolding. Less weird, but still alien to human biological experience, would be a life form that does not exploit thermodynamic disequilibria that are largely chemical. Weirder would be a life form that does not exploit water as its liquid milieu. Still weirder would be a life form that exists in the solid or gas phase.[12] In a different direction, yet also outside the scope of life that most communities think possible, would be a life form that lacks a history of Darwinian evolution.

Some features of terran life are almost certainly universal, however. In particular, the requirement for thermodynamic disequilibrium is so deeply rooted in our understanding of physics and chemistry that it is not disputable as a requirement for life. Other criteria are not absolute. Terran biology contains clear examples of the use of nonchemical energy; photosynthesis is the best known, although energy from light is soon converted to chemical energy. Silicon, in some environments, can conceivably support the scaffolding of large molecules. This report explicitly considers nonaqueous environments.

Even Darwinian evolution is presumably not an absolute. For example, depending on how human civilization applies gene therapy, our particular form of life could be able to evolve via Lamarckian,[a] as opposed to Darwinian, processes. Humankind will be able to perceive and solve problems in human biology without needing to select among random events, thus sparing the species the need to remove unavoidable genetic defects through the death of individuals. That will make the human biosphere no less living, even to those who make Darwinian evolution central in their concept of life.

Likewise, we can easily conceive of robots that are self-reproducing or computer-based processes that grow and replicate.[13] Here, information transfer is not based on a specific molecular replication but on a replication involving information on a matrix. Whether such entities will be called life remains to be seen.

What is clear is that the scientific community does not believe that Lamarckian, robotic, or informational "life" could have arisen spontaneously from inanimate matter. At the very least, its matrix would have to be constructed initially by a chemical, Darwinian life form arising from processes similar to those seen on Earth. Again, it is not clear whether those views are constrained by our inability to conceive broadly from what we know or whether they reflect true constraints on the processes by which life might emerge in natural history.

[a]Lamarck recognized a similar principle of evolution referred to as "inheritance of acquired characters," stating that variations in characteristics seen in organisms were acquired in response to the environment.

Those thoughts introduce a subsidiary theme of this report. It is conceivable that chemistry, structure, or environments able to *support* life were not suited for the *initiation* of life. For example, Earth can support life today, but prevailing views hold that life could not have originated in an atmosphere that is as oxidizing as Earth's today. If that is true, the surface of Earth would be an environment that is habitable but not able to give rise to life.

1.5 STRATEGIES TO MITIGATE ANTHROPOCENTRICITY

We have only one example of biomolecular structures that solve problems posed by requirements for life, and the human mind finds it difficult to create ideas truly different from what it already knows. It is thus difficult for us to imagine how life might look in planetary environments very different from what we find on Earth. Recognizing that difficulty, the committee chose to embrace it. The committee exploited a strategy that began with characterization of the terran life that humankind has known well, first because of its macroscopic visibility and then through microscopic observation that began in earnest 4 centuries ago. This, of course, is like life that is associated with humankind. As the next step in the strategic process, the committee assembled a set of observations about life that is considered exotic when compared with human-like life. Exploration of Earth has taken researchers to environments that human-like organisms find extreme, to the highest temperatures at which liquid water is possible, to the lowest temperatures at which water is liquid, to the depths of the ocean where pressures are high, to extremes of acidity and alkalinity, to places where the energy flux is too high for human-like life to survive, to locales where thermodynamic disequilibria are too scarce to support human-like life, and to locations where the chemical environment is toxic to human-like life.

The committee then asked, Can we identify environments on Earth where Darwinian processes that exploit human-like biochemistry cannot exploit available thermodynamic disequilibria? The answer is an only slightly qualified no. It appears that wherever the thermodynamic minimum for life is met on Earth and water is found, life is found. Furthermore, the life that is found appears to be descendant from an ancestral life form that also served as the ancestor of humankind (perhaps we would not necessarily have recognized it if its ancestry were otherwise) and exploits fundamentally human-like biochemistry.

The committee then reviewed evidence of abiotic processes that manipulate organic material in a planetary environment. It asked whether the molecules that we see in contemporary terran life might be understood as the inevitable consequences of abiotic reactivity. Although signatures of such predecessor reactivity can be adumbrated within contemporary biochemistry, they are generally faint.[14] Some 4 billion years of biological evolution have attached a strong Darwinian signature to whatever went before; hypotheses regarding evidence of our inanimate ancestry within modern biostructures are the subject of intense dispute.

If life originated first on Earth, it was long ago when conditions on the surface of this planet were very different from what they are today. We do not know what those conditions were, and we may never know. Furthermore, the organisms around today are all highly evolved descendants of the first life forms and probably contributed long ago to the demise of their less fit, more primitive competitors. The historical slate has been wiped clean both geologically and biologically. Finally, because life forms replicate, singular events can have enormous impacts on future developments. Life does not have to be a probable outcome of spontaneous physicochemical processes, although it may well be. Arguments based on probability are not as powerful in this sphere as they usually are in the physical sciences.

The committee surveyed the inventory of environments in the solar system and asked which non-Earth ones might be suited to life of the terran type. Such locales are few, unless there are laws not now understood that could govern the early stages of the self-organization of biochemical structures and processes that could lead inevitably to evolving life forms.[15] Subsurface Mars and the putative sub-ice oceans of the Galilean satellites are the only locales in the solar system (other than Earth itself) that are clearly compatible with terran biochemistry.

The committee's survey made clear, however, that most locales in the solar system are at thermodynamic disequilibrium—an absolute requirement for chemical life. Furthermore, many locales that have thermodynamic disequilibrium also have solvents in liquid form and environments where the covalent bonds between carbon and other lighter elements are stable. Those are weaker requirements for life, but the three together would appear, perhaps simplistically, to be sufficient for life. The committee asked whether it could conceive of biochemistry adapted to those exotic environments, much as human-like biochemistry is adapted to terran environments. Few detailed hypotheses are available; the committee reviewed what is known, or might be speculated, and considered research directions that might expand or constrain understanding about the possibility of life in such exotic environments.

Finally, the committee considered more exotic solutions to problems that must be solved to create the emergent properties that we agree characterize life. It considered a hierarchy of "weirdness":

- Is the linear dimensionality of biological molecules essential? Or can a monomer collection or two-dimensional molecules support Darwinian evolution?
- Must a standard liquid of some kind serve as the matrix for life? Can a supercritical fluid serve as well? Can life exist in the gas phase? In solid bodies, including ice?
- Must the information content of a living system be held in a polymer? If so, must it be a standard biopolymer? Or can the information to support life be placed in a mineral form or in a matrix that is not molecularly related to Darwinian processes?
- Are Darwinian processes and their inherent struggle to the death essential for living systems? Can altruistic processes that do not require death and extinctions and their associated molecular structures support the development of complex life?

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2

A Sketch of the Chemistry Behind Known Carbon-based Life on Earth

To support an informed analysis of the possibilities for life in the cosmos, this chapter summarizes chemical concepts needed to evaluate the plausibility of a potential exotic life form and shows how they relate to the chemistry of the life that we know. This summary is necessarily selective. Students of chemistry and related areas develop their understanding of molecular behavior and chemical reactivity through years of study that cannot be condensed into a few pages. The committee has chosen topics for emphasis to permit a discussion of terran life to support its later discussion of exotic life.

2.1 MOLECULAR STRUCTURE AND PHYSICAL PROPERTIES

2.1.1 Pairs of Electrons Form Bonds Between Atoms

Covalent bonds between two atoms are formed when the two atoms share a pair of electrons. In the simplest of molecules, dihydrogen (often written as H—H, or H_2), a single line represents the pair of electrons that forms a single bond between the two hydrogen atoms. In water (H—O—H), the lines connecting each hydrogen atom to the oxygen atom each represent a pair of electrons that form a single bond holding the hydrogen atoms to the oxygen. In formaldehyde ($H_2C{=}O$), the double line between the carbon atom and the oxygen atom represents two pairs of electrons, four electrons in total, joining the carbon atom to the oxygen atom.

A complete structural model for a molecule also shows the positions of electrons *not* involved in covalent bonding. For example, in the Lewis structures of formaldehyde and water (Figure 2.1), the oxygen atom in each carries two pairs of unshared electrons from the outer valence shell. Each of these electrons, not involved in a covalent bond, is represented by a dot. The oxygen atom in water has four nonbonding electrons, and the oxygen atom in formaldehyde carries two pairs of unshared electrons, represented by four dots on the oxygen atoms of the two molecules in the Lewis structure.

2.1.2 Distribution of Charge Is Key to the Physical Properties of Molecules

An electron carries a negative charge; a proton in a nucleus carries a positive charge. In many molecular species, the number of electrons is not equal to the number of protons. In this case, the molecule carries a charge and is called an ion. In sodium chloride (table salt), the sodium ion carries a positive charge and the chloride ion carries a negative charge (Figure 2.2).

FIGURE 2.1 Lewis structures for formaldehyde (left) and water (right) show the presence of four electrons (each represented by a dot) on the oxygen atoms of the two molecules. These electrons are not involved in the formation of any bond. The pairs of electrons involved in holding the molecule together are represented by lines.

Molecules with equal numbers of protons and electrons are not charged. But the distribution of electrons in three dimensions need not be uniform in the molecule with respect to the distribution of the nuclei. In this case, the distribution of negative charge in a molecule may be different from the distribution of positive charge, and the molecule has one or more bond dipole moments, the magnitude of which depends on these distributions. The more uneven the distributions of charge in space, the more polar the molecule is.[a]

In general, atoms with similar electronegativities share electrons equally. Carbon and hydrogen, as atoms, have similar electronegativities. Hence, the carbon-hydrogen bond is not generally associated with a large bond dipole moment, and molecules that contain carbon-hydrogen units are not particularly polar. In contrast, bonds joining carbon and hydrogen atoms to heteroatoms (atoms other than carbon or hydrogen) are often quite polar. For example, the oxygen-hydrogen bond, a bond between atoms with very different electronegativities, is generally associated with a large dipole moment. As a consequence, molecules that contain —OH groups are generally polar. Molecules that contain many unshared pairs of electrons are also more polar.

The nature and distribution of its charge are the dominant features in determining a molecular structure's bulk physical properties. Knowing that a molecule is an ion is generally more important than almost any other piece of information for understanding the physical behavior of the molecule. If a molecule lacks a charge, then a statement about the magnitude of the dipole moment is most informative about its physical properties.

One of the more prominent features of polar molecules and ionic species is their ability to dissolve in polar solvents. Water, in turn, is one of the more polar solvents, because the distribution of electrons is quite different from the spatial arrangement of protons; the oxygen atom of H_2O carries more negative charge, while the hydrogen atoms carry more positive charge (Figure 2.3).

As a consequence, water dissolves many salts and many molecules that have large dipole moments. Water does not dissolve molecules that lack a charge or a substantial dipole moment. Nonpolar molecules that contain many carbon-hydrogen and carbon-carbon units are called hydrocarbons and are well known as oils and fats. Their nonpolarity is the general reason that oil (petroleum oil and vegetable oil alike) and water do not mix.

2.1.3 Distribution of Charge Can Be Inferred from Molecular Structures

The polarities of molecules can be estimated from their molecular structures. The electronegativities of the constituent atoms are the key to making those estimates. The structure of the organic molecule is scanned, polar bonds between atoms with different electronegativities are identified, and the number of those polar bonds present in the molecule relative to the nonpolar bonds between carbon and hydrogen, or between

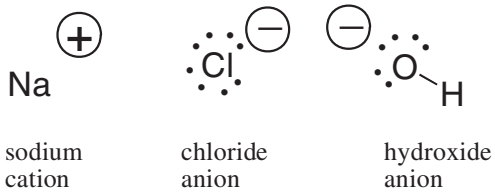
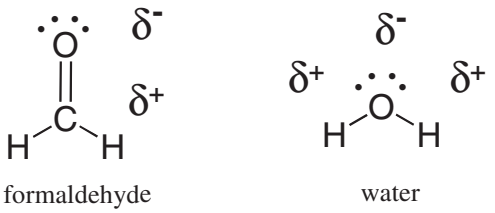


FIGURE 2.2 The sodium cation, the chloride anion, and the hydroxide anion, with their charges represented by the sign in a circle, have different numbers of protons and electrons.

[a]The bond dipole moment is a measure for the polarity of a chemical bond between atoms within a molecule. For a complete molecule the total molecular dipole moment may be approximated as the vector sum of individual bond dipole moments.



formaldehyde water

FIGURE 2.3 The oxygen atom is more electronegative than either carbon or hydrogen. Therefore, the bonds between oxygen and these atoms are polarized to place a fractional negative charge (indicated by δ−) on the oxygen atom and a fractional positive charge (indicated by δ+) on the carbon or hydrogen atoms.

carbon and another carbon, is determined. For example, it can be predicted that glucose, whose molecular structure (Figure 2.4) is easily seen to have many carbon-oxygen and oxygen-hydrogen polar bonds, is polar and easily dissolved in water, even though it is not an ion. Such molecules are called hydrophilic (water loving).

Conversely, the molecule octane, a component of gasoline, has only carbon and hydrogen atoms, 8 and 18, respectively, and thus only nonpolar carbon-carbon and carbon-hydrogen bonds. Octane is therefore predicted to be nonpolar and insoluble in water, but soluble in other oils. Such molecules are called hydrophobic (averse to water).

2.2 MOLECULAR REACTIVITY

Molecules that contain only carbon-carbon and carbon-hydrogen covalent bonds are relatively unreactive at standard temperatures.[b] Even terran life does not generally break unactivated carbon-carbon bonds directly and requires highly reactive species when it does so. The issue is slightly complicated because bonds can break in different ways. A bond strength is usually reported as a homolytic disassociation, in which one of the two electrons forming the bond remains on each of the atoms. Bond dissociation energies describe a bond's strength after the bond has been broken and no new bonds are being formed. Reactivity is a different concept not unrelated to bond strength but often dependent on environment, because atoms that are no longer bonded can form bonds elsewhere.

The key to chemical reactions, including terran biochemical reactions, at standard temperatures and pressures is the reactivity of carbon-carbon and carbon-hydrogen bonds in molecules that also contain carbon-heteroatom (any atom other than carbon or hydrogen) bonds. Bonds to heteroatoms are often said to activate carbon-carbon and carbon-hydrogen bonds. In terran metabolism, the most important heteroatoms are oxygen and nitrogen, although sulfur is also important, and other heteroatoms such as phosphorus occasionally play a role.

The influence of these heteroatoms on the reactivity of carbon-scaffolded molecules is briefly reviewed in Section 2.4.1. As one unifying principle, hydrogen-heteroatom bonds break and re-form dynamically in water. In contrast, carbon-heteroatom bonds are rarely broken at standard temperatures unless another bond is formed at the same time. In general, for a chemical reaction to proceed under standard conditions at a rate metabolically useful for terran organisms, new bonds must be formed as old bonds are broken, or soon thereafter.

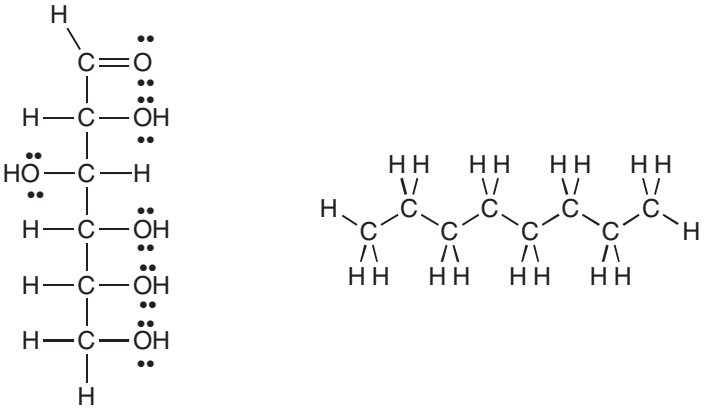


FIGURE 2.4 Structures of glucose (left) and octane (right). Given the large number of dipolar C-O bonds and the large number of nonbonding electrons (the dots in the structure), one expects glucose to be a hydrophilic molecule that dissolves well in water. In contrast, octane can be seen, by its structure, to contain only nondipolar C-C and C-H bonds, and no unshared electron pairs. Therefore, octane is expected to be a hydrophobic compound insoluble in water.

[b] Standard temperatures are temperatures where water is liquid at 1 atmosphere Earth pressure.

2.2.1 Reactive Centers in the Structure of a Molecule

The movement of pairs of electrons is key to understanding chemical reactions that involve the making and breaking of bonds (Box 2.1). Nucleophilic and electrophilic centers in a molecular structure are recognized by examining that structure. Nucleophilic centers (Box 2.2) that bear an electron pair prominently in a Lewis structure are easy to spot. Electrophilic centers are frequently less so, especially when the electrophilic center is a carbon atom. For example, the carbon of formaldehyde (H_2CO) has all four of its valences occupied and does not seem to have a valence available to form a new bond with anything.

If, however, one of the two bonds between carbon and oxygen breaks, with the electron pair moving from a position between the carbon and the oxygen to a new position on the oxygen, then the carbon center has a valence free. It then welcomes nucleophilic attack from an oxygen atom, such as from any nearby H_2O molecule. This process is shown in Figure 2.5.

The nucleophilic and electrophilic centers in a molecule are defined with respect to a specific reaction. Many molecules contain both nucleophilic and electrophilic centers; which centers react under any given circumstances depends on those circumstances and the availability of reaction partners. Only through experience are chemists able to predict, under any given circumstances, which nucleophilic centers in a system will react with which electrophilic centers.

BOX 2.1
The Movement of Pairs of Electrons in Reactions

Covalent bonds are formed by pairs of electrons. Therefore, the movement of electron pairs is key to understanding chemical reactions that involve the making and breaking of covalent bonds. Any pair of electrons not already involved in a bond is available (at least in principle) to form a new bond. And a pair of electrons originally occupied as part of a bond can form a new bond if the original bond is broken.

Organic chemists use curved arrows to describe reactions between nucleophilic and electrophilic centers that produce a new bond. The curved arrow begins with the pair of electrons that will form a new bond in the product. The arrow is drawn to end at a position (on the structures of the reactants) where the electron pair will be after the bond is formed.

Figure 2.1.1 shows the reaction of the unshared pair of electrons on the oxygen atom of water (in red) with H^+ (a proton) to give H_3O^+ (the hydronium ion). This simple bond-forming reaction represents the process by which protons move freely in water.

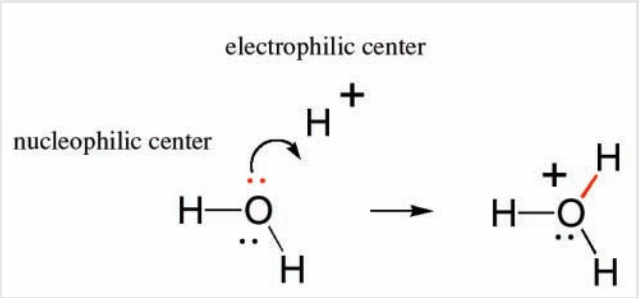


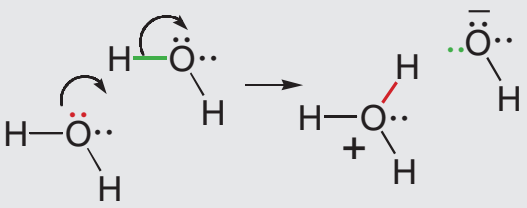
FIGURE 2.1.1 The formation of a new bond between the oxygen atom of a water molecule and a proton to give the hydronium ion. The red pair of electrons on the oxygen is originally unshared and is the pair of electrons used to form the new bond, which is also shown in red.

BOX 2.2
Nucleophilic Centers and the Formation of New Bonds

In a chemical reaction, the molecule that contributes the pair of electrons that forms a new bond is called the nucleophile. To form a bond, the electron pair on the nucleophile must find an atom that lacks a bond in its reaction partner, or will soon lack a bond through breakage. This partner is called the electrophile. Thus, in the reaction shown in Figure 2.5, the oxygen in the water molecule was the nucleophilic center, while the hydrogen in H^+ was the electrophilic center.

A more complex reaction with water that leads to the motion of protons involves the formation of a new bond between the oxygen atom of water, acting as the nucleophile, and a hydrogen atom of another water molecule acting as the electrophile (Figure 2.2.1). Here, the hydrogen that is acting as an electrophile is originally bonded; to form a new bond to the incoming nucleophile, it must lose the original bond.

FIGURE 2.2.1 Two curved arrows can be written to show the transfer of a proton from one water molecule to another to give the hydronium ion (H_3O^+) and the hydroxide ion (HO^-). The oxygen carrying the red electrons is the nucleophilic center. It brings the electron pair that is to form the new bond (red). The hydrogen attached to the oxygen in the second water molecule via the green bond is the electrophilic center.



2.2.2 The Reactivity of Water

As suggested by its structure and the discussion above, water is itself reactive, presenting both a nucleophilic oxygen atom and an acidic hydrogen atom. This has both advantages and disadvantages for a biosolvent. First, because of the availability of an acidic hydrogen, reactions in water never lack for this species. Thus, reactions that require the equivalent of an H^+ can always find one from water, so easily that many chemists ignore the movement of protons when they consider reactivity in biology. Protons can always move easily in water. They can always be gained, and they can always be lost.

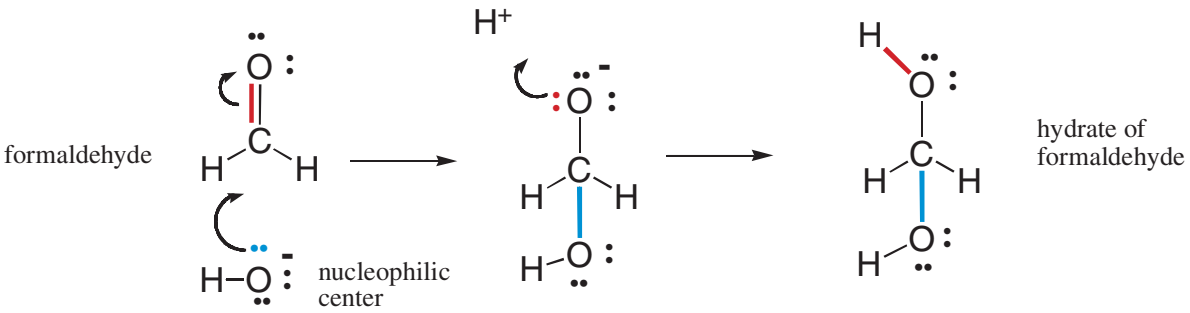


FIGURE 2.5 Reaction of the electrophilic carbon of formaldehyde with the nucleophilic oxygen of hydroxide gives the hydrate of formaldehyde. Note the concerted formation of a new bond (blue) using the unshared pair of electrons (also blue) on the oxygen nucleophilic center with the breaking of a bond (red). This bond breaking delivers an unshared pair of electrons (red) to the oxygen intermediate. These electrons can form a new bond (red) to an electrophilic proton.

The reactivity of water creates problems as well. In particular, many molecules are unstable in water. This generalization applies to many molecules important in terran metabolism, catalysis, and genetics. In some cases, molecules simply decompose through reaction in water, and require another round of metabolism for replacement. For genetic molecules, damage by water must be repaired.

2.3 MOLECULAR STABILITY

2.3.1 Chemical Bonds Have Different Strengths

The formalism outlined above that describes chemical bonding concerns covalent bonds. Table 2.1 lists the strengths of typical covalent bonds found frequently in terran biochemistry. The precise value of the energies associated with bond breaking depends on the context of the molecule.

To place the numbers in Table 2.1 in perspective, at one atmosphere of pressure at room temperature in a balloon filled with dihydrogen gas, the volume must be on the order of the size of the Milky Way Galaxy for there to be a 50 percent chance that it will contain one dissociated molecule. In human terms, the covalent bond between two hydrogen atoms is thus very strong. At reduced pressures, dihydrogen is more easily dissociated into two constituent atoms at equilibrium. Thus, the equilibrium $H_2 \rightleftharpoons H\cdot + H\cdot$ (where $\cdot$ represents a single electron, and $H\cdot$ represents a hydrogen atom) is equally distributed between species on each side of the equation at concentrations of 10^{-71} molar.

Molecules do not need to have formal covalent bonds to associate. For example, solids are associates of molecules held together by weaker bonds. Liquids, including water, also arise through weak association of molecules that do not involve intermolecular covalent bonding. Some interactions, such as between Na^+ and Cl^- in table salt, are strong until the compound comes into contact with a solvent such as water, which interacts strongly with the separated ions.

Chemists have a variety of names to describe these weaker bonds. Bonds between Na^+ and Cl^- in table salt are often referred to as ionic bonds. Weaker interactions between the positively and negatively charged ends of dipolar molecules are also possible. In particular, a hydrogen atom attached to a heteroatom (in terran biochemistry, the nitrogen-hydrogen and oxygen-hydrogen bonds are particularly important) generally carries a significant fraction of a positive charge. Therefore, the hydrogen interacts strongly with the negatively charged ends of dipolar molecules (such as the oxygen atom of water). This type of interaction is often called a hydrogen bond. Hydrogen bonds are typically 5 percent as strong as a typical covalent bond. Hydrogen bonds between the hydrogen-oxygen and oxygen units of different water molecules account for the ability of water to form a liquid at standard terran temperatures and pressures (Figure 2.6).

TABLE 2.1 Bond Energies of Typical Covalent Bonds Found Frequently in Terran Biochemistry

Covalent Bond	Energy (kilojoules/mol)
H—H	436
H—C	413
C—C	348
H—N	391
N—N	170
H—O	366
O—O	145
C═C	614
C—S	272
N≡N	945

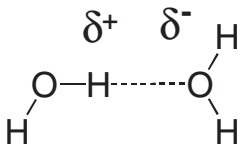


FIGURE 2.6 A hydrogen bond between two water molecules accounts for the liquid phase of water at standard temperatures and pressures.

Even the concept of covalent bonding has a degree of anthropocentricity. A covalent bond is, operationally, a bond that is largely stable at temperatures and pressures where humans live. At lower temperatures, a single hydrogen bond can hold together a molecular complex for as long a time as a covalent bond holds together a complex at room temperature.

2.3.2 Temperature Limits on Organic Molecular Stability

The strength of bonds between atoms is undoubtedly a universal feature, the same on Earth as elsewhere in the solar system, in other systems in the galaxy, and indeed in other galaxies throughout the universe. This implies a universal range for the temperature at which life based on carbon, hydrogen, oxygen, and nitrogen is possible.

Bond strengths make it difficult to imagine life based on carbon-backboned chemistry at temperatures above 600 K, which corresponds to 327°C, or 620°F—not dramatically higher than the temperature of a typical kitchen oven set at its highest setting. This expectation applies strictly to life at terran sea-level pressure, given that some decomposition reactions are significantly slower at higher pressure. But presumably it is possible to rule out carbon-based life at temperatures over 800 K at any pressure.

Carbohydrates are especially unstable, even at temperatures well below the boiling point of water. This instability arises from the fact that they contain a C═O unit. Because of this instability, some have proposed that carbohydrates could not have been part of genetic molecules in early life, as they are in modern life, at least until advanced metabolic repair, and sequestration became available to manage their reactivity. Indeed, until recently, no nonbiological process was well established that would yield carbohydrates under plausibly prebiotic conditions and in sufficient concentrations before the carbohydrates were then destroyed under the same conditions in which they were formed.[1] The simplest carbohydrate that has been observed in the interstellar medium is formaldehyde; the most complex, glycolaldehyde (Figure 2.7).

There is no similar low-temperature limit for carbon-based life. The temperature throughout most of the cosmos is much colder than standard temperature, and organic species are expected to be abundant. Indeed, microwave spectroscopy has established the presence of a large number of these in interstellar gases (Figure 2.8).

2.4 MOLECULAR REACTIVITY IN TERRAN LIFE: METABOLISM

Metabolism includes the set of all chemical reactions that occur within a living system. It is often formally limited to those reactions that are controlled by catalysts encoded by the genome of that system. These reactions result in the extraction of energy and raw materials from the environment. They also lead to synthesis of macro-

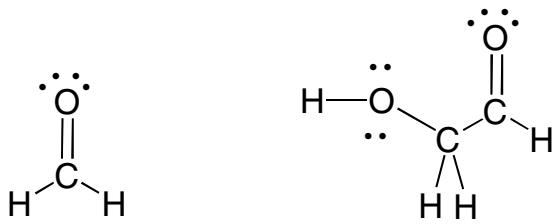


FIGURE 2.7 Formaldehyde and glycolaldehyde.

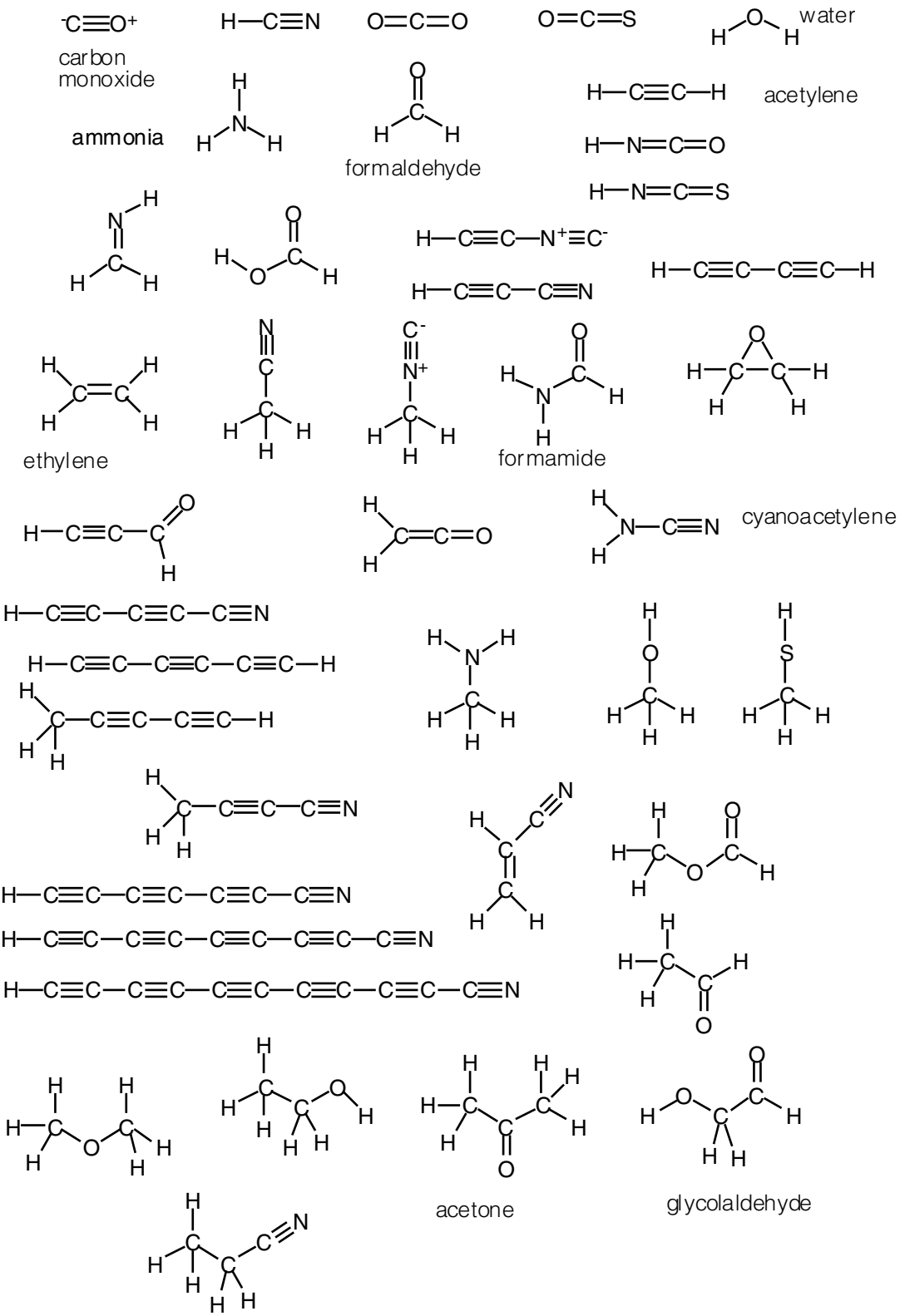


FIGURE 2.8 Some molecules observed by microwave spectroscopy in interstellar space.

molecules required for cellular function and to synthesis of compounds that allow an organism to manipulate its environment and interact effectively with other organisms in the environment.

2.4.1 Heteroatoms Confer Reactivity to Hydrocarbons to Enable Metabolism

The stability of carbon-carbon and carbon-hydrogen bonds at standard temperature and pressure means that stable organic compounds are easily available to forms of life in such environments. It also means that compounds built exclusively from carbon and hydrogen do not easily react and—in biological terms—that organic compounds containing only carbon and hydrogen atoms are not easily metabolized.

Heteroatoms create opportunities for reactivity by activating carbon-carbon and carbon-hydrogen bonds, in general by offering a place for electrons to go during a reaction sequence. Oxygen and nitrogen are frequently used in terran life for this purpose. Indeed, virtually every pathway in central metabolism exploits the electrophilicity of carbon doubly bonded to nitrogen or oxygen (C═N or C═O), or the electrophilicity of phosphorus doubly bonded to oxygen (the core of phosphate metabolism and terran bioenergetics).

Many reactions in terran metabolism are simple variants of a few basic reactions of carbon compounds containing a carbon atom doubly bonded to oxygen. For example, glycolaldehyde has a proton attached to a carbon next to its C═O carbonyl group. This proton can be removed by a base (calcium hydroxide is useful) in a reaction represented by curved arrows indicating delivery of electrons to the electronegative oxygen atom (Figure 2.9).

The curved arrows in Figure 2.10 show how electrons move in this process, which is commonly called enolization. In the figure, green electrons originally used to form the bond between the proton and the rest of the glycolaldehyde form a second bond between the two carbons of glycolaldehyde. The red pair of electrons, which originally formed the second bond between carbon and oxygen in the glycolaldehyde, goes to the oxygen atom, giving it a negative charge. To remove the proton, a pair of electrons (blue in Figure 2.10) from a hydroxide forms a new bond to the moving proton and generates a water molecule. This process is the enolization of glycolaldehyde.

The carbon that lost a proton is now a nucleophilic center and can therefore react with formaldehyde. As mentioned above, formaldehyde has an electrophilic center on carbon. Therefore, the carbon of formaldehyde can react with the nucleophilic carbon on the enolate of glycolaldehyde to form a new compound containing three carbon atoms, a three-carbon carbohydrate called glyceraldehyde. The overall reaction sequence is often called an aldol addition reaction, here of formaldehyde and glycolaldehyde.

Much of terran metabolism includes variants of these basic processes.

2.4.2 The Energetic Requirements for Metabolism

Metabolism cannot occur in a system that is at thermodynamic equilibrium. The synthesis of molecules and the construction of cellular structures require energy that an organism must obtain from the environment and that

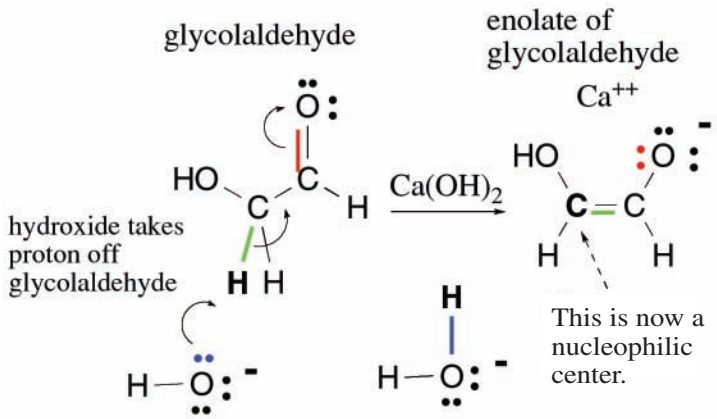


FIGURE 2.9 An archetypal set of reactions in terran metabolism, illustrating how the presence of bonds to heteroatoms (here, a double bond to oxygen) activates a C—H bond for breakage, a bond that would not break under standard conditions without that activation.

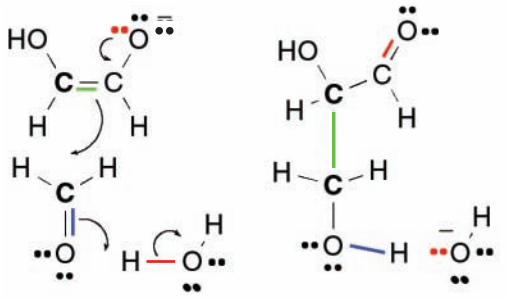


FIGURE 2.10 The enolate of glycolaldehyde, containing a new nucleophilic center, can react with an electrophilic center on formaldehyde to form a new C—C bond. This is another archetypal reaction in terran metabolism.

must be coupled to processes within the organism, rather than dissipated as heat or used to stimulate the formation of unreactive substances. One useful form of coupling leads to the formation of more-reactive compounds.

Except in the unusual case where the energetic compounds are used immediately, that energy must be stored in a chemical or physical form. Energy is stored in two general ways in terran organisms, either as a gradient of concentrations of species across a physical structure (e.g., a biological membrane) or in a molecule that has a high free energy of reaction under the conditions that prevail inside the organism. Adenosine triphosphate (ATP) is a well-known example of the latter.

Terran life uses photons from the Sun and thermal energy from the chemical disequilibria generated by Earth's internal heat sources as its primary sources of external energy. Photosynthesis is the primary process for obtaining energy from the Sun. Moving along the food chain, nonphotosynthetic systems must obtain their energy from high-energy compounds that they consume.

Often, this energy is transferred along the food chain via high-energy species. Whether obtained directly or from the environment, the energy in those species is used by their transformation to lower-energy species. These transformations often involve the movement of atoms and electrons to other organic or inorganic acceptors. The energy can also be transferred to a new molecule by metabolic coupling with a synthetic process that requires energy.

Certain patterns of metabolism have emerged on Earth. Multicellular organisms, without exception, use O_2 as a terminal electron acceptor. Plants obtain energy from light and carbon from CO_2, and animals obtain both energy and carbon from metabolizing ingested organic compounds. Microbes display a much greater diversity in metabolic strategies. Table 2.2, which shows examples of the range of electron donors and acceptors used by microbes, illustrates the range of possible strategies for harnessing energy available from redox couples in the environment.

2.4.3 Terran Life Has a Common Set of Reactions That Form a Core Metabolism

A century of study of metabolism in terran molecules has revealed a common core of metabolic processes through terran life. This commonality exists, in part, because of common features throughout terran genetics and biocatalysis. The core includes the tricarboxylic acid cycle, glycolysis, and synthetic pathways for construction of simple amino acids, sugars, purines, and pyrimidines.

This core was presumably required for primitive terran organisms to conduct their fundamental metabolism. A subset of the core processes furnished the building blocks of cellular macromolecules. Many organisms have shed pieces of the universal core as a consequence of adaptation to particular ecological niches in which certain metabolic abilities are dispensable.

Some variations on the universal core are seen. For example, there are five pathways for biosynthesis of the key amino acid lysine, three beginning with aspartate and two beginning with α-ketoglutarate. In both cases, the early steps in the pathway are identical, and variations occur in the final steps.

Nevertheless, the universality of this metabolic core that is still distinguishable 3.8 billion years after the emergence of life on Earth is consistent with the ribosomal RNA evidence that all known forms of life on Earth have descended from a common progenitor.

TABLE 2.2 Examples of Electron Donors and Acceptors Used by Microbes

Electron Donor	Electron Acceptor	Example Organisms
Phosphite (HPO_3^{-2})	Sulfate (SO_4^{-2})	*Desulfotignum phosphitoxidans*
Hydrogen	O_2	*Ralstonia eutrophus*
Hydrogen	Fe^{+3}	*Acidothiobacillus ferrooxidans*
Sulfide	O_2	*Sulfolobus acidocaldarius*
Sulfide	Nitrate	*Thiobacillus denitrificans*
Sulfur	O_2	*Acidianus ambivalens*
Ammonium (NH_4^+)	O_2	*Nitrosomonas* sp.
Nitrite	O_2	*Nitrobacter* sp.
Fe^{+2}	O_2, nitrate (NO_3^-)	*Acidothiobacillus ferrooxidans*
Organic compounds	Fe^{+3}	*Acidiphilium cryptum*
Organic compounds	Nitrate (NO_3^-)	*Paracoccus denitrificans*
Organic compounds	Arsenate	*Desulfosporosinus* sp. strain Y5
Organic compounds	Mn^{+4}	*Shewanella putrefaciens*
H_2, organic compounds	Sulfate	*Desulfovibrio* sp.
H_2, formate, CO, alcohols	CO_2	Methanogens

2.5 CATALYSIS

Even when organic molecules are activated by heteroatoms, chemical reactions that form and break carbon-carbon bonds are slow at standard temperature and pressure. Thus there is a need for catalysis, a process involving the addition of an exogenous substance (the catalyst) that speeds the rate of chemical reaction, but survives the reaction unchanged.

Catalysts are central to life. First, the catalysts can be derived from inherited information. This permits genetics and, ultimately, Darwinian processes, to determine what reactions occur within the confines of a living system. Conversely, reactions that are sufficiently fast that they do not require catalysis are not under the control of the living system. Therefore, they cannot be part of a Darwinian process that leads to improved adaptation of the organism.

Many of the chemical reactions that are at the core of terran metabolism are extremely slow in the absence of catalysis (see Table 2.3). Simple monomers can generate rate enhancements of 1,000-fold.[2] Highly evolved protein catalysts accelerate reactions by 7 to 19 orders of magnitude.[3]

In addition to simply accelerating chemical reactions, catalysts play an important role in channeling molecules toward formation of particular products. Typical abiotic reactions yield a mix of products. Reactions under the control of biological catalysts generally yield a single product, because the catalysts accelerate the rate of a particular reaction far beyond the rates of competing, uncatalyzed reactions. Indeed, catalysts can foster reactions that simply would not occur in the absence of catalysis because of competing reactions. In terran biocatalysts, this selectivity is achieved by sequestration of reactive intermediates at an active site in which solvent has been excluded, and by orientation of reacting centers to promote interactions between particular atoms.

Polymeric biological catalysts are called enzymes. Terran enzymes are, in most cases, protein molecules. Typical bacteria have on the order of 2,000 protein enzymes. Proteins are excellent catalysts because they fold to give stable yet dynamic conformations, and deliver functionality from the side chains of the 20 amino acids into regions of space where they are most useful for catalysis. The 20 amino acids that make up proteins provide structural diversity that can be used to develop binding pockets that are highly specific and bind substrates with high affinity. Further, many of these amino acids provide functional groups that participate in catalysis (see Figure 2.11). Many enzymes also contain cofactors that expand the range of chemical reactions that can be catalyzed. These include metal ions and metal ion clusters, as well as a range of organic cofactors.

TABLE 2.3 Bond Energies of Typical Covalent Bonds Found Frequently in Terran Biochemistry

Reaction	Half-life for Spontaneous Reaction in Water at 25°C
Hydration of fumarate	7×10^5 years
Isomerization of triose phosphate	2 days
Decarboxylation of orotidine 5'-monophosphate	8×10^7 years
Peptide bond hydrolysis	450 years
Phosphodiester bond hydrolysis	$>13 \times 10^6$ years

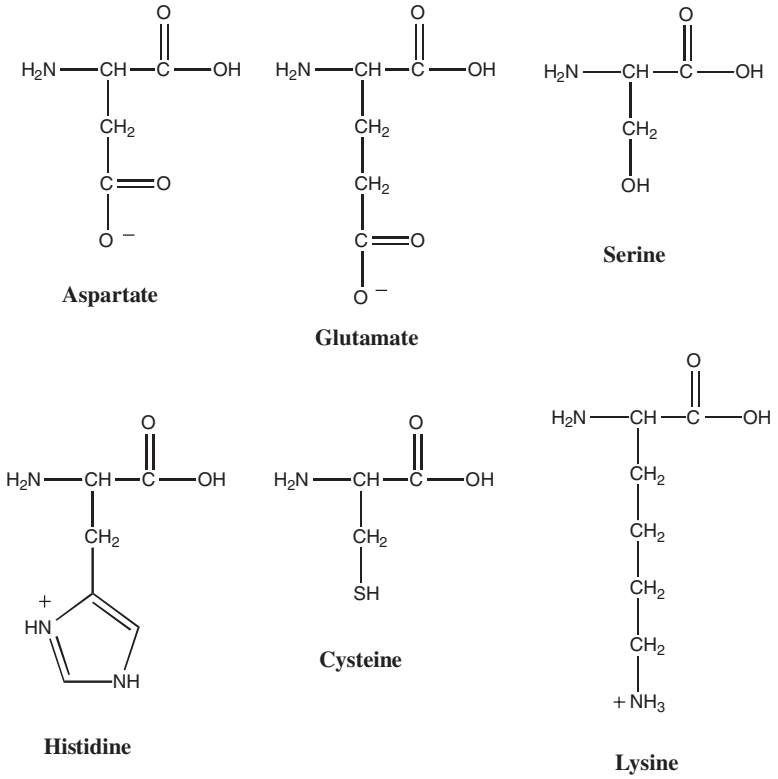


FIGURE 2.11 Amino acids whose side chains are most important in catalysis at enzyme active sites.

2.6 MACROMOLECULAR STRUCTURE IN TERRAN LIFE

Many functions critical for terran organisms are provided by macromolecules. Biological catalysis is performed largely by proteins. Hereditary information is generally stored in DNA. Compartmentalization is usually achieved using lipid bilayer membranes. Structural integrity is provided by polysaccharides and/or proteins. Movement on the nanometer to macroscopic scale is provided by special proteins that undergo specific and directional conformational changes.

Although specific macromolecules have evolved to provide these functions in life on Earth, it is possible that some or all of these functions might be provided by monomers or by different macromolecules in other forms of life. However, all things are not possible, as the physicochemical characteristics of macromolecules conferred by their molecular structures inherently limit their functional properties. Consideration of alternative macromolecules to serve these functions must be realistic in terms of structure-function relationships of particular macromolecules.

For example, polysaccharides are not likely to provide motor molecules, and proteins are not well suited to carry genetic information. Particular features of nucleic acids and proteins make them well suited for the functions they serve in life on Earth. An understanding of these features provides an intellectual framework for thinking about alternative macromolecules in other forms of life.

DNA is well suited to serve as the repository of hereditary information for many reasons. First, it is double-stranded, and the double strands are complementary. This permits a simple mechanism for replication, as noted by Watson and Crick. Further, the resulting redundancy provides correct sequence information in case of damage to one strand of DNA. 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.[4,5] Finally, the interaction between the two complementary strands that is mediated by hydrogen-bonding interactions between the Watson-Crick-model 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.

The connection between DNA and proteins involves the use of RNA as an intermediate message, as well as a triplet genetic code. Codes that utilize a different number of bases or different sizes of codons have been considered and are conceivable. However, if we assume that approximately 20 amino acids are required to create good protein structures, and the encoding molecule (on Earth, DNA) is built from four alphabetic letters, then most other coding strategies have either too little coding capacity or far too much. Only a triplet code using four nucleobases and a doublet code using six nucleobases have coding capacities in a range that might encode 20 amino acids. Of course, larger genetic alphabets, including those with six or more nucleobases, would allow other possibilities.

The life that we know also 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. Further, the backbone has a repeating dipole able to make hydrogen bonds. These structural features are exploited as proteins fold into globular structures, as they promote the formation of stable secondary structures such as alpha helices and beta sheets.

The planarity of the amide linkers in the protein backbone restricts rotation around the carbon-nitrogen bond. This provides some restrictions on the number of conformers that can be adopted. The linkage joining amino acids in a polymer is quite stable, but not infinitely so, and it can be relatively easily hydrolyzed by enzymes to allow 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 carbon-carbon bonds lack one or more these properties.

Life as we know it builds its proteins primarily from the same 20 amino acids, although there are many other amino acids that might 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.

2.7 SUPRAMOLECULAR STRUCTURE IN TERRAN LIFE

Supramolecular structures include aggregates of biomolecules held together by noncovalent bonds. These enable many functions that are key to terran life and are presumed to be key to life generally.

2.7.1 Compartmentalization Arises from Supramolecular Structures

Compartmentalization is one of these key functions. Compartmentalization is global in terran life forms, which are built from cells that span a wide range of sizes. Cellular structure is so widespread on Earth that a central theory in biology is known as cell theory. An unexpected form of life that would be considered "weird" would be a life form that does not exploit cells but achieves a distinction between "self" and "non-self" that is not "cellular" as we define the term.

2.7.1.1 Advantages of Compartmentalization

Compartmentalization allows the concentration of molecules so that the rates of second-order chemical reactions can be increased over what could be achieved in, for example, a bulk ocean. Modern cells concentrate molecules in the cytoplasm to an amazing level; the concentrations of many metabolites are in the high micromolar or even millimolar range.

Compartmentalization also allows protection of sensitive molecules, both small metabolites and the macromolecules that serve structural, catalytic, and genetic functions, from harsh external conditions. This is especially evident for terran organisms that live in environments with a high or a low pH. Organisms that live in such environments maintain their internal environment at nearly neutral pH. The compartment walls allow them to do so.

Further, compartmentalization allows cells to control the import of nutrients and the export of products that manipulate the environment, or excrete waste. These include siderophores that enhance uptake of metal ions, quorum-sensing molecules that inform cells of the presence of others of their kind, molecules that form biofilms that allow attachment to surfaces and protection from mechanical and chemical stress, and toxins that inhibit the growth of competitors.

Important properties of a boundary between inside and outside can be identified in terran cells. Most importantly, the permeability of the boundary must be low enough to prevent loss of valuable metabolites, but high enough to allow the import of nutrients and the export of waste. In terran extant life, the permeability of cell boundaries is low, and transport processes are carried out by proteins embedded in the boundary. The boundary must be fluid rather than solid, to facilitate both transport and incorporation of new components during enlargement prior to cell division. Furthermore, a fluid boundary allows cells to engulf objects in their environments. This ability is important for predation in terran life.

2.7.1.2 Compartmentalization Exploits the Low Polarity of C—C and C—H Bonds

All known life forms on Earth are surrounded by bilayer membranes that exploit the unique behavior of molecular structures that have, in one part, hydrophobic units built primarily from nonpolar carbon-carbon and carbon-hydrogen bonds and, in another part, polar bonds involving heteroatoms. The first part of the molecule is insoluble in water and therefore aggregates. The second part is soluble in water and presents itself to bulk water solvent, sequestering the hydrophobic parts of the molecule to the interior.

The detailed structure of membranes is not uniform in terran life (Figure 2.12). Membrane bilayers in bacteria arise from supramolecular organization of phospholipids, with hydrophobic fatty acids attached to a variety of polar head groups by ester linkages. Eukaryotic membranes also contain phospholipids, but they are distinctive in the inclusion of sterols (such as cholesterol), another class of hydrophobic molecules.

Archaeal membranes, in contrast, contain lipids in which the hydrophobic tails are linked to the head groups by ether linkages, rather than ester linkages. Since ether linkages are more stable to heat and extremes of pH, this feature is presumed to be important to the ability of Archaea to survive in extreme environments.

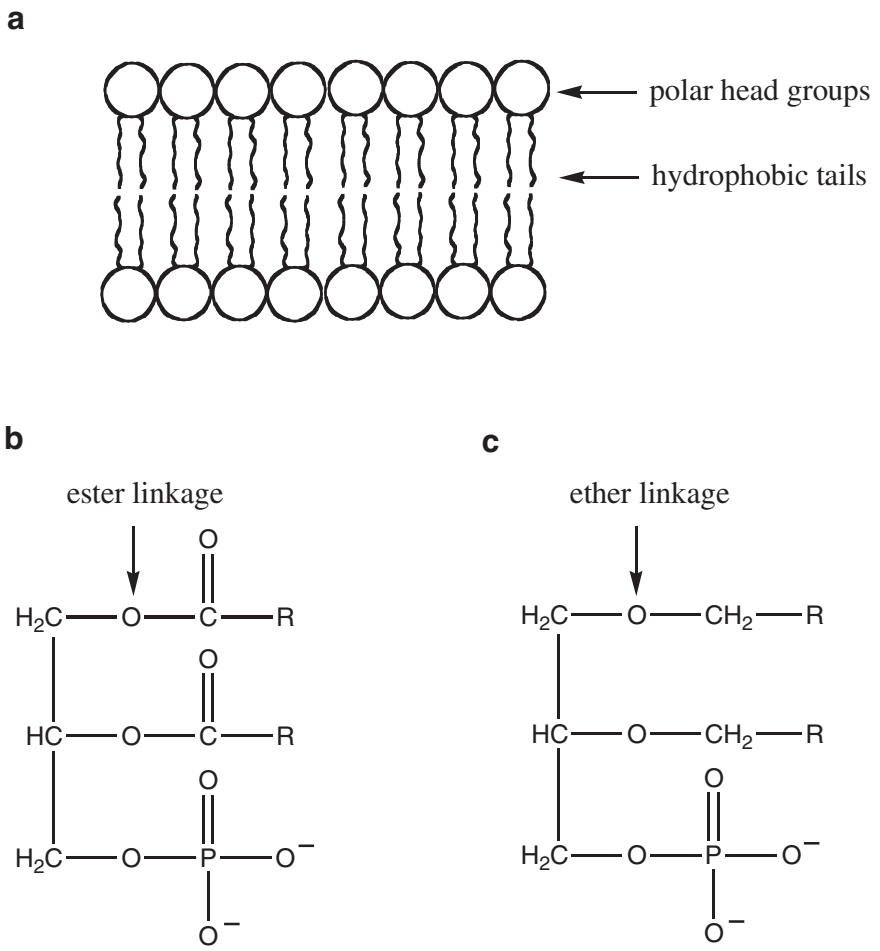


FIGURE 2.12 (a) The structure of a bilayer lipid membrane; (b) the structure of the glyceryl esters that are major components of bacterial and eukaryotic cell membranes; and (c) the structure of the glyceryl ethers that are major components of archaean cell membranes. R is a hydrophilic chain of CH and CH_2 atoms.

Further diversity in terran membrane architecture is found in the presence of a second structure enclosing the cell. Some microbes have only a single membrane. However, some bacteria (referred to as gram-negative bacteria because of their staining properties) have both an inner and an outer membrane, between which is a layer of polymeric material (peptidoglycan) that provides structural rigidity. In contrast, eukaryotic cells contain a number of compartments, each of which is bounded by a membrane. These compartments include the nucleus, the mitochondria, the chloroplast, lysozomes, the Golgi apparatus, and the endoplasmic reticulum. Thus, eukaryotes use membranes to organize the structure and activities of larger and more complex cells. Some of the membranes are the vestiges of endosymbiosis.

2.7.1.3 Compartmentalization Assists in the Generation of High-energy Compounds

The membrane barrier surrounding cells is a key component in energy metabolism, because processes that harvest energy from either light or oxidative reactions result in storage of energy as an electrochemical gradient across the membrane. This gradient is subsequently used to drive energy-requiring processes such as synthesis of ATP or accumulation of nutrients against a concentration gradient. ATP is the fundamental unit of energy currency used by cells; its hydrolysis can be coupled to a large number of endergonic processes to result in an energetically favorable process.

Some, but not all, of the benefits conferred by compartmentalization inside lipid bilayer membranes can be achieved by surfaces. Surfaces allow concentration of metabolites, and, if the surface is a mineral that is not in redox equilibrium with its surroundings, a surface can provide a source of energy. Compartmentalization can also be achieved inside porous minerals. For example, the walls of hydrothermal vents are porous and trap organic material[6] and may indeed have provided the first compartmentalization that allowed the emergence of metabolism and macromolecules in a protected environment.[7] Also, there are tiny pores in rocks, including tubes in chroysotile, and there are microcracks in quartz, both of which could support diffusion and dispersion by currents.

2.7.2 Supramolecular Soluble Structures

Supramolecular structures are found throughout biology. These are assemblages of covalently bound molecules that form functional aggregates using noncovalent bonding.

One particularly important example is also an exception to the rule that catalysis in terran life is carried out by protein enzymes. This is the ribosome, the molecular machine that synthesizes proteins using the information provided by a messenger RNA. It is an ancient machine and a key component of every known form of life on Earth. The ribosome is a large complex of at least three RNA molecules and more than 50 proteins. The structure of the large subunit of the ribosome was solved in 2000,[8] followed closely by the structure of the entire ribosome.[9] Consistent with the "RNA world" model, RNA was present at the site in the ribosome where the peptide bond is synthesized.[10] The active site is composed entirely of RNA. Nearly all catalytic functions in living organisms have been taken over by proteins, which are inherently more suited for catalysis than RNAs. However, at the heart of this centrally important and ancient molecular machine, catalysis is still being executed by the RNA.

2.8 THE RELATIONSHIP BETWEEN WATER AND BIOMOLECULES

2.8.1 Adaptation of Terran Biomolecules to Water

Terran life uses water as a solvent. As expected, terran biomolecules have multiple signatures of their compatibility with water as a solvent. Further, terran biochemistry exploits the distinction between polar molecules, which are soluble in water, and nonpolar molecules, which are not. This is exemplified in the use of hydrophobic interactions as a way to fold proteins and organize supramolecular structures, inter alia.

Water also constrains the structure of carbon-containing biomolecules involved in metabolism. At the very least, for life to exploit the dynamic behavior of species dissolved in a solvent, the biomolecules serving as metabolic intermediates must be soluble in that solvent at appropriate concentrations. This is the simplest explanation for the prevalence of hydroxyl groups in organic molecules central to biological metabolism. Glucose has five and dissolves well in water.

Charge also helps a molecule to dissolve in water. Accordingly, many intermediates in terran metabolism are charged. Most typically, an uncharged molecule acquires a charge through phosphorylation. Other metabolic intermediates are intrinsically charged. For example, the citric acid cycle is based on tri- and dicarboxylic acid intermediates. These are ionized at physiological pH, making them very soluble in water. Terran metabolism also exploits the metabolic reactivity of the C═O carbonyl group, which supports chemical reactions in water well.

The compatibility of a biomolecule with water is seen especially well in genetics. The DNA and RNA molecules that transfer genetic information have a repeating charge in their backbone, carried by the phosphate linkers. This repeating negative charge makes DNA and RNA extremely soluble in water. It also prevents their passing across hydrophobic membranes. Further, the nucleobases that encode the genetic information are, by comparison, hydrophobic. Therefore, they lie inside the double helix, away from water, in base pairs that are well known features of the Watson-Crick model for duplex nucleic acids.

2.8.2 Disadvantages of Water for Terran Biomolecules

Water, however, carries both nucleophilic and electrophilic centers. This means that water reacts with many biomolecules in a way that damages them. In the case of proteins, as noted above, water reacts with the amide backbone to degrade proteins, generating amino acids as hydrolysis products (see Figure 2.13). This can be disadvantageous if the protein is desired, as it requires that the protein be re-synthesized. The turnover of proteins is important, however, in any system living in a dynamic environment. Thus, the hydrolytic instability of proteins in water is key to maintaining life.

The disadvantageous reactivity of water is especially obvious when considering RNA and DNA (see Figure 2.14). Cytidine, for example, hydrolytically deaminates to give uridine with a half-life of ca. 70 years in water at 300 K.[11] Adenosine and guanosine also hydrolytically deaminate in water at only slightly slower rates. A more recent study at the base level affords the following half-lives for the bases at 298 K: cytosine, 340 years; adenine and guanine, about 10,000 years.[12] As a consequence, terran DNA in water is continuously damaged in a way that causes it to lose its genetic information. In modern terran life, this continuous water-generated damage is mitigated through continuous repair.

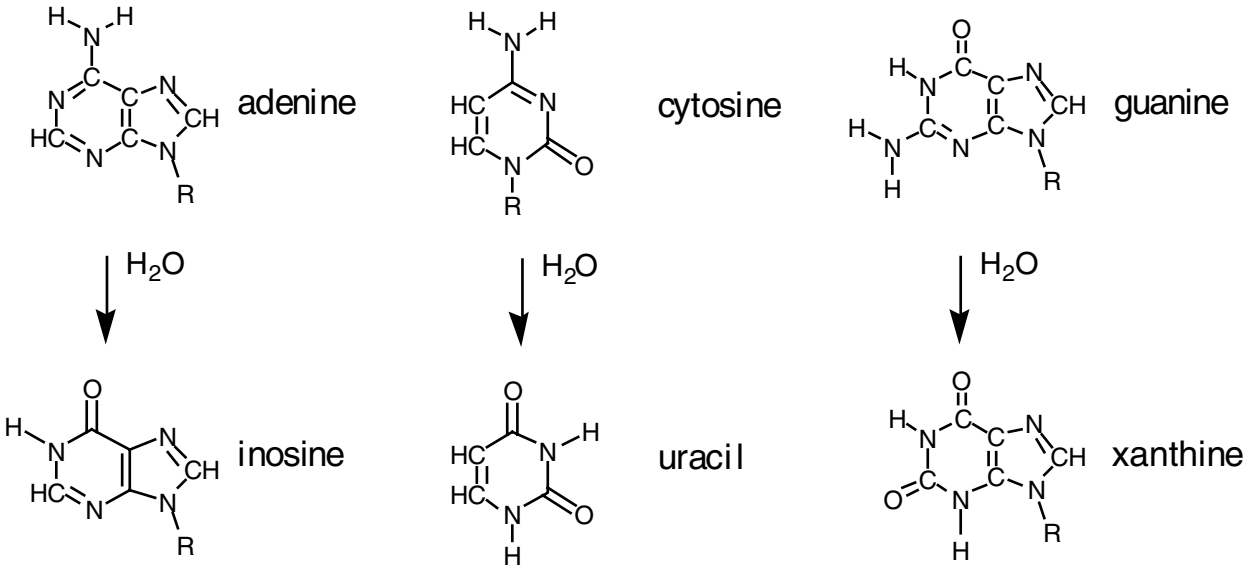


FIGURE 2.13 Hydrolytic degradation of the standard nucleobases.

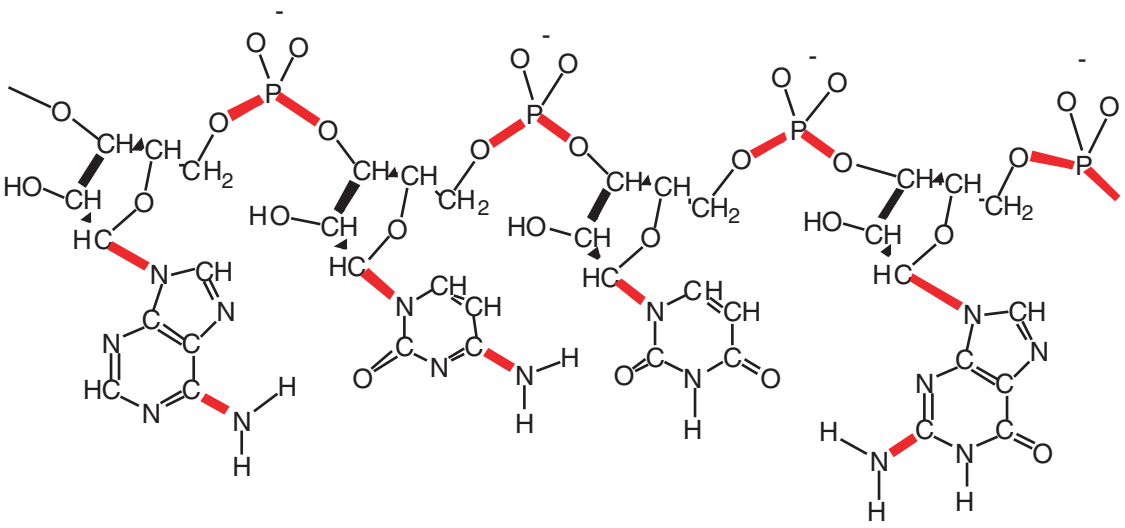


FIGURE 2.14 A generic RNA molecule. The bonds shown in red are thermodynamically unstable with respect to hydrolysis in water. Each of these bonds represents a problem for prebiotic synthesis, as well as the maintenance of the genetic information in RNA in modern life. Today, the aggressive reactivity of water with respect to molecules like RNA and DNA is mitigated by sophisticated repair systems. It is difficult to imagine such complex repair systems having been present at the dawn of life. This conundrum underlies the most significant paradox in the structure of genetic matter with respect to the origin of life. On the one hand, the repeating charge in the backbone suggests that the molecule worked in a hydrophilic solvent such as water. On the other hand, the abundance of easily hydrolyzable bonds suggests that RNA could not have been easily assembled in water.

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3

Pushing the Boundaries of Life

Following its strategic plan, the committee examined the wealth of information concerning the life that we know on Earth, especially in what (from a human perspective) are extreme environments.

3.1 THE LIMITS OF EARTH LIFE

The search for extraterrestrial life is intimately linked with current understanding of Earth life. That is not to say that only Earth-like life could exist on other planets and moons, but it is important to know the limits of environmental conditions that can support the growth of Earth life as a first-order set of criteria for the identification of potential extraterrestrial habitats. The limits of life on Earth may help to define the limits of life elsewhere even though different life forms may have different biochemistries and different origins. In addition, Earth life is possible outside the bounds of extreme conditions found on Earth (for example, the bacterium *Deinococcus radiodurans* can tolerate levels of radiation not found naturally on present-day Earth, and *Escherichia coli* apparently can tolerate hydrostatic pressures greater than 10 times the pressures in the deepest ocean trenches[1,2]). Because we know about only one kind of life, it is logical to look first for life elsewhere that resembles Earth life. Life as we know it requires liquid water, either light or a chemical energy source, and nutrients, including nitrogen, phosphorus, sulfur, iron, and many elements in trace concentration; 70 elements are either required by or interacted with by Earth life.[3] Thus, the current search for extraterrestrial life is focused on planets and moons that have or have had liquid water, that have a history of geological and geophysical properties that favor the synthesis of organic compounds and their polymerization, and that provide the energy sources and nutrients needed to sustain life.

Once life appears on a planet, regardless of the circumstances under which it appeared, evolution will push toward occupying every conceivable niche, even when the environments in some niches are very different from that in which life first arose.

In using Earth life as a point of comparison, we are limited by what we know about it. Two issues are being addressed by the astrobiology community: our incomplete understanding of the physiological diversity of Earth life, and the near absence of data on possible alternative forms of carbon-based biochemistries. First, it is clear that little or nothing is known about the physiological diversity of most microorganisms in most Earth environments. For example, examination of the physiological characteristics of some of the organisms in the "unknown majority" has provided new insights into their adaptations to "extreme" environments, those at the fringes of habitability as we know it. A novel marine photosynthetic microorganism that was isolated from deep-sea hydrothermal vents

might be using the black-body radiation from hot sulfides for photosynthesis.[4] Newly isolated microorganisms have extended the upper temperature for growth to 121°C and extended the pH limit to below 0. One hyperthermophilic microorganism lacks consensus sequences (nucleotide base sequences in the 16S rRNA gene that are universal in known organisms) in its 16S rRNA and, incidentally, is parasitic on another archaean species.[5]

To address the second issue, researchers begin by assuming carbon-based life. The key arguments for carbon-based life are the ubiquity of organic (carbon-containing) compounds in the universe and the ability of carbon to form stable compounds with many elements, thus creating the wide variety of structural, catalytic, and informational macromolecules (very large molecules, such as DNA and proteins) that make up Earth life. But how versatile and adequate is the carbon-based life model for environmental conditions that either have not been adequately explored on Earth or extend beyond the bounds found on Earth? Are there alternative carbon-based biochemistries that would allow organisms to exist under more extreme conditions than Earth life can? Embedded in that question are two others: What are the limits of evolutionary innovations in carbon-based life? How do environmental characteristics or extrinsic factors, such as hydrostatic pressure and solute concentrations, affect the limits?

Powerful new molecular methods allow the construction of novel enzymes and, eventually, novel organisms. A viral genome has already been constructed from synthetic oligonucleotides.[6] Those "directed evolution" methods have the potential to explore whether it is possible to construct life with novel physiologies, including new metabolic pathways that exploit energy sources not used by extant Earth organisms—such energy sources as ultraviolet radiation, gravity, and thermal gradients. Furthermore, exploration of deep subsurface environments has uncovered microbial communities potentially decoupled from photosynthetic reactions that occur at Earth's surface. Those communities use magmatic degassing, radiolysis of water, or serpentinization reactions (involving the aqueous alteration of mantle material) to drive their metabolism. The ecosystems persist in environments that have remained essentially unchanged for millions or billions of years and that overlap with conditions favorable for abiotic organic synthesis. Do organisms in those ecosystems contain relict biochemistries that have eluded evolutionary pressures?

Thus, two questions are associated with understanding the limits of carbon-based life: What are the limits of extant Earth life? What are the limits of carbon-based life? With respect to the second question, one of the implications of using Earth-based life as a point of comparison is the need to understand the full range of habitat conditions that can support carbon-based life, including conditions not found on Earth. The tendency is to look for Earth organisms that might be best suited to live under the extreme conditions found on other planets, but that assumes that an extraterrestrial organism would have the same physiological characteristics of an Earth organism if the environmental conditions were the same in the two places. In thinking that way, we assume that life originated in the other place and then evolved physiologies to take advantage of the available habitat conditions. But any planet or moon that has or has had environmental conditions that could support an Earth-like organism might never have had the conditions necessary for a separate origin of life and could be sterile. That may be particularly true for icy planets with liquid water even if they have the cache of chemicals necessary to support life. Could life originate de novo, or would it have to be seeded from a neighboring planet or moon that during its early history had more suitable conditions for spawning life? How many types of environments can lead to the origin of life?

In the case of Earth, various models provide clues to geophysical conditions that may have favored early organic and biochemical stages leading to life. Some models rely on subsurface hydrothermal settings because they can provide all the chemical precursors and catalysts essential to generating complex carbon chemistry. Other models exploit alternative settings, such as meteoritic input, reduced atmospheres, or freshwater ponds. The origin of life is likely to involve multiple environmental conditions that span spatial and temporal dimensions. However, we cannot answer the general question of whether there could be multiple settings for creating different carbon-based life forms. Would a separate origin of life under conditions different from the ones that produced life on Earth create a carbon-based life form capable of different evolutionary innovations, or do rules of organic chemistry limit carbon-based life to the physiological diversity represented by extant Earth life?

Earth may be just one of many models of planets that can evolve complex life. We do not know whether it is even practical or logical to assume that planets that exist outside our perception of a habitable zone could harbor life, particularly life that we know nothing about. Our practical search for extraterrestrial life is focused on water-rich planets and moons because of the possibility that they can support Earth-like life. That does not preclude

other strategies for carbon-based life to thrive in nonaqueous solvents, such as exist on Titan or in the hot sulfuric acid atmosphere of Venus. Even more radical is the possibility of seriously "weird life" on extremely hot, cold, or gaseous giant planets.[7,8] We know about only one kind of life, and it is carbon-based, requires liquid water, can evolve, and, given "optimal" environmental conditions, can evolve into complex organisms.

3.2 EXTREMOPHILES AND THE LIMITS OF LIFE

Extreme conditions that limit growth or prove lethal to most organisms can be ideal habitats for others. Extremes of high temperature, high and low pH, high salt concentration, concentrations of toxic metals and organic compounds, and high levels of radiation kill the overwhelming majority of Earth's organisms. However, organisms in all three domains of life have adapted to many terrestrial extremes. High-temperature, low-pH, and high-salinity environments are probably ancient, as may be frozen environments. Those extreme environments are not rare: most of the ocean is cold and deep, and a vast portion of the subsurface is hot.

There are few natural environments on Earth where life is absent—life is the rule rather than the exception. Microbial life on Earth has proliferated in habitats that span nearly every imaginable physicochemical variable. Until recently, only the very highest temperatures or lowest water activities (desiccation) were thought to render terrestrial environments unsuitable for growth. Now there is evidence that environments that have $MgCl_2$ at concentrations greater than 2.3 M, such as a high-brine lake on the Mediterranean Sea floor, may inhibit life and that this inhibition is due to the ability of $MgCl_2$ to denature biological macromolecules.[9] However, such conditions do not necessarily render the environments sterile; many organisms have adapted mechanisms for long-term survival at temperatures more than 100°C above their maximal growth temperature or in a desiccated state. Few of the supposedly sterile environments are actually free of surviving life. Viable microorganisms have been detected, albeit in low numbers, in Chile's Atacama Desert, perhaps the driest environment on Earth and thought to be an analogue of sterile Martian soil.[10] In contrast, although they are rare, some environments with liquid water do not appear to support life; they include water over 400°C at submarine hydrothermal vents that is kept liquid by hydrostatic pressure[11] and the high-brine liquid water found in sea-ice inclusions at −30°C. Even in those extreme cases, there is evidence of viable microorganisms that apparently survive exposure to temperature extremes well outside their growth range.[12]

Several recent review articles discuss the limits of life, the characteristics of extremophiles, and implications for astrobiology.[13-15] Most discussions of the limits of life focus on extremes of single physical or chemical conditions, such as temperature, salinity, heavy-metal concentrations, desiccation, and pH. There are also many excellent reviews of single classes of extremophiles that should be consulted for detailed information on their ecology, physiology, and biochemistry.[16-20]

Individual organisms are often highlighted for their ability to lead the pack in tolerance of or ability to grow under extreme conditions (Table 3.1). In some cases—such as high pH, high hydrostatic pressure, and high metal content—the stated limits for life merely reflect the limits found in natural environments. There appears to be no absolute maximal temperature or minimal concentration of water that will prevent cellular growth. Two distinct classes of extreme environmental conditions are based on how they affect cells. In one, the effects of extremes in pressure and temperature extend into the cytoplasm, and intracellular biosynthesis, metabolism, and macromolecular structures are adapted to function under such conditions. In the other, organisms capable of growing in extremes of pH, salinity, and irradiation and in the presence of high concentrations of organic solvents and toxic metals are adapted either to maintain intracellular conditions that are typical for nonextremophiles or compensate for the extreme conditions. There are some exceptions. A recently described acidophilic archaean, *Picrophilus torridus,* grows optimally at a pH of 0.7 and apparently maintains an intracellular pH of 4.6.[21] Most acidophiles maintain an internal pH near neutrality, and *P. torridus* must have novel intrinsic factors for stabilizing proteins and nucleic acids at low pH. The extremely halophilic archaeans have an absolute requirement for salt and grow best at salt concentrations of 3.5-4.5 M but can also grow in saturated NaCl (5.2 M). The intracellular functional and structural components of haloarchaeans are adapted to high salt concentration (up to 5 M, mainly KCl), and their enzymes require high salt to maintain their active structure. Other moderately halophilic bacteria grow over

TABLE 3.1 Examples of Bacteria, Archaeans, and Eukaryotes That Grow in Extreme Environmental Conditions on Earth

	Extreme Value for Growth or Tolerance		
Condition	Bacteria and Archaeans	Eukaryotes	Examples of Environments
High temperature	121°C, Strain 121; 113°C, *Pyrolobus fumarii*	~60°C algae, e.g., *Cyanidium caldarium* and some fungal species	Submarine hydrothermal systems; geothermal hot springs (e.g., Yellowstone)
Low temperature	About −15°C, bacterial growth detected; pure cultures, e.g., grow at less than −12°C	Algae and protists in snow and ice; fish and invertebrates in −2°C Arctic water; −18°C, Himalayan midge	Brine pockets in sea ice at about −30°C
Acid pH	pH 0, acidophilic archaeans; e.g., *Picrophilus* sp. and *Ferroplasma* sp.	pH 0, fungi, e.g., *Cephalosporium*; pH 0.5, acidophilic algae, e.g., *Cyanidium caldarium*, *Dunaliella acidophila*	Acid mine drainage; geothermal sulfurous sites (e.g., Yellowstone)
Alkaline pH	pH 13, *Plectonema*; pH 10.5, *Natrobacterium*; pH 9-11, *Methanosarcinales* at Lost City hydrothermal vent environment	pH 10, many species of protists and rotifers (e.g., Lake Nakuru, Africa); diatoms (e.g., Mono Lake)	Soda lakes; peridotite-hosted hydrothermal systems (e.g., Lost City)
High hydrostatic pressure	High diversity of bacteria and archaeans in deep ocean trenches, including obligate pressure-requiring organisms (piezophiles)	High diversity of invertebrates and fishes in ocean trenches	11,100 m (Challenger Deep, Marianas Trench)
Low water activity (desiccation)	1. Grow in 35% NaCl, archaeans and bacteria, e.g., *Halobacteria*	Molds and yeasts, e.g., *Zygosaccharomyces rouxi* (grow in high sugar or dried fruit, about 15-20% water)	Deep-sea brines, soda lakes, evaporate ponds, dry soil and rocks, foods with high solute content (jams, honey, dried fruit)
	2. Survive dessication, e.g., *Deinococcus* sp. and *Mycobacteria* sp.	Brine shrimp (*Artemia* sp.), cysts, tardigrades can survive dessication	
Damaging radiation (survival, not growth)	10,000-11,000 grays (gamma radiation), *Deinococcus radiodurans*	German cockroach (*Blatella germanica*) can survive exposure to radiation above 1,000 grays	No natural source of radiation at level tolerated by *D. radiodurans*
Toxic heavy metals	Cd 2-5 mM, bacteria and archaeans; Ni 2.5 mM, Co 20 mM, Zn 12 mM, Cd 2.5mM, *Ralstonia eutrophus*	Algae, e.g., *Euglena* and *Chlorella* can grow in Cd, Zn, and Co at mM concentrations	Submarine hydrothermal vent fluids and sulfides; some high-metal-containing lakes

SOURCES: Adapted from Cox, M.M., and Battista, J.R., 2005, Deinococcus radiodurans—The consummate survivor, *Nat. Rev. Microbiol*. 3:882-892; Deming, J.W., 2002, Psychrophiles and polar regions, *Curr. Opin. Microbiol*. 5:301-309; Holland, M., and Baross, J.A., 2003, Limits of life in hydrothermal systems, pp. 235-250 in *Energy and Mass Transfer in Marine Hydrothermal Systems* (P.E. Halbach, V. Tunnicliffe, and J. Hein, eds.), Proceedings of the 89th Dahlem Conference, Springer-Verlag, Berlin; López-García, P., 2005, Extremophiles, in *Lectures in Astrobiology*, Volume 1 (M. Garguad, B. Barbier, H. Martin, and J. Reisse, eds.), Springer-Verlag, Berlin; Potts, M., 1994, Desiccation tolerance of prokaryotes, *Microbiol. Rev.* 58:755-805; Rothschild, L.J., and Mancinelli, R.L., 2001, Life in extreme environments, *Nature* 409:1092-1101; Silver, S., and Phung, L.T., 1996, Bacterial heavy metal resistance: New surprises, *Annu. Rev. Microbiol.* 50:753-789; Ventoza, A., Nieto, J.J., and Oren, A., 1998, Biology of moderately halophilic aerobic bacteria, *Microbiol. Mol. Biol. Rev.* 62:504-544; Wiegel, J., and Kevbrin, V.V., 2003, Alkalithermophiles, *Biochem. Soc. Trans.* 32:193-198.

a wide range of salt concentrations and maintain internal salt concentrations typical of nonhalophilic organisms (generally about 0.85 percent NaCl).[22]

Some combinations of extreme conditions apparently prevent cells from growing. For example, no organisms have been characterized that can grow in high salt concentrations at the upper and lower limits of temperature and pH. It is not known whether such combinations pose an insurmountable barrier or there has been insufficient sampling or a lack of assessable habitats with those combinations. For example, a marine environment with high temperature (up to 90°C) and high pH (up to 11) that was only recently discovered is teeming with microorganisms.[23] Some other combinations known to exist in natural environments—such as high pressure, high salt, and low temperature—have rarely been studied.[24] There are also combinations of extreme conditions that are known to have a synchronous effect on the growth or survival of cells that are not adapted to them. That is the case for hydrostatic pressure and temperature and for salt and temperature.[25] Low temperature and high hydrostatic pressure affect cell processes in the same way; the result is that the minimal growth temperature of nonpiezophilic microorganisms increases with increasing pressure. Similarly, high salt concentrations will increase the minimal or decrease the maximal growth temperatures of nonhalophilic microorganisms.

Organisms have evolved a wide array of physiological and metabolic strategies for growing in nutrient-poor environments. In the open ocean—particularly in the deep-water column, in deep sediments, and in deep crustal environments—carbon and energy sources are extremely scarce (oligotrophic), but active microorganisms are present (although their growth rates may be extremely low). Recently, *Pelagibacter ubique*, a representative of one of the most cosmopolitan microorganisms in oligotrophic oceans, was found to grow only in the in situ micromolar concentrations of organic carbon. *P. ubique* has one of the smallest genomes, but it includes all the genes essential for existing as a free-living organism (growing without help from other organisms).[26] *P. ubique* and related marine oligotrophs may help to set the lower limits of concentration of organic compounds that can support the growth of heterotrophs. *P. ubique* and related marine oligotrophs could serve as models for designing strategies to detect similar organisms in Europa's ocean, the subsurface of Mars, or Lake Vostok (under the surface of the central Antarctic ice sheet) if it is determined that these ice-covered bodies of water contain low concentrations of dissolved organic compounds.

3.3 WATER, DESICCATION, AND LIFE IN NONAQUEOUS SOLVENTS

Water and temperature are the only single variables known to prevent growth and survival of organisms. Temperature and pressure together determine the boundary conditions for liquid water. The other physical and chemical factors that are thought of as presenting extreme conditions—such as pH, pressure, damaging radiation, and toxic metals—are life-prohibiting factors for most organisms but not all. Over the course of evolution, life has adapted to the entire terrestrial range of those variables (see Table 3.1). However, there are some combinations of physical and chemical conditions in which no organisms have been known to grow. They include environments that have both high salt (over 30 percent NaCl by weight) and low temperature (below 0°C), such as in sea-ice inclusions, and high salt (over 30 percent NaCl) and high temperature (over 90°C), known to exist in brine pools beneath the Red Sea and the Mediterranean Sea. Is life capable of growing in those combinations of stressors, or have we not looked hard enough in the appropriate environments?

Life as we know it is a series of aqueous chemical reactions. There is a point where intracellular water activity[a] decreases to the point where cells die. Desiccation causes DNA to break, lipids to undergo permanent phase changes, and proteins to crystallize, denature, and undergo condensation reactions.[27,28] Saturated brine pools (35 percent salt; water activity, 0.75) are naturally occurring low-water-activity environments that are inhabited by bacteria, archaeans, eukaryotic algae, and brine shrimp. Organisms that grow or survive in dry environments or in solutions with low water activity, such as brines and syrups, match their internal water activity with that of their environment by accumulating compatible solutes, which can be ions, such as K^+, or low-molecular-weight soluble organic compounds that do not interfere with the normal physiological functions of the cell.[29] The obli-

[a] Water activity, a_w, is defined as the ratio of the vapor pressure of a solution to that of pure water at a specified temperature. This is expressed as $a_w = P/P_0$, where P is the vapor pressure of a solution and P_0 is the vapor pressure of pure water.

gate halophilic archaeans (which cannot survive without high salt conditions) accumulate K^+ and Cl^+ ions in the cytoplasm to a concentration similar to that in the environment. That strategy requires evolutionary adaptations of intracellular macromolecules and of metabolic and biosynthetic processes to operate despite high salt concentrations. Most other microorganisms and eukaryotes deal with desiccation by accumulating compatible organic compounds that include organophosphorus compounds, β-amino acids, and glycerol. Some organisms survive desiccation by forming spores or cysts; others, such as the bacterium *Deinococcus radiodurans,* have mechanisms to repair damage to their DNA caused by desiccation. Both bacteria and eukaryotes can grow in Antarctic rocks that have liquid water for only short thaw periods.

An important issue regarding water is the degree to which it is required by carbon-based life or whether an organic substance could replace it as the primary solvent. Another issue is the ability of organisms to survive in environmental conditions that are outside their known limits for growth. These conditions usually involve decreasing the water content of the cell, as in bacterial and fungal spores and some animals, such as tardigrades (water bears). Those issues are important in assessing whether carbon-based life could exist in liquid methane or ethane pools on Titan, survive the harsh physical conditions that would be encountered during transport from one planet to another, or survive long periods in a completely desiccated state and retain the ability to grow if water were eventually introduced.

Is it possible for carbon-based life to exist in solvents other than liquid water? Many organic solvents—including alcohols, phenols, and toluene—are extremely toxic to microorganisms. The degree of antimicrobial action of a solvent depends on its hydrophobicity. The more hydrophobic the solvent, the more readily it can accumulate in cellular membranes. The toxicity of a solvent to cells is due to its ability to permealize the cell membrane, which results in the leakage of macromolecules, including RNA and proteins.[30] Organic solvents kill most microorganisms, but some bacteria can tolerate solvents at relatively high concentrations. Two mechanisms of solvent tolerance have been identified: membranes' limiting the diffusion of solvents into the cell and specialized efflux mechanisms that remove solvents that have diffused into the cell.

Can the carbon-based biochemistry of life as we know it function in organic solvents? Certainly, many enzymes function in organic solvents, and many organic reactions fundamental to biochemistry can occur in nonaqueous solvents.[31,32] However, even enzymes that are active in organic solvents need some bound water to maintain their active structure. Moreover, water is important in other vital biochemical reactions during metabolism and biosynthesis, and water is a product of metabolic reactions. Scientists infer that carbon-based life is not likely to be able to adapt to a pure-solvent milieu unless it has some mechanism to form water from such solvents as alcohols or to produce all the necessary water de novo from biochemical reactions. Are those mechanisms possible, and is there evidence that bacteria could grow in some organic solvent, with or without trace levels of water?

Until recently, it was generally believed that external water passively diffuses through cell membranes. That is not necessarily true, and specific channels in bacterial membranes have been identified that function as pores for the transport of water. Named aquaporins, they can facilitate the rapid transport of water during osmotic stress.[33] The presence of specific water-transport pores makes it imaginable that some organisms have organic-solvent-resistant membranes with specialized aquaporins that extract and selectively transport water at low concentrations from the solvent-water mixture. Moreover, it has been demonstrated with oxygen isotopes that up to 70 percent of the intracellular water in actively growing *Escherichia coli* is generated by metabolic processes, and not derived from the environment.[34] That finding needs to be confirmed, but it leads to the question of whether it is possible for organisms to grow in intracellular water generated exclusively de novo or in water and solvent mixtures in which the concentration of water is 10 percent or less. A cell would have to overcome two obvious problems: If it is heterotrophic and requires transport of organic nutrients from an organic-solvent milieu, a cell will require a membrane that can differentially transport organic nutrients and prevent solvent toxicity. If the organism is a methanogen or some other chemolithoautotroph that requires carbon and energy sources that are gases, only a mechanism for passive diffusion through a solvent-resistant membrane will be necessary. Do such organisms exist, or can carbon-based organisms be engineered to grow under those conditions? Addressing the question would be worthwhile in the context of life in organic ponds on Titan or life in a gaseous milieu of CO_2, H_2, N_2, and so on. (See Chapter 6 for a discussion of biochemical processes that are possible and in some cases favored in organic solvents as compared with water.) However, there is no information about the possible alternative membrane structures that would be stable and function optimally in organic solvents.

3.4 TEMPERATURE

Temperature is a fundamental thermodynamic characteristic that affects all biochemical reactions, in particular setting limits on life because temperature and pressure determine whether water is in the liquid phase. Given the presence of liquid water, what is the allowable range of temperatures for life? Microorganisms have been cultured and growth observed at temperatures as high as 121°C[35] and as low as −15°C (Figure 3.1).[36] There is even evidence of intact microorganisms with DNA and RNA in hydrothermal vent sulfides at temperatures exceeding 200°C.[37] Eukaryotic organisms, however, are not known to live above 60°C. Despite the wide gap in maximal growth temperatures between prokaryotic and eukaryotic cells, eukaryotes share all other extremes of life with bacteria and archaeans, including growth in environments of high acidity, high salt, high pressure, and high concentrations of toxic metals (see Table 3.1).

Microorganisms that grow best at temperatures above 80°C are called hyperthermophiles. The maximal temperature at which growth of cultured hyperthermophiles occurs can vary from 80°C to 121°C, and the minimal growth temperature can vary from about 40°C to about 80°C, depending on the organism.[38,39] Hyperthermophiles include bacteria and archaeans, aerobes and anaerobes, and heterotrophs and autotrophs. Some acidophiles (which grow at low pH), alkalophiles (which grow at high pH), and radiation-resistant organisms are also hyperthermophiles. Hyperthermophiles have protein and lipid structures that are adapted to high temperature. No generalizations can be made about why enzymes and other proteins are thermally stable, but there are some recurrent characteristics.[40] Protein structures are stabilized at high temperature through amino acid substitutions and, most important, the increased use of disulfide bonds for structural stabilization.[41] Heat-stable, ether-linked lipids are universal in hyperthermophilic archaeans and in some hyperthermophilic bacteria. Fundamental changes in protein and lipid structure compensate for the increased mobility and fluidity at high temperatures. All hyperthermophiles studied have a reverse gyrase that positively supercoiles DNA. (The DNA of all other organisms is negatively supercoiled.) Supercoiling with cationic proteins increases the thermal stability of DNA.[42]

Is 121°C the highest temperature for growth of life? The chemical properties of water and biological macromolecules are affected by high temperature. Theoretically, it should be possible to engineer biological macromolecules to maintain their three-dimensional structure with increasing temperature by compensating for temperature effects with higher pressure or with increasing salt concentrations. That appears to be the case with hyperthermophilic proteins.[43,44] Important cofactors, such as adenosine triphosphate (ATP) and nicotine-adenine dinucleotide (NAD), are thermally labile (subject to chemical modifications to enable coping). Hyperthermophiles stabilize ATP and NAD by increasing their turnover rates (rates of synthesis), by employing extrinsic factors, such as high ion concentrations, or by using intrinsically more stable replacements.[45] Neither protein, nor DNA, nor cofactor lability precludes life at higher temperatures, and the upper temperature for life is still to be determined.

Freezing temperatures can kill cells if ice crystals are formed internally. Cells survive freezing if frozen quickly; viable cells can be preserved for long periods if frozen quickly in liquid nitrogen (−196°C). Slow freezing or slow thawing of cells favors ice-crystal formation that can damage macromolecules and structural polymers and lead to death. Cellular adaptations for preventing ice-crystal formation during slow freezing include increasing intracellular solute concentrations (similar to low-water-activity adaptation), production of exopolysaccharides, and modification of lipids and proteins to increase the fluidity of membranes and mobility of the enzymes.

Is there a lower temperature limit for life? Microbial activity has been measured at −20°C in ice, and photosynthesis has been observed in Antarctic cryptoendolithic (living hidden in the rock) lichens at −20°C.[46] Water can remain liquid at temperatures lower than −30°C in the presence of salts or other solutes and at even lower temperatures in combination with soluble organic solvents. Enzyme activity has been measured at −100°C in a mixture of methanol, ethylene glycol, and water.[47] There is also evidence of electron transfer and enzyme activity at −80°C in a marine bacterium.[48] The process of vitrification (liquid water moving directly into the glassy state without ice-crystal formation), facilitated by the presence of salts and exopolymers (insoluble high-molecular-weight organic compounds, usually carbohydrates) in the starting solution, may be essential for such activity. It is possible that there is no low-temperature limit for enzyme activity or even cell growth if a suitable solvent or solvent mixture is available.[49]

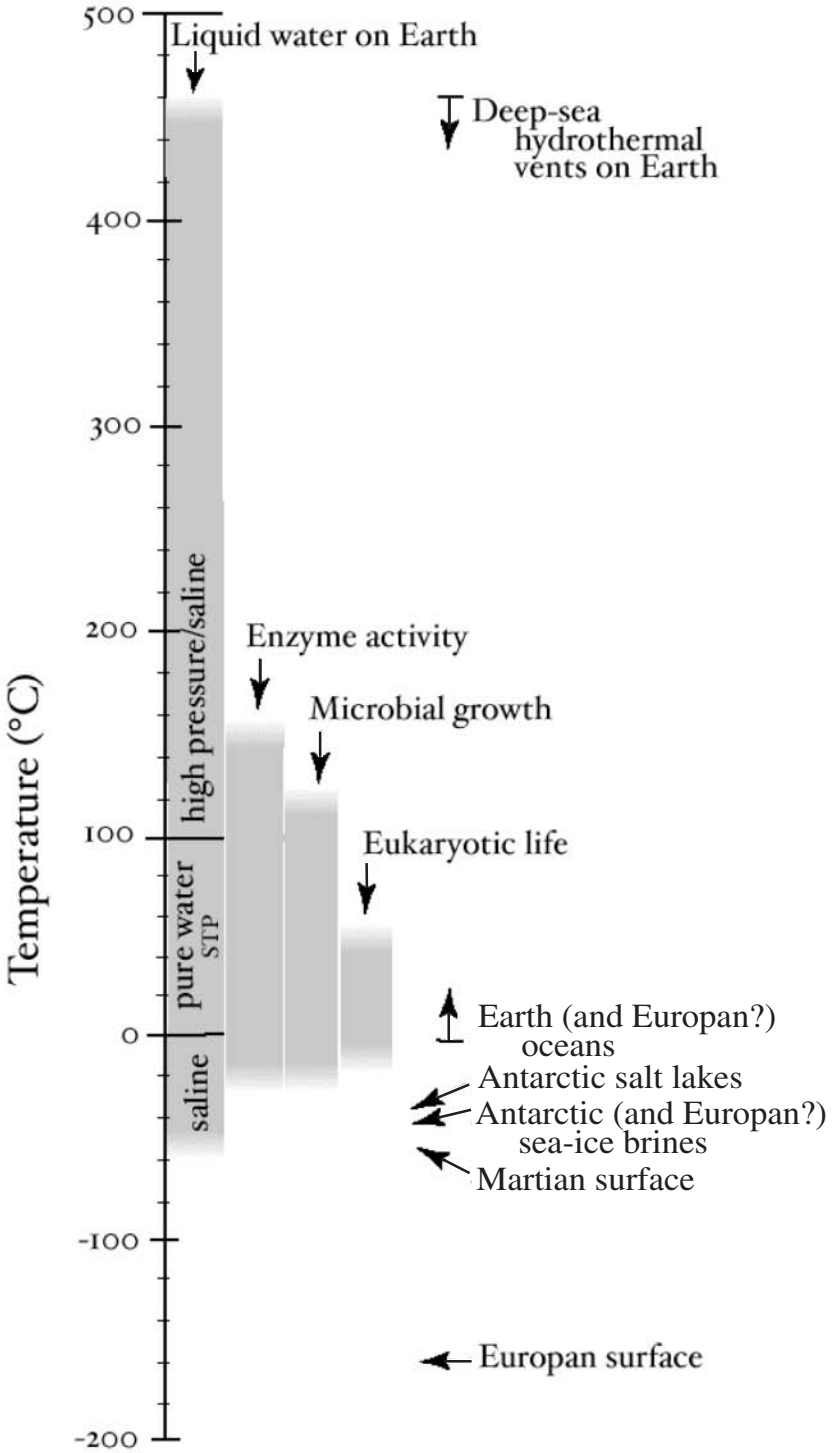

FIGURE 3.1 Liquid water and life on planets. Presence of liquid water and observed functioning on Earth of enzymes and microorganisms (Bacteria and Archaea). Note that more complex organisms (Eukarya) occupy a more restrictive thermal range. Average surface temperatures on Mars (−55°C) and Europa (−160°C) are also shown. SOURCE: Deming, J.W., and Eicken, H. Life in ice. In *Planets and Life: The Emerging Science of Astrobiology* (W. Sullivan III and J. Baross, eds.). Cambridge University Press, in press.

3.5 SURVIVAL STRATEGIES AND INTERPLANETARY TRANSFER

The ability of microorganisms to survive extreme environmental conditions—such as high and low temperature, desiccation, radiation, and extremes in pressures and pH—increases the probability that they could survive transport to other planets and moons and thereby effect panspermia, the transfer of life from one world to another. Interplanetary transfer of life would be likely only within a solar system, and transfer to a planet orbiting another star would be extremely unlikely. Panspermia is an important issue in the context of this report because the presence of life in any extraterrestrial setting would require that it have an origin—de novo or by panspermia. Moreover, if panspermia is how life began on Earth, it is likely to be how life could arise elsewhere, and life forms found elsewhere would then resemble Earth life at the fundamental, biochemical level. Two groups of microorganisms have received most of the attention as possible successful space travelers: spore-forming bacteria and radiation-resistant microorganisms. Spore formation by bacteria and fungi is usually in response to stress, such as in limiting of nutrients, dessication, and heat shock. Spores are capable of long-term survival and have been recovered from environmental samples that are more than 1 million years old.[50] There is even a report of viable spores from the gut contents of a bee entombed in amber estimated to be more than 25 million years old.[51] Spores are also known to be resistant to heat and radiation; and, because of their small size and stable dormancy, they can travel through the atmosphere to distant locations or slowly settle on the floors of deep ocean trenches. Some spores can survive more than an hour of exposure to dry heat at 150°C, although survival is greatly reduced in moist heat.[52] Spores from thermophilic bacteria are more resistant to heat than are mesophilic spores.[53] Similarly, spores are much more resistant to ultraviolet radiation than are vegetative cells (those capable of growing).[54]

If microorganisms are to be transported from one planet to another, they must have the ability to resist the lethal effects of radiation. High-energy electromagnetic radiation (ultraviolet, x-ray, and gamma radiation) and high-energy alpha and beta particles damage DNA and have cytotoxic and mutagenic effects on cells. Ultraviolet radiation is the most abundant form of damaging radiation and probably the most common natural mutagen. Ultraviolet light can also kill cells as a result of dimerization of thymidine residues in the DNA, which prevents replication. Ionizing radiation kills cells by causing multiple breaks in the double-stranded DNA. Most organisms have mechanisms for protection from damaging radiation, such as radiation-absorbing pigments and DNA-repair mechanisms.

The most radiation-resistant microorganisms include bacteria and archaeans. *Deinococcus radiodurans* is one of the most studied of those organisms and can survive radiation levels up to 10 kGy.[b] That level of radiation is much higher than is found naturally anywhere on Earth. Radiation resistance in *D. radiodurans* is the result of extremely efficient DNA-repair mechanisms that can reassemble, with high fidelity, DNA that has been sheared into multiple double-stranded pieces.[55] It is believed that this repair mechanism evolved in response to DNA damage due to desiccation rather than radiation. Recently, a hyperthermophilic archaean, from a submarine hydrothermal vent environment, was found to be resistant to radiation at a level of 8 kGy.[56] The German cockroach (*Blattella germanica*) is the most radiation-resistant metazoan, although the tardigrade (*Milnesium tardigradum*) in its desiccation-resistant "tun" state can survive high-level exposures to x rays.

Besides being desiccated and irradiated, microorganisms traveling in space will be exposed to space vacuum that can reach 10^{-14} pascal (a unit of pressure—100 Pa = 1 mbar).[57] The result is extreme dehydration, and naked spores can survive for only days if exposed to space vacuum. Survival of spores is increased if they are associated with various chemicals such as sugars, or are embedded in salt crystals. Nicholson et al. (2000) discuss the various stresses that a microbial cell or spore would have to endure to survive interplanetary travel.[58] They include the process that transports them out of Earth's atmosphere, such as volcanic eruptions and bolide impacts, long periods of transit in the cold of space, and atmospheric entry into a new planetary home. Spores have been shown to survive the shock conditions of a meteorite impact and the ultraviolet radiation and low temperature of space.[59] It is clear that panspermia is possible and even probable if bacterial spores become embedded in rocks that are ejected from one planet and eventually enter the atmosphere of another. Bacterial

[b] A gray (Gy) is a unit of absorbed dose of ionizing radiation corresponding to the absorption of 1 J/kg of absorbing material. 1 gray = 100 rads. On Earth, a typical middle-latitude, sea-level natural background level is about 0.3 mGy per year, whereas on the surface of Europa the level is almost 10^{10} times higher, enough to kill humans in about 1 minute of exposure.

spores have received most of the attention with respect to panspermia, but they may not be the only candidates for long-term survival in space. There is evidence that microorganisms that are attached to surfaces, such as minerals, have enhanced survival of a variety of stress conditions.[60] In many cases, the attached organisms form biofilms in which the cells are encapsulated in exopolysaccharides and often reach high densities.[61] Stress resistance has been attributed to two features of the biofilm communities: their ability to limit rates of diffusion, and the presence of multiple physiological states, including "persister" cells with recalcitrant physiologies.[62] More research is needed to assess the full range of microorganisms that can survive interplanetary travel, particularly those known to form biofilms on minerals.

3.6 THE PLASTICITY OF HUMAN-LIKE BIOCHEMISTRY

As the exploration of the biosphere has continued, environments on Earth have been discovered that are quite different from and alien to human-like life. Nevertheless, where an environment has been found to contain a chemical disequilibrium, if liquid water is also present and the temperature does not exceed the upper limit for covalent bonding of core biomolecules, life is present. Active life has been found in deep-ocean thermal vents at temperatures in excess of 112°C. Life has been found in Antarctica where liquid water exists only transiently. It has been found 5 km below Earth's surface in mine shafts, and in the effluents of mining operations at Rio Tinto, Spain, that are as acidic as dilute sulfuric acid. Several of those environments are summarized in Table 3.1.

The life that is present in those environments is, in many of its molecular aspects, identical with human life. It exploits the same DNA for genetics, the same amino acids in its proteins, and the same core metabolism. Furthermore, analysis of its chemical structure leaves no doubt that these exotic terran life forms are related to humans by common ancestry.

Those observations compel the conclusion that the standard macromolecular, genetic, catalytic, and metabolic structures are versatile enough to support life in exotic environments. How life adapted to such environments and what its primitive environment was remain topics of research. The details of how human-like forms of life have adapted to exotic environments give some clues to those questions and suggest environments where human-like life could *not* survive.

3.7 LIMITS OF ANTHROPOCENTRIC BIOCHEMISTRY

As plastic as standard terran biochemistry is, it is easy to find on Earth environments with features whose exotic or extreme nature cannot be directly accommodated by human-like biomolecules. For example, the nucleobases of DNA and RNA are, as their name implies, chemical bases. They can absorb a proton from their environment to become positively charged. In adenine and cytosine, for example, the nucleobases are more than 50 percent protonated below a pH of 4, the acidity of dilute vinegar. That would argue against an origin of life in an acidic environment.

Because the protonated forms of adenine and cytosine have different arrangements of hydrogen atoms, they form different patterns of hydrogen bonding. Thus, for example, adenine is no longer complementary to thymine, and cytosine is no longer complementary to guanine. That, in turn, means that the Watson-Crick nucleobase pairing essential for DNA duplex formation and genetics is no longer possible.

The motion of protons in water is extremely rapid, and no catalyst can manage this problem at low pH. Therefore, the known life that is found in acidic environments on Earth manages the low pH of its environment by pumping protons out of the cell. Although the pH of Rio Tinto outside the cells that live there is about 2, the pH inside the cells is well above 6, as it is in human cells.

The same considerations limit the intrinsic adaptability of standard terran biochemistry at high pH.[63] Guanine and thymidine and the RNA analogue uracil are weak acids. They lose a proton to water at a pH above 10. The deprotonated forms of those nucleobases have different arrangements of hydrogen atoms and form different patterns of hydrogen bonding. That means that they are no longer complementary to their standard Watson-Crick partners, and that the nucleobase pairing essential for DNA duplex formation and genetics is thus no longer possible.

Any forms of life that are found at high pH must either pump protons to maintain an intracellular pH close to neutrality or adopt a genetic structure that is different from the chemical structures found in standard DNA. The first is the strategy for organisms that live in such alkaline environments as Lake Mono.

High pH has other limits as well. The hydroxide anion, arising from water at a high pH, is a powerful nucleophile and destroys many molecules that are part of core metabolism. Thioesters have short half-lives in neutral water; their half-lives at a pH of 12 are measured in seconds. They play central roles in the manipulation of organic acids, including the formation of new organic acids by the formation of new carbon-carbon bonds and the degradation of organic acids through the breakage of carbon-carbon bonds.

The second set of organic molecules that become unacceptably unstable at high pH are the mixed anhydrides of organic acids and phosphoric acid. They are central in the management of high-energy phosphate esters in living systems, including the generation of ATP from glucose.

At still higher pH, standard oxygen esters, such as those found in lipids (see Figure 2.12), become unacceptably unstable. Standard oxygen esters are key components of many hydrophobic structures, including membrane structures in normal bacteria. That is why human skin, for example, suffers a reaction when it comes into contact with lye. It would be difficult to imagine life containing fatty acid esters surviving, for example, in water-ammonia eutectics on Titan. An alternative hydrophobic system would be required.

It is clear that biological systems can manage the chemical reactivity of unstable species. For example, oxaloacetate—a metabolic intermediate in terran metabolism that is a precursor of citric acid, malic acid, and the amino acid aspartic acid—decarboxylates readily, with a half-life measured in minutes at room temperature at neutral pH. The half-life for the decarboxylation of oxaloacetate drops to seconds at high temperatures in pure water. It is not clear how microorganisms that live at high temperatures manage the instability of oxaloacetate, which is a key intermediate in standard biochemistry for the formation of amino acids, such as aspartate, and asparagine.

3.8 EARLY ENVIRONMENTS OF LIFE ON EARTH

That RNA and DNA could not work well if bathed in water having a pH much higher than 9 or much lower than 5 suggests that these are the bounds for the pH of the environment where RNA and DNA first functioned as genetic molecules. If we assume that the most primitive forms of life on Earth could not have been sophisticated enough to pump protons across membranes that would not leak protons and if we assume that RNA and DNA worked in the first living systems, we are compelled to conclude that the first living systems lived at a pH near 7. An alternative possibility is that RNA and DNA were first used by terran organisms after they evolved sufficiently to control their internal pH. How replication would have occurred until that happened remains uncertain.

Other constraints on the temperature at which earlier forms of life lived come from experiments. For example, Gaucher et al.[64] compared the sequences of elongation factors from a variety of bacteria to infer the sequences of ancestral elongation factors that may have been present in now-extinct bacteria lying deep in the eubacterial tree. With recombinant-DNA technology, several of those candidate ancestral elongation factors were resurrected for study in the laboratory. They were found to function best at a temperature of about 67°C, typical for modern thermophilic bacteria.

Despite their antiquity (perhaps 2.5 billion years old), the resurrected ancestral elongation factors are inferred for bacteria that lived long after the origin of life. Nevertheless, the notion that early life lived at high temperatures, in water, and at nearly neutral pH is consistent with available data. For example, a recent reconstruction of the phylogenetic tree of life based on 31 common gene families supports the notion that the last common ancestor lived at high temperatures.[65]

3.9 OPPORTUNITIES FOR RESEARCH

The exploration of the terran biosphere has been a continuous source of insights and surprises, even to the present date. The development of high-throughput DNA sequencing techniques and sequencing surveys of habitat microorganisms makes it possible to continue to expand our understanding of the inventory of metabolic diversity on the planet. Molecular methods are not likely to find any life that is not related by common ancestry to the life

that we already know, and so it remains important to continue to push our search for the limits of life on Earth. For example, if polyelectrolytes are universal features of genetic biopolymers, the search for them might uncover life forms that are not identified with standard polymerase chain reaction primers directed at ribosomal RNA genes.[66] Laboratory research and field studies are needed so that we would recognize, on Earth, life that is not related to us by common ancestry.[67] Concepts and instruments that are designed to do that would be applicable to flight missions as well.

Carbon-based life is extremely versatile and has adapted to grow in most of the physicochemical environments on Earth. Only high temperatures and low water activities limit the growth of organisms. Even in those limiting conditions, some organisms have adapted strategies for long-term survival.

There is still much to be understood about the limits of carbon-based life. Some extreme terran environments have not been adequately explored for microbes or for evidence of microbial interactions. They include the hot subseafloor crust, hot brine sediments and crustal aquifers, and rock-hosted microbial communities, particularly those affected by hydrothermal activity or subjected to changing environmental extremes, such as hydrating and dehydrating conditions. Even in relatively well-studied environments, including extreme environments, very little is known about the microbial inhabitants other than their identities based on molecular signatures. There is also a need to understand how different physiological characteristics of organisms, such as biofilm formation and production of exopolysaccharides, affect growth and survival in extreme environments. It is also probable that the limits of extant terran organisms might not be indicative of limits possible for organisms with terran biochemistry. Life occupies most known habitats and in some cases can tolerate extreme conditions, such as irradiation and hydrostatic pressure, that exceed those found in natural environments. Important unanswered questions include these: What is the maximal growth temperature for life? Are there extrinsic or intrinsic factors—such as high hydrostatic pressure, high concentrations of salt, or high concentrations of intracellular inorganic and organic compounds and macromolecules—that facilitate growth above 120°C? Can organisms adapt to grow with much lower amounts of water or higher concentrations of organic solvents than are now accepted as limits? The ability of cells to grow under such conditions would change our perception of the lower and upper temperature limits for life. Can organisms grow in environments that are deficient in one or more essential nutrients, such as phosphate, by substituting arsenic, for example?

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4

Alternatives to Terran Biochemistry in Water

All that we know about terran life suggests that it exists in water at nearly neutral internal pH and at a range of temperatures that permits water to be in the liquid state. That range includes temperatures higher than 100°C, the boiling point of pure water at sea level, if pressures higher than 1 atm prevent boiling.

The committee found that it has proved difficult to find extant life in environments that do not contain liquid water, although searches for life in very dry, very cold, and very hot places continue. That suggests that water is key to the life that we know. Furthermore, the exploitation of the polar versus nonpolar, hydrophilic versus hydrophobic, and water-soluble versus water-insoluble dichotomies in the construction of terran metabolites, macromolecules, and supramolecular structures appears to be an elegant way to solve problems that are presumably presented to life generally.

The evident compatibility between terran biochemistry and water makes it difficult to distinguish cause and effect. First, the global features of terran biochemistry may indicate that the biochemistry we know is the only chemical solution to particular challenges posed by life in general. Second, the compatibility may reflect that terran biochemistry is the *only* chemical approach to particular challenges posed by life in water. Third, it is possible that alternative chemistry could support life, even in water, but did not originate on early Earth or did not survive in competition with the life we know. Finally, the observed facts might indicate nothing about what environments might support life but only that all life on Earth is related by a common ancestry; that is, the compatibility might reflect neither the optimal nor the universal, but simply a shared historical accident.

If the first is the case, then our observations of terran life should determine our view of life generally. In particular, if features of terran biochemistry are presumed to be the only possible solutions to problems that must be solved for life to exist and if those solutions are possible only in water, we would have to conclude that life can exist only in water. The "follow the water" strategy would thus be uniquely suited for the search for life in the cosmos.

If the second is the case, we would be called on to creatively define chemistries that might support life in nonaqueous solvents. They might be used to support NASA missions to locales, such as Titan and Venus, where liquids other than water are abundant.

If the global structures of terran life reflect origins, we must turn to models of early Earth and consider its chemistry in an effort to guess the potential for life in the solar system. Other planets and moons in our solar system have had histories different from Earth's. We would be called on to creatively define chemistries that might have originated in the early histories of those other bodies.

Last, if the global features of terran life reflect nothing more than the common ancestry of the life that we know, we could infer little from a study of life on Earth about the nature of alien life that does not share an ancestry. Indeed, as Cleland and Copley have recently discussed,[1] it is conceivable that Earth harbors yet undiscovered forms of life that are not related by common ancestry to the life that we know, have quite different biochemistry, and may have been overlooked for precisely that reason.

4.1 SYNTHETIC BIOLOGY AS A STRATEGY FOR UNDERSTANDING ALTERNATIVES TO TERRAN BIOMOLECULES

The goal of synthetic biology is to create a more comprehensive understanding of life by integrating different areas of research, such as engineering, physics, and chemistry, so as to design and construct novel biological and biochemical functional systems. Since biological systems are composed of organic compounds, synthetic biology has become more connected to synthetic chemistry (the shift from studying nature's chemistry to the design and synthesis of new chemistry). The result is novel biochemistry. One area of current emphasis in synthetic biology that is germane to this report is the design and synthesis of new biochemicals that can lead to the synthesis of novel but functional structural, informational, and catalytic biochemical systems. Significant progress has also been made using synthetic biology approaches to origin of life studies and particularly in designing biochemical systems that might better reflect early stages in the synthesis of information macromolecules, replicators, and cell-like structures. One of the goals is to find alternative biochemical systems that undergo evolution.

The committee considered a variety of approaches to determine whether the biochemical structures found in terran life are unique. One was derived from synthetic organic chemistry and is sometimes referred to as synthetic biology.[2,3] Much of contemporary biological research deconstructs living systems, but the ability of chemists to synthesize new forms of matter (i.e., new arrangements of atoms in new molecules) offers an alternative approach, especially if the aim is to ask whether alternative chemistries can support biomolecular function. It is possible for chemists to synthesize alternative chemistries, to ask Why not? and What if? questions about biomolecular structure, and to determine whether the alternative structures might function as alternative genetic molecules, membrane components, catalytic species, or metabolites. That is directly related to the question, Are the biomolecular structures that we know in terran life the only structures that can possibly meet the functional demands of living systems?

4.1.1 Terran Nucleic Acids Are Not the Only Structures That Can Support Genetic-like Behavior

In some cases, direct experimental evidence shows that the molecules found globally in terran biochemistry are *not* the only structures that can perform the functions that they perform in the life that we know. For example, in terran DNA, the Watson-Crick nucleobase pairs obey two rules of complementarity: size complementarity (large purines pair with small pyrimidines) and hydrogen-bonding complementarity (hydrogen-bond donors from one nucleobase pair with hydrogen-bond acceptors from the other). Those rules enable the specificity that gives rise to the simple rules for base pairing (A pairs with T, G pairs with C) that underlie terran genetics and molecular biology.

It is possible through synthesis to show that the DNA alphabet is not limited to the four standard nucleotides known in terran DNA.[4,5] Rather, 12 nucleobases forming six base pairs joined by mutually exclusive hydrogen-bonding patterns are within the geometry of the Watson-Crick base pair. Figure 4.1 shows some of the standard and nonstandard nucleobase pairs and the nomenclature to designate them. Those nucleobase analogues presenting non-standard hydrogen-bonding patterns are part of an artificially expanded genetic information system (AEGIS).

On the basis of simple binding studies, it is clear that the AEGIS components work as well as the natural nucleotides. Each nucleotide pairs with its partner, and mismatches between the 12 destabilize the duplex about as much as mismatches between the standard four nucleotides. Indeed, the specificity of the synthetic biological genetic molecules is so good that they are incorporated into diagnostic tools that first received Food and Drug Administration approval in 2002. Each year, artificial genetic molecules exploiting an expanded genetic alphabet improve the health care of some 400,000 patients infected with HIV or the hepatitis B or hepatitis C virus.

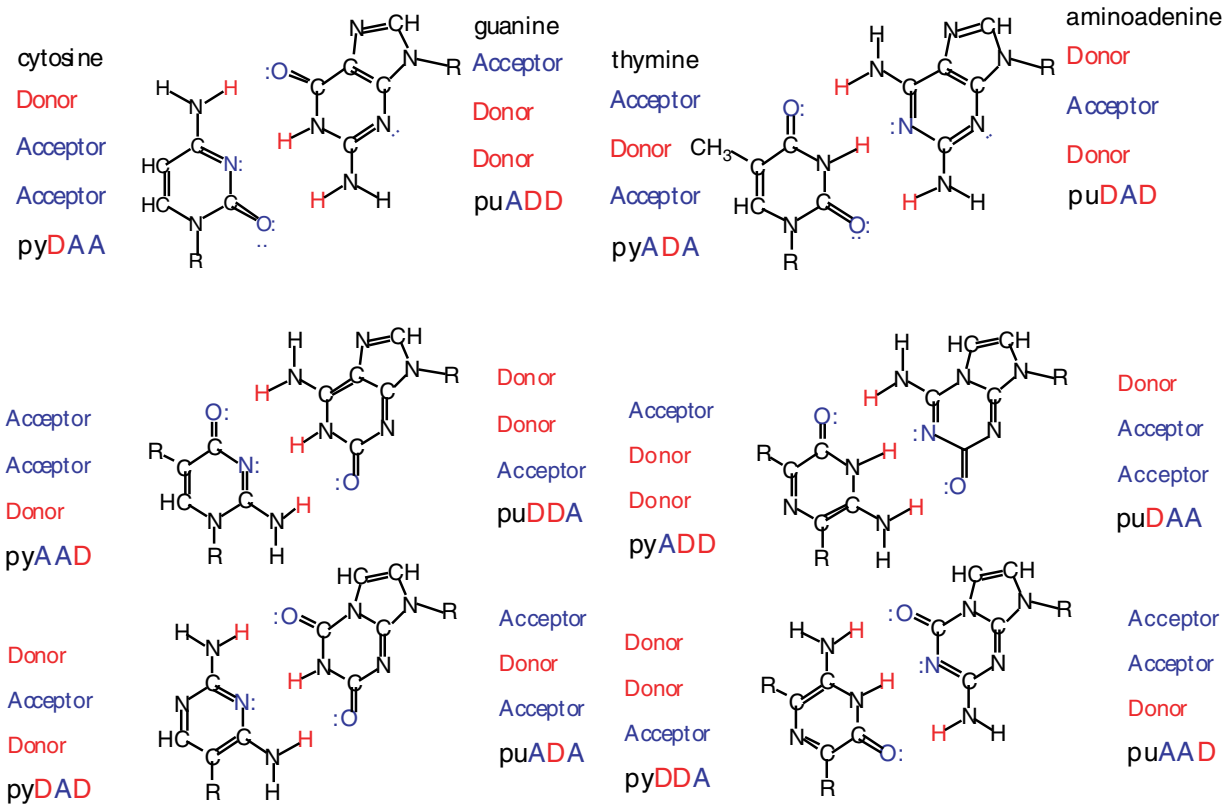

FIGURE 4.1 Twelve possible nucleobases in a DNA- or RNA-based "alphabet" that can form specific base pairs within the constraints of the Watson-Crick base-pair geometry and artificially expanded genetic information system (AEGIS). Pyrimidine base analogues are designated by "py," purine by "pu." The upper case letters following the designation indicate the hydrogen-bonding pattern of acceptor (A) and donor (D) groups. Thus, the standard nucleobase cytosine is pyDAA, and guanosine is puADD.

Artificial chemical systems capable of Darwinian evolution have also been prepared from artificial laboratory genetic systems. Such systems were created in the laboratory by using an artificial DNA that contained six nucleotide letters rather than the four in standard terran DNA.[6,7] These were chosen from the structures shown in Figure 4.1. The artificial systems can support the basic elements of Darwinian evolution (reproduction, mutation, and inheritance of mutated forms) even if the enzymes that support the evolution of artificial genetic systems are the natural terran enzymes that have evolved for billions of years to handle standard nucleobases.

Standard tools to detect genetic molecules are designed to detect standard DNA, containing the four nucleosides adenosine, guanosine, cytidine and thymidine. They cannot detect DNA built from nonstandard building blocks. This creates a special challenge for those seeking to design instruments to detect molecules on other bodies, such as Mars or Europa. Indeed, it is even possible that a form of life based on nonstandard genetic molecules might be present on Earth, undetected by standard tools that detect standard DNA.

Similar efforts in synthetic biology have shown that the ribose and deoxyribose sugars are not unique solutions to the need for a scaffolding in a linear genetic biopolymer. Starting in the 1980s, researchers in groups across the world—including the Benner and Eschenmoser groups in Switzerland, the Herdewijn group in Belgium, the Wengel group in Denmark, and most recently the Krishnamurthy group in La Jolla—have joined researchers in industry attempting to make nucleic acid analogs that might serve as drugs to determine what other types of sugars

might function in the backbone of nucleic acids. Summaries of the work can be found in Freier and Altmann,[8] Eschenmoser,[9] and Benner.[10]

The results were surprising. Replacing the ribose with another sugar generally gave a DNA analogue that formed less stable duplexes. In many cases, the replacement sugar generally diminished the stability of the duplex. For example, a variety of hexopyranosyl-(6′-4′) oligonucleotide analogues of RNA derived from the hexose sugars known as allose, altrose, and glucose displayed Watson-Crick nucleobase pairing that was inferior to that displayed by RNA with respect to both pairing strength and pairing mode specificity.

Other backbone replacements displayed pairing strengths comparable with that seen in RNA but did not cross-bind with natural RNA and DNA. The exceptional cases are the locked nucleic acids of Wengel et al.[11] and the threose nucleic acid analogues of Krishnamurthy and Eschenmoser.[12]

4.1.2 Terran Amino Acids Are Not the Only Structures That Can Be Incorporated into Proteins

An analogous series of experiments has shown that unnatural amino acids function in proteins as well as the standard 20 amino acids found globally in terran proteins. The natural ribosome found globally on Earth was able to incorporate these into proteins. In that way, synthetic biologists have expanded the amino acid repertoire of proteins.[13] The experiments showed no reason to exclude alternative sets of amino acids from hypothetical proteins in hypothetical alien life forms.

The synthetic genetic system has even been coupled to unnatural protein synthesis. Adding extra letters to the genetic alphabet has been shown to increase the number of triplet codons that are accessible to a messenger RNA. In 1993, Bain et al. showed that additional triplet codons made possible by extra nucleobases and delivered by synthetic biological efforts could encode extra amino acids.[14] That result was obtained with the terran ribosome.

4.1.3 Implications of Synthetic Biology for Our View of the Universality of Global Terran Proteins and Nucleic Acids

Work with synthetic biology makes it clear that the core set of nucleotides and the core set of amino acids found in all terran life inspected to date are not the only nucleotides and amino acids that can function in genetic and catalytic systems. Indeed, synthetic alternatives to standard terran biochemistry function in the terran machinery that has evolved to handle the core sets.

It is not clear whether alterative nucleotides and amino acids were available prebiotically. Studies of the Murchison meteorite (see Chapter 5) have revealed a large number of nonstandard amino acids; many are accepted by terran machinery. It is clear that advanced organisms, such as those found in modern life, are fully capable of synthesizing extra nucleotides for RNA and DNA and extra amino acids for proteins.

Those facts mean that alternative nucleotides and amino acids could have been accessible to terran life. The observation that terran life did not exploit them can be explained in various ways. It may be that some terran life forms do exploit alternative nucleotides or amino acids but have not yet been discovered; indeed, hypothetical terran life that uses alternative nucleotides or amino acids would not necessarily be detected by probes designed to look for terran life. It is conceivable that some terran life forms did use alternative nucleotides or amino acids but were less fit than life forms that used the standard set and thus did not survive in Darwinian competition for us to discover them. Alternatively, we might assume that chemical processes needed to make the extra nucleotides or amino acids did not happen in the 4 billion years of life on Earth.

Regardless of which explanation is favored, we have no immediate grounds to exclude alternative nucleotides or amino acids in nonterran life if we encounter it. Somewhat complex arguments based on the chemistry of some of the alternative nucleotides were proposed in the committee's discussion to disfavor them relative to the standard four nucleotides, but it is not clear that equally compelling counterarguments would not be offered if human-like genetics exploited the nonstandard alternatives.

4.2 WHAT FEATURES OF TERRAN GENETIC MOLECULES MIGHT BE UNIVERSAL IN GENETIC MOLECULES ACTING IN WATER?

Synthetic biological experiments are available to help in identifying universal structures in terran biomolecules.

4.2.1 A Repeating Charge May Be Universal in Genetic Polymers in Water

As discussed above, it is important for metabolites to be soluble in water if life is adapted to water. RNA and DNA, by virtue of their repeating charge, are very soluble in water.[15] The DNA duplex in which a polyanion binds another polyanion, appears to disregard Coulomb's law, however. One might think (indeed, many have thought[16,17]) that the duplex would be more stable if one strand were uncharged, or polycationic.[18]

Synthetic biologists have undertaken many efforts to create nonionic analogues of DNA and RNA. For example, replacing the anionic phosphate diester linker with the uncharged dimethylenesulfone linker generated DNA and RNA analogues that were rough isosteres of the phosphate analogue (Figure 4.2).[19] Short sulfone-linked DNA analogues (SNAs) displayed molecular recognition of the Watson-Crick type. In longer species, however, the loss of the repeating charge damaged the capacity for rule-based molecular recognition. Furthermore, SNAs differing by only one nucleobase displayed different levels of solubility, aggregation, folding, and chemical reactivity, a characteristic displayed by many polyfunctional molecules. That implies that SNAs of any length could not support genetics.

Those results suggest three hypotheses for why charged phosphate linkages are important for molecular recognition in DNA. First, the repeating charges in the backbone force interstrand interactions away from the backbone, causing the strands to make contact at the Watson-Crick edge of the heterocycles (Figure 4.3). Without the polyanionic backbone, interstrand contacts can be anywhere.[20]

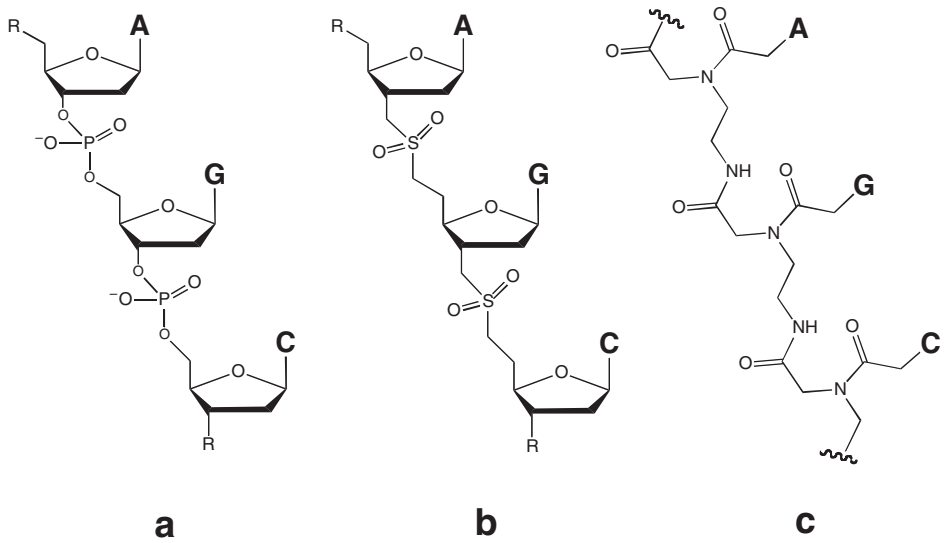

FIGURE 4.2 Replacing the anionic phosphate diester linker (a) with the uncharged dimethylenesulfone linker (b) generates DNA and RNA analogues that are rough isosteres of the phosphate analogue. (c) PNA is a DNA or RNA mimic in which the phosphate-sugar backbone has been replaced with uncharged *N*-(2-aminoethyl)glycine linkages in which the nucleobases are attached through methylene carbonyl linkages to the glycine amino group. The markings at the ends of the chain indicate that the chain continues.

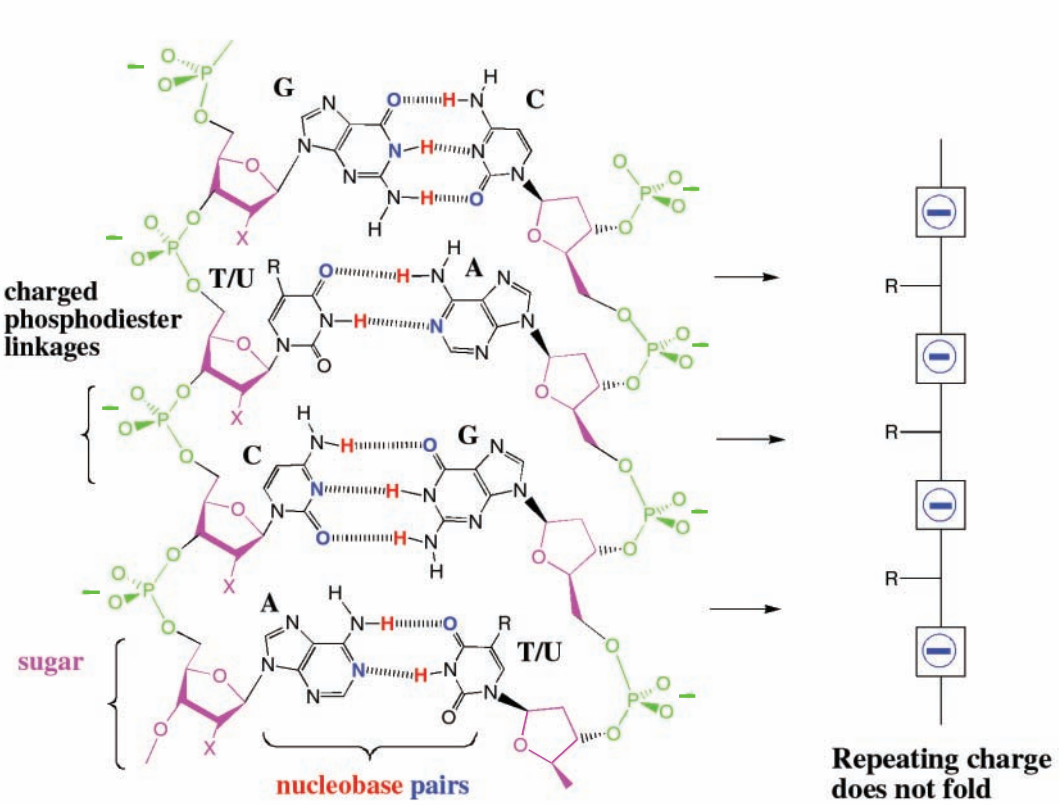

FIGURE 4.3 The repeating charges in the backbone force interstrand interactions away from the backbone, causing the strands to make contact at the Watson-Crick edge of the heterocycles. Without the polyanionic backbone, interstrand contacts can be anywhere. "X" is H in DNA and OH in RNA.

Furthermore, the repeating charges in the backbone keep DNA strands from folding. A flexible polyanion is more likely to adopt an extended conformation suitable for templating than is a flexible neutral polymer, which is more likely to fold.[21] The Flory dimension of a polyelectrolyte (like DNA), other things being similar, is much larger than that of a biopolymer with, for example, a repeating dipole.[22] As noted above, replication is not sufficient for a genetic molecule to support Darwinian evolution. The Darwinian system must also generate inexact replicates—descendants whose chemical structures are different from those of their parents—and the differences must then be replicable themselves. Self-replicating systems are well known in chemistry, but ones that generate inexact copies, with the inexactness itself replicable, are not.[23] Indeed, small changes in molecular structure often lead to large changes in the physical properties of a system. That means that inexact replicates need not retain the general physicochemical properties of their ancestors, especially properties that are essential for replication.

In DNA, the polyanionic backbone dominates the physical properties of DNA. Replacing one nucleobase with another, therefore, has only a second-order effect on the physical behavior of the molecule. That allows nucleobases to be replaced during Darwinian evolution without losing properties essential for replication. Therefore, a repeating charge may be a universal structural feature of any genetic molecule that supports Darwinian evolution in water. Polycationic backbones may be as satisfactory as polyanionic backbones, however. Thus, if NASA missions detect life in water on other planets, its genetic system is likely to be based on polyanionic or polycationic backbones even if its nucleobases differ from those found on Earth. That structural feature can be easily detected with simple analytical devices.

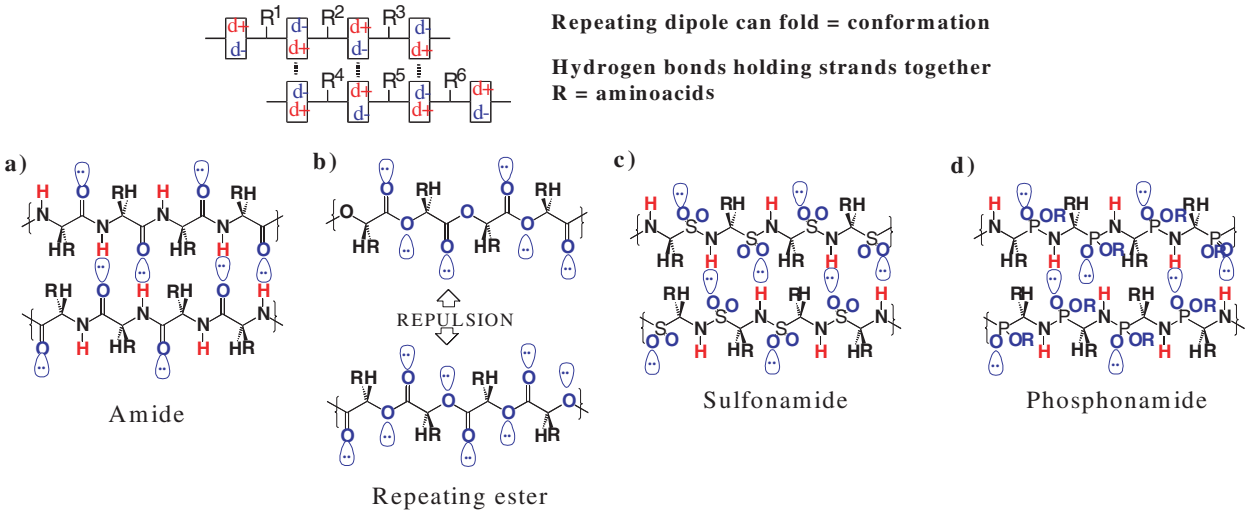

FIGURE 4.4 Some alternative backbone biopolymers with and without repeating dipoles. Red hydrogens can serve as hydrogen-bond donors because of their partial positive charge. Hydrogen-bond acceptors, atoms bearing a partial negative charge, are shown in blue. The polyesters (b) do not have a repeating dipole and therefore cannot form self-structure as easily. The lobes on the oxygen atoms represent unshared pairs of electrons.

4.2.2 A Repeating Dipole May Be Universal in Polymeric Catalytic Molecules in Water

As noted above, the specific 20 amino acids that are common in standard terran proteins need not be universal. But what about the amide (the unit that links amino acids in a polypeptide, a short protein) backbone? Unlike DNA and RNA, in which the repeating element is a monopole (a charge), a polypeptide chain has a repeating element that is a dipole. That is ideal for folding: The positive end of one dipole has a favorable energy of interaction with the negative end of another dipole. In contrast, a biopolymer based on a repeating ester linkage would not fold via backbone-backbone interactions (Figure 4.4). Folding is almost certainly required for efficient catalysis, so one might expect repeating dipoles, as found in polyamides, to be found throughout the galaxy in biospheres that are based on water.

Exobiological research offers reasons to expect that amino acids might be universal. Amino acids appear to be products of prebiotic synthesis. They are famously the products of the Miller process by which methane, ammonia, and water were subjected to electrical discharge. And amino acids are found in carbonaceous meteorites both as alpha amino acids that are standard in terran life and as the nonstandard alpha-methylamino acids found by Cronin and Pizzarello.[24]

The uniqueness of bonding between carbon, oxygen, and nitrogen makes it difficult to conceive of an alternative backbone that contains a repeating dipole. If one selects from the third row of the periodic table, repeating sulfonamide would be one possibility.[25] Repeating phosphonamides, in which the negative charge is blocked, would be another.[26]

Thus, whereas a repeating charge is important for a genetic molecule in water, a repeating dipole is important for the folding of a catalytic molecule, such as a protein enzyme, in water.

4.3 IS WATER UNIQUELY SUITED AS A BIOSOLVENT?

It is clear from abundant experiments in organic chemistry that assembly involving the interaction of dipoles is a good way to create supramolecular structure in many solvents that are not water. Indeed, dipolar interactions are generally stronger in nonaqueous, nonpolar solvents than they are in water. In water, the solvent molecules

interact with dipoles, competing with supramolecular assembly. Thus, even if repeating dipoles are a universal requirement for catalytic molecules, this does not exclude nonaqueous liquids as biosolvents.

That is not the case for a repeating charge. A repeating charge generally confers water solubility on a polymer, but it nearly always makes the polymer insoluble in nonpolar solvents. That means that the repeating charge cannot be used to manage the difficult combination of Darwinian evolution and general chemistry. As noted above, small changes in molecular structure generally create large, often chaotic, changes in molecular behavior. That is not tolerable in an encoding biopolymer that must support Darwinian evolution, in which case, the physical properties of the molecule must remain relatively constant when the informational content changes. The repeating charge in RNA and DNA allows that. The repeating charge (the monopole) dominates the physical properties so greatly that one can change the nucleobases, including their hydrogen-bonding dipolar units, with very little effect on the physical properties of the biopolymer. That strategy cannot be exploited in nonpolar solvents in which a polycharged polymer is insoluble.

Thus, if we regard the repeating charge as the only approach to enabling Darwinian evolution of an encoding genetic molecule, molecular genetics is possible only in polar solvents, such as water.

4.4 OPPORTUNITIES FOR RESEARCH

It is clear that the potential diversity of backbones, recognition elements, and linker groups that are structurally quite different from those known so far, but may nevertheless support genetic-like behavior, has only begun to be explored. There are abundant opportunities for research following the paradigm set forth in the emerging field of synthetic biology. The application of synthetic biology to construct biopolymers that could be suitable to planetary environments radically different from Earth's, such as at Titan, will require NASA missions that emphasize measurements of environmental properties that narrow the options for biopolymers.

The principal challenge in designing research programs in this field is in the number of possible alternative structures that can be drawn on paper. Chemical theory only imperfectly constrains the behavior of molecules whose structures can be drawn and therefore only modestly constrains the array of structures that can be studied. Clearly, high priority should be placed on exploring polyelectrolytes as alternative genetic materials and in developing instruments to detect these that might fly in the future to locales in the solar system where liquid water may be present. Polyelectrolytes detected there would be considered strong biosignatures, especially if they are thermodynamically unstable in their environment.

The rules for potential nucleobases have become better defined by synthetic biologists.[27] It is appropriate to couple design to experimental and theoretical considerations related to heterocycles that might be generated under prebiotic conditions. NASA should place high priority on Earth-based experiments that define what heterocycles might have been formed, especially in cometary bodies and on early Earth.

The same is appropriate for the exploration of potential alternative sugars. The prebiotic origin of sugars is still poorly defined. Recommended research activities focus on constraining possible sugar backbones in alien genetic material to the ones that are more plausibly generated under prebiotic conditions. Synthetic biologists are advised to explore alternative genetic systems that combine the heterocycles and sugars.

Alternative amino acids are easily conceived, both in theory and from experiment. Many alternative amino acids are known in meteorites (Figure 4.5). Several classes, including alpha-methylamino acids, form secondary structures more easily than do standard terran amino acids. Little is known, however, about the ability of polymers built from them to support catalysis.

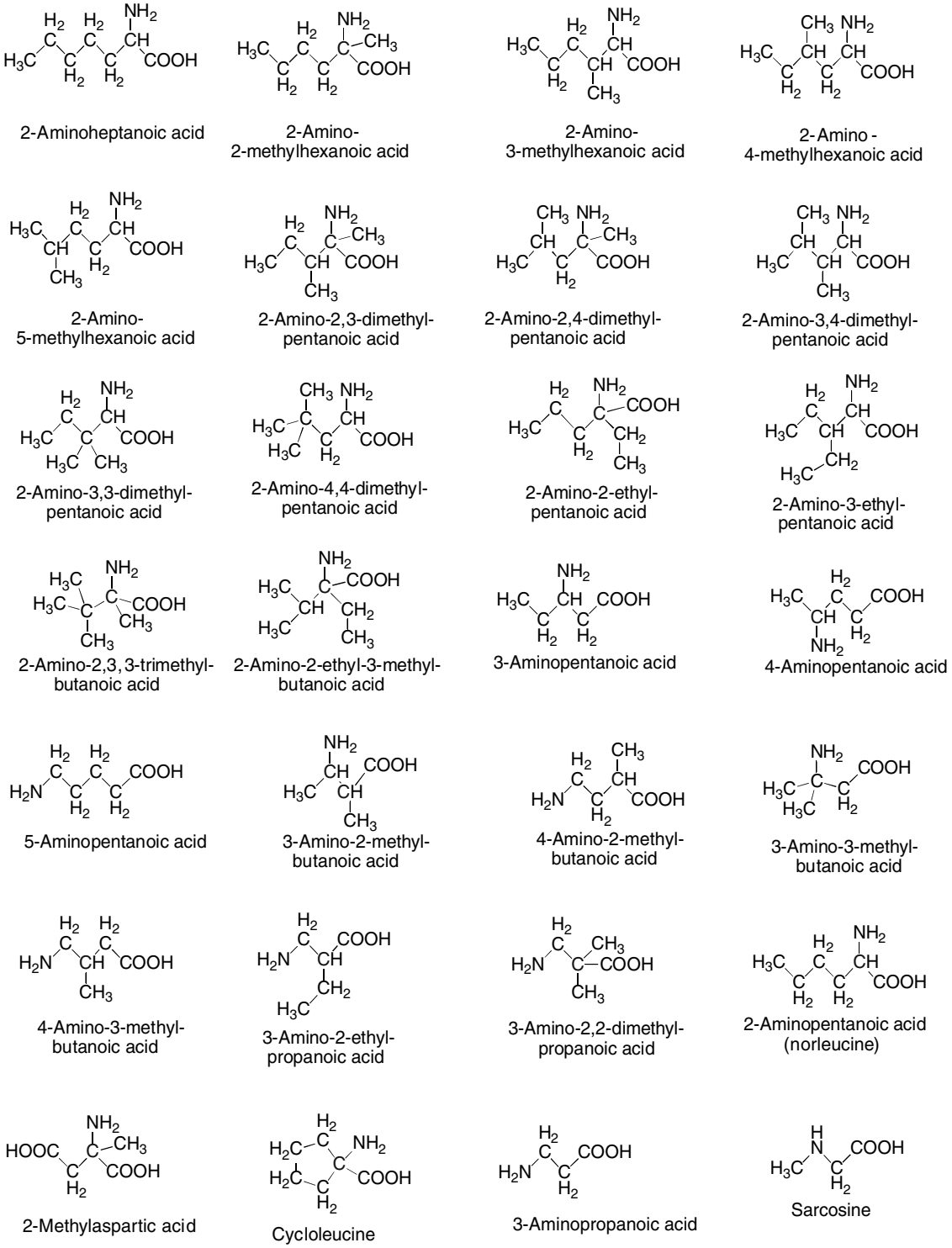

FIGURE 4.5 Amino acids reported from carbonaceous chondrite meteorites.

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5

Origin of Life

To search for additional constraints on the limits of life, the committee considered topics related to the origin of life. There is a clear distinction between environments that are habitable and environments that might support the emergence of animate matter from inanimate matter. Indeed, many observers believe that although the surface of modern Earth is a habitable environment, life could not emerge here now. According to that thinking, the dioxygen that is present in today's terran atmosphere would be toxic to any primitive life form that might emerge spontaneously.

It is conceivable that if we understood the processes by which life arises, we might constrain the existence of life to a small number of locales, to a similar array of organic species, or to a smaller number of liquid phases than the more general thermodynamic-structure-solvent trichotomy, discussed above, would tolerate. That would, in turn, be considered alongside emerging models of planetary formation to provide better direction for the targets of National Aeronautics and Space Administration (NASA) missions to the solar system.

Fifty years' worth of effort has shown that it is difficult to model how life might have originated in any specific environment. The committee recognized that it is still more difficult to consider how life might have arisen in a generic environment. The details of an environment almost certainly determine how life might emerge.

The environment on early Earth is not well defined. The committee recommends that more information be obtained from missions, especially to comets, and that models of planetary formation continue to be developed. It is not certain even that terran life originated on Earth. On the basis of various considerations—including the abundance of water, the paucity of some minerals, and the nature of the atmosphere modeled for early Earth—various authors have suggested that life on Earth originated elsewhere, including locales as nearby as Mars and as distant as galactic nebulas. The geological record is considered to be intact for 4.5 Ga on Mars and Ceres, so there may still be mineralogical and isotopic signatures indicating past life. However, the detection of signs of past life would not be evidence that Mars or Ceres had an origin of life separate from Earth's origin, nor would it provide evidence that panspermia occurred. In the absence of firm information concerning the requirements for the origin of life as we know it and the mechanisms of its formation, such speculation seems premature. Our knowledge of the environments in those remote locales several billion years ago is even less complete than our understanding of early Earth.

It is also not clear that terran life originated on Earth in the chemical form that we know now. Respectable hypotheses suggest, for example, that the three-biopolymer (DNA-RNA-proteins) system that characterizes all life that we know on Earth, a system in which nucleic acids serve roles predominantly in genetics and information transfer and proteins serve roles predominantly in catalysis, may not have been characteristic of life as it first originated. Only one of these complex chemical entities may have been represented in primitive life, or perhaps none at all.

Several hypotheses argue that RNA was the only genetically encoded component of biological catalysis during an earlier episode of life on Earth. Some others view that statement as true for the very first form of life on Earth (the RNA-first hypothesis). Others have argued that the first form of life on Earth was supported by genetic molecules that had structures quite different from the structure of DNA or RNA.

Some have even argued that the original genetic material was mineral, not organic.[1,2] They suggest that a truly primitive replicator might have been a layered inorganic mineral, crystallizing from solution and in the process amplifying some particular permutation of stacking: either identical layers stacked on top of each other in different orientations or stacks of two or more chemically different layers. The "information" would be the particular stacking sequence of a crystal displayed like a bar code on its edges and maintained and extended through crystal growth with ions, or small molecular units, adding only to the edges. The stacking sequences would also specify particular phenotypic properties that would allow Darwinian competition.

As is evident in Section 5.7, a case can be made that the earliest forms of life on Earth contained no macromolecules at all and that heredity was carried by monomers[3]—still another route for future exploration.

5.1 LABORATORY SYNTHESIS OF ORGANIC MONOMERS

It has been more than 50 years since Stanley Miller first explored electrically induced chemical reactions that might convert simple gases into small organic molecules.[4] The production of amino acids was especially easily demonstrated. More recently, the highly reducing atmosphere used by Miller has fallen out of favor as representative of the likely atmosphere on early Earth (although Kasting has shown that the impact of a large asteroid with iron causes a transient reducing atmosphere[5]). Even with more contemporary models of early planetary atmospheres, however, electrical discharge, ultraviolet radiation, and other sources of energy are suitable for creating organic species. For example, Box 5.1 lists compounds, called "tholins," produced from relatively oxidizing environments under these conditions.

BOX 5.1
Organic Compounds Identified in Tholin Mixtures

Hydrogen sulfide	Hexene	Formamide
Hydrogen cyanide	Heptene	Pyridine
Ammonia	Butadiene	Styrene
Ethane	Benzene	2,3 Pentadiene
Propane	Toluene	2-Methylpyrimidine
Butane	Thiophene	4-Methylpyrmidine
Ethene	2-Methylthiophene	3-Butenenitrile
Propene	Methylmercaptan	Butyne
Butene	Ethylmercaptan	Acetonitrile
Pentene	Propylmercaptan	Carbon dioxide
Carbon disulfide	Methylisocyanate	Acetamide

SOURCE: Derived from Sagan, C., Khare, N.B., Bandurski, L.E., and Batholomew, N., 1978, Ultraviolet-photoproduced organic solids synthesized under simulated Jovian conditions: Molecular analysis, *Science* 199:1199-1201; Sagan, C., and Khare, N.B., 1979, Tholins: Organic chemistry of interstellar grains and gas, *Nature* 277:102-107; and Pietrogrande, M.C., Coll, P., Sternberg, R., Szopa, C., Navarro-Gonzalez, R., Vidal-Madjar, C., and Dondi, F., 2001, Analysis of complex mixtures recovered from space missions: Statistical approach to the study of Titan atmosphere analogues tholins, *J. Chromatogr. A.* 939:69-77.

TABLE 5.1 Carbon in the Murchison Meteorite

Total carbon	2.12%, 1.96%
Carbon as interstellar grains	
Diamond	400 ppm
Silicon carbide	7 ppm
Graphite	<2 ppm
Carbonate minerals	2-10% of total carbon
Macromolecular carbon	70-80% of total carbon

SOURCE: Modified after J.R. Cronin, "Clues from the Origin of the Solar System: Meteorites," pp. 119-146 in *The Molecular Origins of Life: Assembling Pieces of the Puzzle,* A. Brack A. (ed.), Cambridge University Press, Cambridge, U.K., 1998.

Similar experiments have generated nonbiological routes for the synthesis of other organic molecules, including some molecules that are used in our own biochemistry. For example, the Oró-Orgel synthesis exploits the reactivity of HCN to make adenine ($C_5H_5N_5$), one of the five nucleobases used to store information in DNA and RNA. Analogous synthesis generates adenine from formamide.

The complexity of the products of adding energy to simple organic mixtures, including the complexity of tholins, has a disadvantage. The diversity of products is so great in such experiments in prebiotic chemistry that they do not greatly limit the inventory of organic species that might have been present on early Earth.

5.2 NATURAL AVAILABILITY OF BIOLOGICAL-LIKE MOLECULES

5.2.1 Biological-like Molecules from the Cosmos

There is little doubt that natural processes generate organic molecules analogous to those generated by the laboratory experiments described above. Amino acids are found in natural specimens, including meteorites, that are almost certainly not influenced by biological processes. They include many amino acids that are not part of the human-like standard collection of encoded amino acids.

Some chemical fragments of DNA and RNA can also be found in meteorites (Tables 5.1 and 5.2). For example, some meteorites have been reported to contain small amounts of adenine, one of the nucleobases found in RNA and DNA. The current view is that the Murchison meteorite contained adenine, guanine, their hydrolysis products hypoxanthine and xanthine, and uracil. The reported concentration of all those substances, however, is low, about 1.3 ppm. The Murchison and other meteorites may also contain ribitol and ribonic acid, the reduced and oxidized forms of ribose, respectively, but ribose itself has not been found.[6]

TABLE 5.2 Organic Compounds in the Murchison Meteorite

Amino acids	60 ppm	Purines and pyrimidines	1.3 ppm
Aliphatic hydrocarbons	>35 ppm	Basic *N*-heterocycles	7 ppm
Aromatic hydrocarbons	15-28 ppm	Amines	8 ppm
Carboxylic acids	>300 ppm	Amides	55-70 ppm
Dicarboxylic acids	>30 ppm	Alcohols	11 ppm
Hydroxycarboxylic acids	15 ppm	Aldehydes and ketones	27 ppm

SOURCE: Data from Cronin, J.R., and Pizzarello, S. 1986. Amino acids of the Murchison meteorite. III. Seven carbon acyclic primary alpha-amino alkanoic acids. *Geochim. Cosmochim. Acta* 50:2419-2427.

TABLE 5.3 The Organic Content of the Tagish Lake Meteorite

Aliphatic hydrocarbons	5 ppm	Dicarboximides	5.5 ppm
Aromatic hydrocarbons	≥1 ppm	Sulfonic acids	20.0 ppm
Dicarboxylic acids	17.5 ppm	Amino acids	≥0.1 ppm
Carboxylic acids	40 ppm	Amines	<0.1 ppm
Pyridine carboxylic acids	7.5 ppm	Amides	<0.1 ppm

SOURCE: Data from Pizzarello, S., Huang, Y.S., Becker, L., Poreda, R.J., Nieman, R.A., Cooper, G., and Williams, M. 2001. The organic content of the Tagish Lake meteorite. *Science* 293:2236.

We do not know the extent to which the Murchison organics reflect what was available on early Earth before life emerged. The rich inventory of amino acids does not appear to be universal in carbonaceous chondrites (although the number that have been examined in detail is very small). For example, only a few amino acids (glycine, alanine, α-aminoisobutyric acid, α-amino-*n*-butyric acid, γ-aminobutyric acid) are found in the meteorite that fell in 2000 on Tagish Lake, Canada (Table 5.3).[7] The near absence of complex amino acids is significant, inasmuch as the meteorite was captured in a pristine condition soon after it fell.

It is also significant that no discovery of a dipeptide in meteorites has yet been reported. Joining two amino acids is the first step toward the synthesis of proteins, such as those found in contemporary terran life. If the meteorite organics analyzed to date are representative of planetary processing of primitive organic compounds, the process of assembling amino acids into polypeptides (short strings) may have been carried out first within living cells.

5.2.2 Biological-like Molecules from Planetary Processes

Current research is showing the interactions between organic molecules and a wide array of minerals. These include the formation of carboxylic acids in thermal vent chemistry and the formation of reduced chemical species through photochemistry involving semiconducting minerals.[8]

5.2.3 The Origin of Phosphorus

Phosphorus is an important component of terran life, but its synthesis in stars is not simple. It is produced as phosphorus-31 in stars with 15 protons and 16 neutrons and hence is an "odd-Z" element. Odd-Z elements are more difficult to produce than "even-Z" elements having an equal number of protons and neutrons; these can be produced from other even-Z elements via an "alpha chain" from helium. Odd-Z elements like P-31 are generally produced in abundance only when there is an excess of neutrons to protons. This excess emerges only as the universe ages, implying that life based on phosphorus cannot have emerged early in the life of the universe. Odd-Z elements are also less abundant in the Sun than common elements, such as carbon and oxygen, by factors in excess of 100, although current models with sophisticated stellar nucleosynthesis account rather well for the observed phosphorus abundance in the Sun.[9]

Phosphorus is abundant on Earth, both as an element (the 11th-most abundant atom in Earth's crust) and as phosphate. Meteorites hold a variety of phosphate-containing minerals and some phosphide minerals.[10] Scientists at the University of Arizona have recently suggested that Fe_3P, the mineral schreibersite, leads to the formation of phosphate and phosphite when corroded in water. Although phosphorylation of alcohols was not demonstrated, mechanistic considerations suggest that it should be possible. It is noteworthy that a clear prebiotic pathway for the chemical incorporation of phosphate into RNA or DNA has not been found. No nucleosides (nucleobases joined to sugars) have been reported from meteorites. Nor has evidence been found in any meteorite of the presence of nucleosides or nucleotides (nucleosides attached to phosphates). That suggests that nucleic acids were first formed as products of metabolism.

5.2.4 The Origin of Metabolism

Considerations of the emergence of life on Earth have often focused on the spontaneous abiotic formation of RNA, which is both a genetic polymer and a catalytic polymer. The chemical complexity of this molecule suggests that the probability of such an event, although not zero, is extremely small, given the absence on present-day Earth of conditions that would favor its formation.[11]

Catalysts may have played an important role in establishing the early metabolism that ultimately led to the biosynthesis of RNA. An intriguing possibility is that modern metabolic pathways emerged by a stepwise process of recruitment of ever more effective catalysts to catalyze steps in a primordial chemical-reaction network. Transition-metal sulfides and mineral surfaces are known to be able to catalyze the formation of simple organic compounds. Later, small molecules—such as amino acids, short peptides, and cofactors—may have catalyzed reactions required to produce more complicated organic compounds. Although their catalytic abilities are known to be limited in both acceleration and specificity compared with later macromolecular RNA or protein catalysts, some small molecules are remarkably effective catalysts. For example, pyridoxal phosphate catalyzes the rate of decarboxylation of amino acids by 10 orders of magnitude.[12] That cofactor has been retained at the active site of many modern enzymes. Iron-sulfur clusters are also found in many modern enzymes and may be relics of a time in which they catalyzed similar reactions but without the context of the protein. A stage in which RNA provided the best function, either alone or in combination with peptides that helped to stabilize folded RNA structures, may have come next.[13] What is clear is that of the synthesis of polypeptides by the translation of encoded RNA eventually became a focus of natural selection, in which the fitness of organisms depended critically on the catalytic abilities of enzymes involved in metabolic processes. In that transformation, protein enzymes replaced most RNA enzymes. An alternative possibility is that RNA catalysis never exceeded the extent to which it is present in modern biochemistry and that short peptides and cofactors carried the catalytic burden until the development of translation.

That diversity of possibilities creates ample opportunities for Earth-based experimental work in the origin of life, and the committee recommends enhanced efforts to exploit them. Such Earth-based research is critical for informing the design of planetary missions whose payloads can detect the conditions for the emergence of life and also detect primitive life, especially life that has not yet evolved to the point where it synthesizes peptides by translation.

5.3 THERMODYNAMIC EQUILIBRIA

Given a source of organic precursors, the question remains, Which reactions might have occurred with and between the precursors on early Earth, and in what quantities would they have been found? To address that question, the committee looked at the thermodynamic properties of molecules.

First, it considered the concept of the reduction-oxidation (redox) state, which is often used to describe organic and other molecules. The rules used to calculate an oxidation state are different between inorganic species and organic species. For example, $Fe++$ and $Fe+++$ have different redox states; the second lacks an electron that the first has. For organic molecules, however, the redox state is generally described with the ratio of the number of hydrogen atoms in a molecule to the number of heteroatoms.

Because its carbon is bonded to two oxygen atoms and no hydrogen atoms, carbon dioxide is as oxidized as a carbon atom can be. Methane, in which carbon is bonded only to hydrogen, is as reduced as a carbon atom can be. Formaldehyde is at the same oxidation level as elemental carbon (because it has an equal number of bonds to hydrogen and oxygen). Viewed alternatively, the ratio of hydrogen atoms (2) to oxygen atoms (1) is the same in formaldehyde as in water. Thus, compounds of the formula $C_n(H_2O)_n$ can be converted to elemental carbon by heating, which extrudes water without a net change in the redox state of the carbons.

At one level, understanding the thermodynamics of carbon-containing molecules with respect to oxidation or reduction is as simple as asking whether hydrogen or oxygen is more abundant in the environment. In the modern terran atmosphere, which contains abundant dioxygen, essentially all compounds that contain reduced carbon are thermodynamically unstable with respect to oxidation to carbon dioxide. From a thermodynamic perspective, virtually all organic matter placed in today's atmosphere will eventually "burn" and yield carbon dioxide

and water. The *rate* of the burning, however, can be very low at 20-40°C and at today's atmospheric oxygen partial pressure.

In the absence of oxygen and in the presence of H_2, reduced carbon is thermodynamically preferred. That is certainly true deep in the ocean, for example, near hydrothermal vents, where the synthesis of reduced organic compounds is thermodynamically favored. Shock, Cody, and others have exploited that fact to propose net synthesis of organic molecules in anoxic environments.[14,15]

A reaction that is thermodynamically "uphill" (not energetically favored) in one direction can become "downhill" in the same direction if the environmental conditions are changed. If (A + B) ⇔ (C + D), the reaction can be pulled to the right if D is removed, converting all (A + B) to C. Conversely, if excess D is added, C will be driven to (A + B). That behavior of equilibria often appears in textbooks as Le Chatelier's principle.

It is important to note that no biological compound can ever be said to be universally "high in energy." Each reaction has a free energy, or ΔG^0, which is measured at a standard, arbitrarily defined, concentration. ΔG^0 does not determine whether the corresponding chemical reaction runs in the forward or reverse direction, however. This is determined as well by the concentrations of the reactants and the products, and the direction in which the state is out of equilibrium. This is captured by ΔG, which reflects both ΔG^0 as well as the concentration of the reactants and products.

For this reason, it is not useful to speak of the "energy" of any particular compound. Rather, the free energy ΔG of a system, which makes a statement about whether it can do work, is determined by the degree to which the system is out of equilibrium. This, in turn, is defined by the equation $\Delta G = \Delta G^0 + RT \ln [\text{product}]/[\text{reactant}]$.

In that context, adenosine triphosphate (ATP), the currency of energy in all cells, is viewed as "high energy" only because at equilibrium the reaction ATP + water ⇔ ADP + inorganic phosphate contains more ADP and inorganic phosphate than ATP. If, however, the initial state contains ADP + inorganic phosphate and *no* ATP, the process spontaneously proceeds in the direction of the synthesis of ATP from ADP and inorganic phosphate. In that case, ADP and inorganic phosphate are the "high-energy" compounds.

Other generalizations concerning reactivity are based on the principles of thermodynamics. For example, organic molecules contain hydrogen atoms, which, given an appropriate catalyst or source of energy (ultraviolet light, for example), might generate H_2. Because H_2 molecules have a lower mass than other molecules, they move faster on average and therefore preferentially escape from planetary bodies, especially those with low mass and, consequentially, weak gravitational attraction. Although both the formation and the loss of H_2 may be slow, cosmic processes have time. A collection of organic molecules slowly becomes more oxidized through loss of H_2.

That is presumably what is occurring today on the surface of Mars. Above Mars, water is dissociated by ultraviolet radiation to yield H· and ·OH, the hydrogen radical and the hydroxyl radical. Two H· units can combine to give H_2. The H_2 then escapes from Mars, leaving behind HOOH, hydrogen peroxide. Under typical conditions on Earth, hydrogen peroxide might be viewed as a high-energy compound; on Mars, escape of H_2 leads to its formation over time. On an aqueous body, such as Europa, the hydrogen peroxide formed by radiation will decompose into water and oxygen. The oxygen would then be available for the biological oxidation of other organic compounds formed by radiation or water-rock reactions, such as methane and formaldehyde. The concentrations of formaldehyde and oxygen from radiation were considered sufficient to support a microbial ecosystem on Europa.[16]

Carbon is likely to congeal to high-molecular-weight polymers as H_2 distills off. In extraterrestrial environments, we expect lower hydrocarbons eventually to transform into pure carbon, either diamond (in which all the carbons are singly bonded to other carbons), fullerenes and graphite (in which each interaction between a pair of carbons is the approximate equivalent of 1.5 bonds), or carbon bonded to other elements that cannot be converted to a volatile form.

Polycyclic aromatic hydrocarbons can be viewed as "carbon on the way to forming graphite." They are common in extraterrestrial environments. Their central structures are fragments of graphite with bonding to hydrogen atoms at the edges. They become larger and larger, and more and more like graphite, as more hydrogen distills away.

5.4 PROBLEMS IN ORIGINS

Chemists' objection to the notion that life is a natural consequence of organic reactivity is simple and comes from broadly based empirical experience in organic-chemistry laboratories. Addition of energy to mixtures of

organic species makes the mixtures more complex and less likely to support life. Shapiro has provided a thoughtful and detailed discussion of the difficulties.[17-22] Briefly summarized, it suggests that existing prebiotic chemistry experiments do not offer plausible hypotheses for routes to complex biomolecules. In the complex chemical mixtures generated under prebiotic conditions, one may be able to find trace amounts of amino acids and perhaps nucleobases. Some might indeed catalyze reactions that have some utility. But other compounds may well inhibit catalysis or catalyze undesired reactions. For example, Joyce and Orgel pointed out that the clay-catalyzed condensation of nucleotides to yield small chains performed best, under the conditions that they considered, if only one enantiomer of the starting material was present. If both were present, the desired reaction with the desired enantiomer might be inhibited by the other enantiomer.[23] Furthermore, the combination of any bifunctional molecule into an information-bearing polymer would be expected to be terminated at an early stage by the presence of an excess of molecules that bear only one functionality.[24]

Even crystallization, a well-documented method of obtaining order through self-organization, is not a particularly powerful way to separate mixtures of organic chemicals into their constituents. Normally, an organic compound must be relatively pure before crystallization occurs. That salts crystallize better may explain why crystals are more common in the mineral world than in the organic world. Even organic salts can have problems in crystallizing from an impure mixture.

Those facts generate the central problem in prebiotic chemistry. Spontaneous self-organization is not known to be an intrinsic property of most organic matter, at least as observed in the laboratory. It can be driven only by an external source of free energy that is coupled to the organic system.

5.4.1 Nucleophilic and Electrophilic Reactions Can Destroy as Well as Create

As described above, formidable chemical obstacles oppose the abiotic synthesis of such biopolymers as RNA, DNA, and protein despite their prominence in life today. Numerous degradative processes would also hinder any event of that kind. The same inherent reactivities that generate organic molecules can convert them into complex mixtures. An example can be seen in the processes that might have generated the sugar ribose, a key component of RNA and DNA, under prebiotic conditions. A reaction called the formose reaction is known to produce ribose by converting formaldehyde in the presence of calcium hydroxide into several sugars, including ribose.[25-27]

The formose reaction exploits the natural electrophilicity of formaldehyde and the natural nucleophilicity of the enediolate of glycolaldehyde, a carbohydrate that has been detected in interstellar clouds.[28] That species reacts as a nucleophile with formaldehyde (acting as an electrophile) to yield glyceraldehyde. Reaction of glyceraldehyde with a second equivalent of the enediolate generates a pentose sugar (ribose, arabinose, xylose, or lyxose, depending on stereochemistry). A curved-arrow mechanism describes this process in Figure 5.1.

Despite the reactivity inherent in glycolaldehyde and formaldehyde, the formose reaction does not offer a compelling source of prebiotic ribose. Under typical formose reaction conditions, ribose not only forms but also decomposes. In the presence of calcium hydroxide, ribose is rapidly converted to a mixture of organic species; this mixture has never been thoroughly characterized, but it does not appear to contain much ribose, and it is not an auspicious precursor of life. The further reaction of ribose in the presence of calcium hydroxide occurs because ribose itself has both electrophilic and nucleophilic sites, respectively, at the aldehyde carbon and at the carbon directly bonded to the aldehyde (after enolization—see Figure 5.1). Molecules having both reactivities tend, as expected, to polymerize as the nucleophilic sites and electrophilic sites react with each other, with more formaldehyde, with water, or with other electrophiles in the increasingly complex mixture. Those reactivities undoubtedly cause the rapid destruction of the ribose formed under formose conditions. On the basis of those reactivities, Larralde, Robertson, and Miller concluded that "ribose and other sugars were not components of the first genetic material."[29]

A solution to the instability of ribose has been offered by Eschenmoser, Arrhenius, and others. It has focused on the generation of sugar phosphates, which have long been known to be more stable to degradation under alkaline conditions. A possible mechanism for forming them is shown in Figure 5.2.

For those reasons, some have suggested that life may have begun with an alternative organic compound as a genetic material, not RNA, but have been based on molecules that are less fragile.[30] They are commonly suggested to be molecules that do not have carbohydrates in their backbones. Underlying that concept is the notion

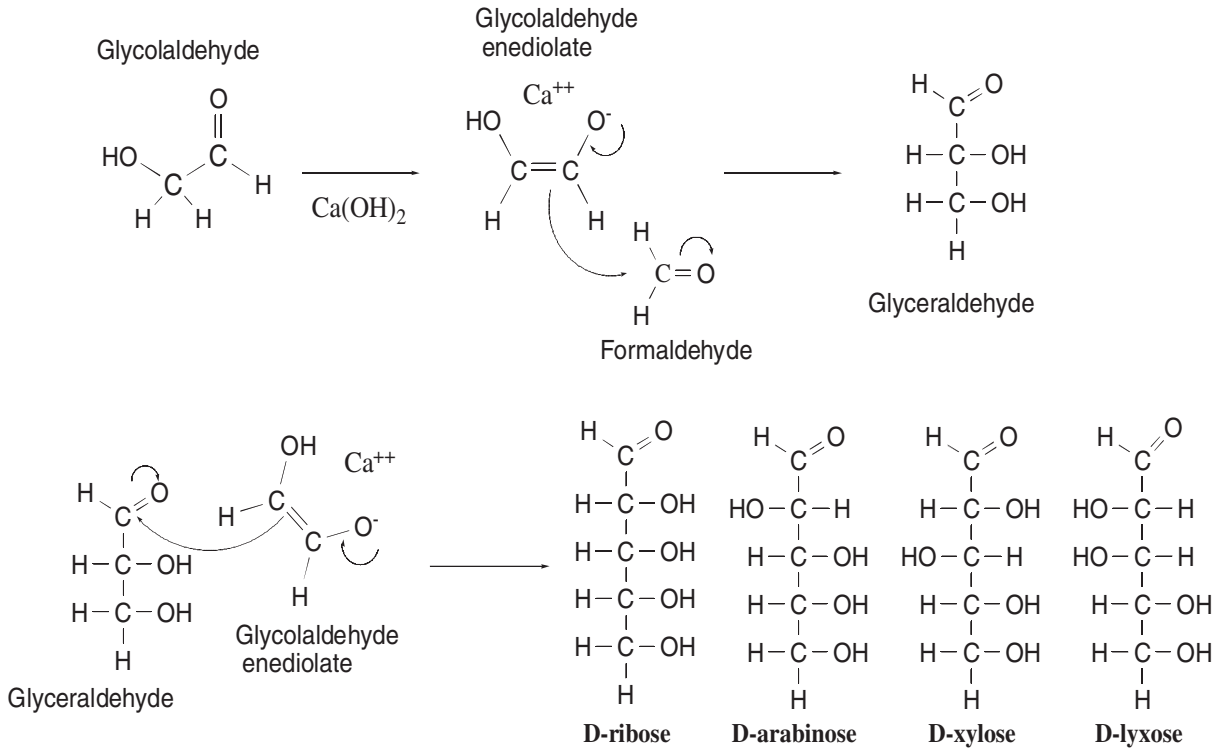

FIGURE 5.1 The aldol addition reaction, called an addition because the reaction adds one molecule to another. This addition reaction yields D,L-pentoses (only D-pentoses shown; note that the L-pentoses arise from reaction of L-glyceraldehyde and that all the species formed are formed as racemic mixtures) by combination of the enediolate of glycolaldehyde and glyceraldehyde.

of a "genetic takeover," in which delicate RNA or DNA molecules arose rather late in the development of life, supplanting a hardier genetic molecule on which life was founded.[31]

The reactivity of nucleophilic and electrophilic centers can convert molecules that were plausibly present on early Earth into biologically interesting products. But the products themselves often have nucleophilic and electrophilic centers and will therefore react further and yield less interesting products. That is the central paradox associated with theories positing that the origin of life involved a polymeric replicator, even given plausible mechanisms for the creation of its components.

5.4.2 The Reactivity of Water Constrains Routes to Origins

As noted above, the nucleophilicity of water allows it to enter into reactions that cause the degradation of biological macromolecules, including DNA and proteins. Analogous problems are associated with the assembly of biopolymers. In water, the assembly of nucleosides from component sugars and nucleobases, the assembly of nucleotides from nucleosides and phosphate, and the assembly of oligonucleotides from nucleotides are all thermodynamically uphill in water.

That is also true for polypeptide chains that join amino acids. Two amino acids do not spontaneously join in water. Rather, the opposite reaction is thermodynamically favored at any plausible concentrations: polypeptide chains spontaneously hydrolyze in water, yielding their constituent amino acids. Those obstacles can be avoided if we adopt an alternative explanation: that life began with a mixture of small molecules rather than with a biopolymer.

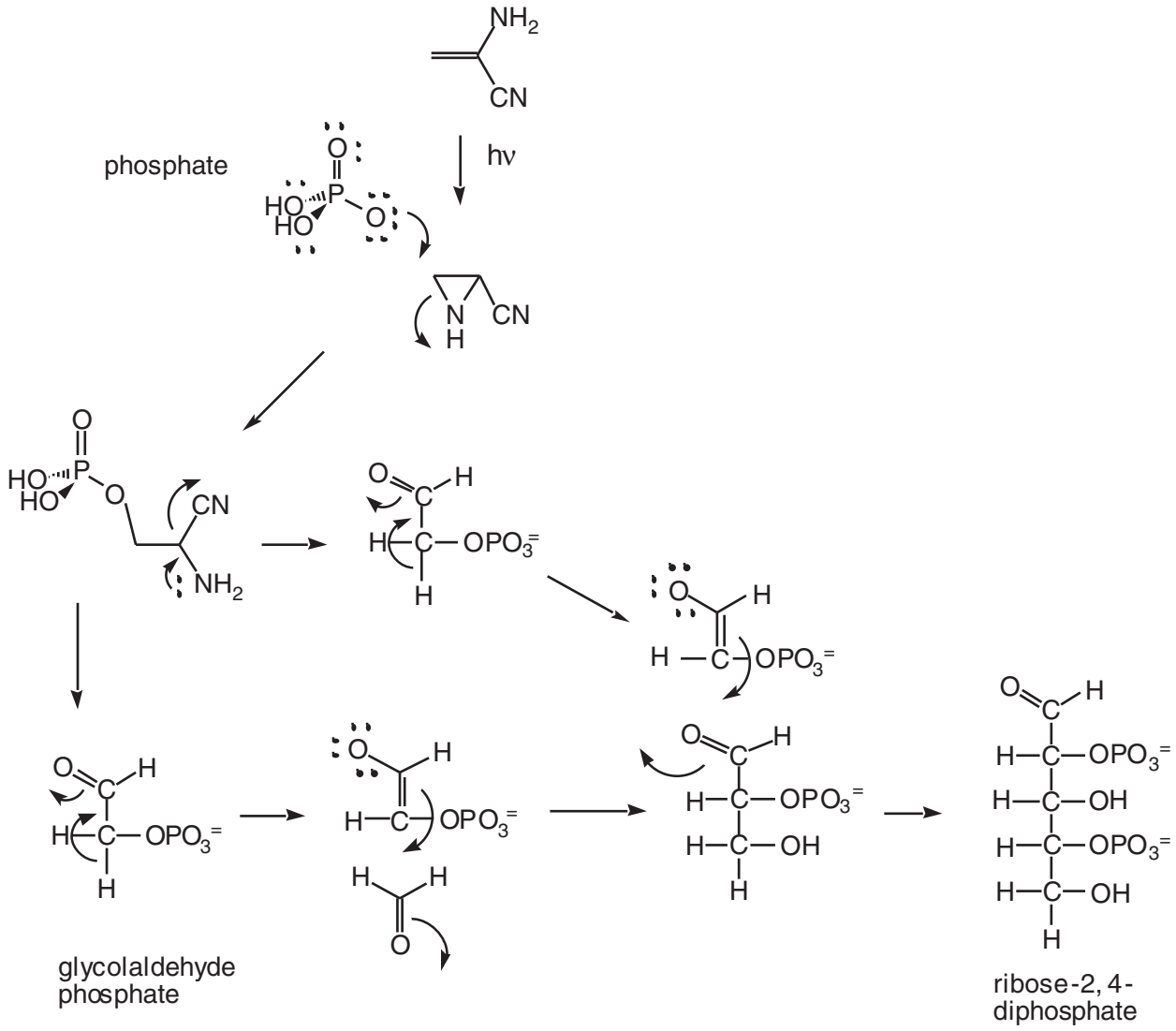

FIGURE 5.2 Synthesis of ribose-2,4-diphosphate from glycolaldehyde phosphate, as proposed by Eschenmoser.

5.5 MINERALS AS A POSSIBLE SOLUTION TO THE INSTABILITY OF RIBOSE

Despite the difficulties outlined above, the intellectual elegance of the "RNA world" theory of the origin of life[32] has led many scientists to seek abiotic routes to the components of RNA. That can be illustrated with the chemical instability of ribose.

One approach to stabilize ribose exploits the fact that borate forms complexes with 1,2-dihydroxy units in organic molecules. The borate complex carries a negative charge. The anionic nature of the complex should prevent glyceraldehyde from losing a proton to create a nucleophilic enolate but not prevent glyceraldehyde from reacting as an electrophile with the enediolate of glycolaldehyde to generate pentoses.

Furthermore, the 1,2-dihydroxy unit of the cyclic form of ribose should form a stable complex with borate. That would stabilize the cyclic form of ribose at the expense of the aldehyde form. It should render ribose largely

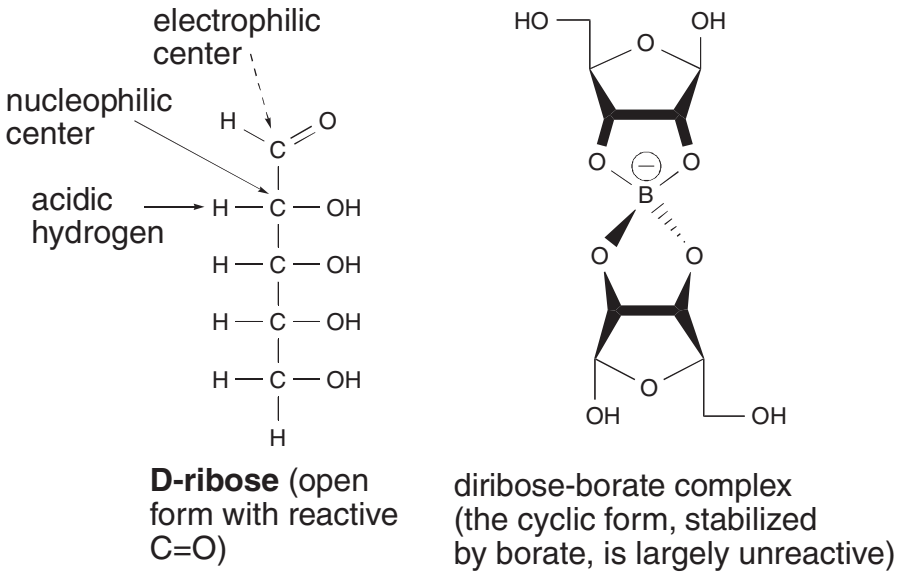

FIGURE 5.3 Borate minerals stabilize ribose under conditions in which it is formed from glycolaldehyde and glyceraldehyde. Wedges are used to describe bonds that project above and below the plane of the paper.

unreactive as either a nucleophile or an electrophile, in that the cyclic form lacks a C═O carbonyl group, which is the center of this molecule's electrophilicity (Figure 5.3).

Experiments confirm that reasoning. In the presence of $Ca(OH)_2$ under formose conditions at temperatures of 25-85°C, a solution of glycolaldehyde and glyceraldehyde rapidly turns brown. The brown mixture holds little ribose. When the same incubation takes place in the presence of the borate-containing mineral colemanite ($Ca_2B_6O_{11}\cdot 5H_2O$), however, the solution does not turn brown, and ribose, with other five-carbon carbohydrates, is found.[33] Borate has evidently constrained the intrinsic reactivity of glyceraldehyde to form tar, causing it to enter a productive reaction with glycolaldehyde.

Boron is not abundant in the solar system, although it is known in carbonaceous chondrites, where it is almost certainly present as borate.[34] Borate is, however, excluded from many mineral-forming processes, appearing in tourmalines, minerals best known as gemstones. Tourmaline weathers to generate borates, which have appreciable solubility in water. As a consequence, colemanite and other borate-containing minerals are delivered to deserts by runoff from the weathering mountains and are crystallized from water as it evaporates to yield evaporites. Such conditions are close to what is needed to generate ribose and related sugars.

Curiously, those conditions are also found in the most recent explorations on Mars. Although the instrument package delivered to Mars was not configured to detect either boron or ribose, the ratio of chloride to bromide salts is consistent with the formation of evaporite minerals on Mars. Given the appropriate source rocks, these should include alkaline borate minerals, analogous to those found in, for example, Death Valley. (The key mechanism for obtaining alkaline water on Earth is the conversion of olivine minerals to brucite in a process known as serpentinization; this is the process that raises the pH of Lake Mono, for example.) These are exactly the conditions described above that generate ribose. Indeed, noting that such conditions may not have been present on early Earth, Kirschvink has suggested that terran life originated from ribose formed on Mars.[35]

Solutions of that type are improbable. In exchange for a simplification mixture of the formose reaction, we must specify the presence of glycolaldehyde, glyceraldehyde, and borate minerals (and the absence of many potentially interfering substances, such as cyanide) and a route by which the protective borate will be removed so that the route to oligonucleotide formation remains open. Ribose is only one of the six components present in RNA. An extended number of steps involving the synthesis, purification, transportation, and highly specific

connection of the six components would be required to generate even a short RNA molecule, let alone one with biological function.

5.6 MINERALS INVOLVED IN THE CONSTRUCTION OF BIOMOLECULES

Minerals can participate in many ways in the synthesis and interconversion of organic species. For example, Martin and colleagues at Harvard have noted that sphalerite can convert photochemical energy into chemical energy.[36] Minerals can also provide a direct source of redox chemical energy, such as in the conversion of sulfides to disulfides and vice versa. In addition, they may catalyze reactions and provide compartments to house evolving chemical systems.

5.7 SMALL-MOLECULE ("METABOLISM FIRST") THEORIES OF LIFE'S ORIGIN

5.7.1 Life Without a Replicator

Replicator theories, which state that life began with the spontaneous formation of RNA or another information-rich genetic polymer that could direct its own replication, face difficulties in fundamental chemistry. These obstacles have long been thought to place them in the category of extremely improbable events. The appearance of such a replicator appears to require the combination of many chemicals in a long reaction sequence in a specific order, interspersed with a number of complicated separations, purifications, and changes of location.[37] Physical law does not forbid such a process, but if a replicator initiated life and no natural environments can be found that make its generation favored, life may be very rare in the universe, and life that we encounter elsewhere is likely to be a result of panspermia.

It is reasonable to consider the assumption that life began, somehow, among one of the mixtures of small organic molecules that are produced by abiotic processes. The only natural examples in hand today are the components of meteorites that have fallen to Earth (see Section 5.2.1) and particles returned by the Stardust mission. Spectroscopy has also yielded partial lists of the organic molecules in interstellar space and interplanetary dust clouds.

For such a mixture to move in the direction of life, self-organization would be necessary. That process would increase the concentration of some components of the mixture either at the expense of others or by new synthesis from raw materials, such as carbon monoxide or carbon dioxide. An external source of free energy would be needed to drive the changes, which otherwise would involve an overall negative change in entropy.

That view of the origin of life has commonly been called "metabolism first"; the absence of a genetic polymer has been equated with the lack of any mechanism for heredity. As we have seen, replicator theories center on the spontaneous formation of large, information-bearing organic polymers endowed with the ability to copy themselves. The hereditary information carried in the sequence of such a polymer is called a genome.

In the words of Lancet and colleagues, a "fundamentally different approach has envisaged primordial self-replication as the collective property of ensembles of relatively simple molecules, interconnected by networks of mutually catalytic interactions."[38] The hereditary information in this case would be represented by the identity and concentration of its components. The term *compositional genome* has been used to describe this system, in which genetic information is not stored in a list, as in DNA, but is represented by the presence or absence of organic components.[39,40] As an analogy, consider DNA to be the equivalent of a class list that records the full possible enrollment in a course. The information in a compositional genome would be represented by the presence of students who have turned up on a particular day.

A molecular assembly with a compositional genome would adapt to changes in the surrounding environment by altering the composition of the system and in the reactions used to sustain it. Growth of the system would take place through the acquisition or synthesis of additional quantities of the key components, and reproduction would occur when physical forces split the enlarged system into two or more fragments. For successful reproduction, each "daughter" fragment resulting from the division should contain a sufficient quantity of the key molecules to enable the networks of mutually catalytic interactions to continue. Such networks have been demonstrated in computer simulations,[41,42] and "an experimental demonstration that amphiphilic assemblies display self-replication behavior" has been carried out.[43] According to Lancet and colleagues, when competition for material and

energy was established between the fragments, Darwinian natural selection would then provide a driving force for further evolution.[44]

Those concepts are not new. They were initiated in the ideas of Alexander Oparin during the first half of the 20th century, and a limited but accurate scheme of this type was presented by Eakin.[45] A summary of the ideas of a number of contributors—including Harold Morowitz, Christian de Duve, Gunther Wächtershäuser, Stuart Kauffmann, Freeman Dyson, Michael Russell, and Doron Lancet—can be found in a review by Fry.[46]

The experts do not agree, however, on specific details of the type of energy to be used, the source of the organic raw materials, the identity of the responsive chemical system, and the most suitable location on the early Earth for chemical self-organization. A consensus exists that some barrier is needed to protect the evolving entity from dispersal by diffusion, but the experts differ on the nature of the barrier. The committee can provide only a brief account of the more prominent suggestions here, and the listed references (and in some cases other sections of this report) should be consulted for more details. Some of the key variables are summarized below.

- *The energy source.* A variety of possibilities were available on early Earth.[47,48] Morowitz has considered solar radiation and chemical redox energy to be the most significant and favored the latter because of the difficulty of effectively harnessing solar radiation.[49] The redox energy is derived primarily from encounters between the effluents of a reduced mantle and the more oxidized atmosphere and lithosphere. The oxidized regions are produced by the photochemical decomposition of water and the loss of hydrogen to space. Reduced and oxidized species are brought into contact by volcanism and other geological processes, which produce a supply of available free energy.[50] Section 2.4.2 provides an overview of the most prominent energy sources currently supporting terran life.
- *The location.* In the past, the suggestions have varied from Darwin's "warm little pond" to the global ocean as a gigantic "prebiotic soup." Three sites have received special attention recently: hydrothermal deep-sea vents[51] and mounds[52] and the ocean-atmosphere interface.[53,54]
- *The diffusion barrier.* Much attention has been directed toward primitive amphiphile vesicles, inasmuch as they self-assemble from simple components and have an obvious ancestral connection with the more complex membranes that enclose modern cells. A review has been provided by Monnard and Deamer.[55] The papers by Segre et al. and Hanczyc et al. contain additional discussion.[56,57] Other prominent alternatives that would limit loss by diffusion have been electrostatic forces at mineral surfaces,[58] iron sulfide membranes,[59] and aerosols at the ocean-atmosphere interface.[60] Section 2.7.1 discusses the function of compartmentalization in Earth life today.
- *The source of organic materials.* Possible sources cited have included mineral-catalyzed hydrothermal synthesis, atmospheric syntheses driven by radiation or electrical discharges (see Section 5.1), and delivery from outer space (see Section 5.2). Cleaves and Chalmers have summarized the alternatives.[61]
- *The reactive chemical system.* Some scientists have attempted to specify key components of such a system but not the entire reactive system. See, for example, de Duve[62,63] and Weber.[64,65] Others have suggested complete chemical cycles. Modified versions of the reductive citric acid cycle, a carbon-fixation pathway that is used by several organisms today, have been proposed.[66-70]

5.7.2 Coupling to an Energy Source as a Driver of Chemical Self-organization

Some versions of "metabolism first" schemes have drawn criticism in the literature.[71,72] The schemes have assumed that a self-sustaining metabolic network must be autotrophic;[73] that is, it must obtain its carbon supply entirely from carbon dioxide, rather than using carbon compounds made by abiotic processes (Sections 5.2.1 and 5.2.2). The assumption that chemicals in proposed reactions in the central metabolic cycle will be catalyzed by other compounds that participate in the cycle has also been questioned.[74] Such features are attractive but not essential to the idea of life with small molecules. It has been argued that the objections can be met in principle by introducing a small number of assumptions:[75]

- A thermodynamically favorable, irreversible "driver" reaction is coupled directly to an external source of available free energy and can occur in a plausible abiotic setting. The term *coupled* indicates that one process cannot occur without the other. Many examples of coupled reactions occur in modern biochemistry. In an abiotic setting,

such coupling would make a particular reaction favorable from a thermodynamic point of view. Other reversible processes that could produce the starting material for the driver reaction would be shifted in that direction.

- A multistep reversible pathway can convert the product of the driver reaction back to the starting material, completing a cycle. If such a pathway existed, the synthesis of its members would be favored at the expense of competing reactions to maximize the discharge of the free-energy source. Energy-driven self-organization would take place.
- The cycle functions at a "profit" in its environment: the gain of carbon by the cycle exceeds its loss by all mechanisms. Some losses of material from the central cycle are inevitable, either by diffusion or by irreversible side reactions that lead to such products as unreactive molecules or insoluble tars. For the metabolic network (the central cycle and associated reactions that feed material into it) to grow, the losses must be compensated for by the absorption of carbon dioxide or organic substances provided by the environment.

The diversity of organic chemistry, with its harvest of competing, interconnected reactions,[76,77] would become an asset rather than a liability in the case of the energy-driven system described above. The existence of side-reaction paths could provide the network with the capacity of reacting to changes in the environment, for example by providing an alternative path for closure of the cycle if one central step were hindered. If alternative pathways were explored, a variety of catalysts and substances that hinder loss of material might be encountered and added to the network, enhancing its capability.[78]

5.7.3 Significance and Implications for Astrobiology

The above discussion uses organic chemistry as a reference point, but no specific compounds are identified. Any abundant set of interconnected reactions that is compatible with a specific geochemical environment might qualify as a starting point for metabolism. Although it is logically possible that only a single solution exists, which corresponds to the start of life on Earth, there is no reason to believe that this must be the case. Some requirements for the small-molecule scenario have been specified, but they are far more permissive than those required by the polymeric-replicator theory. The proposal involving metabolism suggests a universe in which energy-driven self-organization may take place under a variety of circumstances and afford a harvest of "weird" life. The polymeric-replicator theory points to a cosmos that may be barren except for Earth.

5.8 OPPORTUNITIES FOR RESEARCH

5.8.1 Research on Earth

After a long hiatus, research in prebiotic chemistry once again has momentum. For the proposal that life originated in complex mixtures of small molecules ("metabolism first"), there is a framework but not a specific recipe to illustrate how a coupled free-energy source could initiate the process of self-organization. A detailed discussion of the possible further development of one system of this type[79] can be found in Lindahl.[80] In the long run, the question will be answered by space missions, but appropriate laboratory experiments may provide some guidance.

The first priority in such experiments should be to characterize any systems that will self-organize when coupled to appropriate free-energy sources. The principal initial task will be the identification of candidate driver reactions. There should be no need to specify the remainder of the system in advance once a plausible driver reaction has been found. If the materials and coupled energy source needed for the reaction were brought together, perhaps with an input of simple carbon-containing compounds to provide for growth, the metabolic network should establish itself, and its identity could be determined with simple analysis. Nature will be instructing us, rather than our attempting to impose our schemes onto it. The information that we would gather by observing such a system could enable us to identify the later steps, such as compartment formation, in the energy-driven self-organization process that leads to life.

Alternative opportunities await those who continue to explore replicator-based solutions for the origin of life. In the RNA-first tradition, a number of groups are continuing to search for plausible prebiotic syntheses for ribose

and other RNA components. Several involve mineral combinations that are now thought to be likely on Mars. Further research is needed to constrain the inventory of water on early Earth.

The largest remaining paradox in replicator-first theories concerns how to address the unfavorable interactions between water and biological molecules. Even if the heterocycles and sugars can be prepared and stabilized and can accumulate under prebiotic conditions, there are few good approaches to assembling them in water uphill against a thermodynamic gradient favoring hydrolysis. That paradox may be partially resolved by considering alternative solvents.

The possibility of life based entirely on minerals could also be explored on Earth, both in field work and in controlled laboratory studies. Attention could be given to the relationship between mineral morphology and the detailed sequence of layers in the mineral. Examples of edge catalysis of organic reactions by minerals could be sought, as well as edge adsorption of abnormal inorganic cations and organic molecules. Above all, it should be asked whether edge growth could perpetuate stacking irregularities that would afford the minerals that contained them an advantage in survival.

5.8.2 Research in Space

In the search for weird life in space, "replicator first" and "metabolism first" theories share a goal: "follow the carbon." The goal is to identify organic mixtures that differ sharply in composition from the nearly random collections identified in meteorites. For "metabolism first" advocates, such collections would represent key components of metabolic cycles. For "replicator first" supporters, the compounds might include the building blocks of genetic polymers. Such a strategy would be highly preferable to one that assumes that replicator first (RNA in particular) is correct—for example, the inclusion in space missions of experiments specifically designed to detect nucleic acids. The committee recommends that missions planned for Mars should include instruments that detect lighter atoms, simple organic functional groups, and organic carbon. Such instruments were originally planned as part of the Athena payload to Mars but were later removed. A total-carbon analysis instrument was delivered by Beagle 2, but this mission was lost. As a consequence, the Opportunity and Spirit rovers were unable to confirm the presence of borates or simple organic materials even though the locales that they visited would theoretically be very likely to contain them. Similar considerations should guide small-organic-molecule detectors that could function on the surface of Europa and Titan.

That approach offers the opportunity for research that combines the exploration of potential metabolic cycles with the synthetic biology of unnatural nucleic acid analogues and their building blocks. The results of the studies would be used to guide the design of instruments. That may be one of the principal ways in which ground-based research in astrobiology can inform NASA missions of exploration in the cosmos. The committee recommends that NASA fund carefully constructed programs in fundamental organic chemistry and synthetic biology for that purpose and for the design of instruments that might detect weird life on Earth and elsewhere.

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6

Why Water? Toward More Exotic Habitats

A liquid phase facilitates chemical reactions, something that has been known empirically for centuries. As a solvent, a liquid allows dissolved reactants to encounter each other at rates that are higher than the rates of encounter between species in a solid. At the same time, a solvent provides an environment that has some degree of constancy relative to the spectrum of possible environments. In water, for example, the temperature must lie between 273 and 373 K at typical pressures, and the dielectric constant and other physical parameters that influence reaction rates are also within specific ranges. Chemical reactions can take place in the gas and solid phases as well, of course. But each of these phases has disadvantages relative to the liquid phase.

Results obtained from both the emerging field of synthetic biology and the analysis of origins are beginning to be effective in constraining the biomolecular form that life might have taken if it arose in water. Earlier chapters of this report discuss important chemical properties of water and their relevance to life. In this chapter the committee examines alternative perspectives suggesting that water might not be essential for life. Other than preferring a repeating charge in a genetic biopolymer able to support Darwinian evolution subject to the constraints of general chemistry, only a few arguments indicating that water is uniquely suited as a biosolvent survive scrutiny.

6.1 IS WATER UNIQUELY SUITED FOR LIFE?

Many specific properties of water are cited to make the case that water is an ideal biosolvent uniquely suited to support life: Frozen water floats. Water is an excellent solvent for salts. Water is liquid over a broad range of temperature. Indeed, the concept of a habitable zone, a region around a star where life is presumed to be possible, largely posits a region in which a planet's surface or subsurface might support liquid water. One such planet in a habitable zone could export life to other suitable environments via meteoroids.

The committee found that many current views about water in relation to life are geocentric. For example, floating water ice can indeed insulate a body of liquid water below and thus keep it from freezing. However, several types of water ice exist. Ice 1, which is the most stable form of ice at 273 K at standard pressure, is indeed less dense than liquid water at those conditions. In contrast, ice 2, or any of the other form of ice, is denser than water. Those forms of ice sink. Researchers view ice 1 as especially worthy of discussion, because they live on an Earth-sized body. If researchers lived on a substantially more massive body, they might consider ice 1 to be less important than the forms of ice that sink.

Although surface ice does insulate a body of liquid water below from loss of heat, it also has a higher albedo than water liquid. When water ice floats, it reflects more light from the Sun, which leads to more cooling, more ice on the surface, a resultant higher albedo, and still more cooling. Floating water ice amplifies a glaciation event, leading to more glaciation. Indeed, it appears to have done just this many times on Earth, including within the past few million years. Thus, the fact that water ice floats causes water to amplify, not damp, perturbations in energy flux coming to a planet. If a useful property of a bulk solvent is that it supports a stable environment conducive to life, then the fact that water ice floats might be viewed as a disadvantage.

Another example is that a focus on terran sea-level atmospheric pressure can influence researchers' views of the range of temperatures at which water is a liquid. On the surface of Mars liquid water cannot exist, except possibly transiently, because the pressure is too low, and water ice sublimes directly to water vapor without going through an intermediate liquid phase. Further, over most ranges of pressure, formamide has a larger liquid temperature range (255 to 480 K) than water and is also an excellent solvent for polar materials.

The view of water as an ideal biosolvent is geocentric in other ways. Although the temperature range over which water is a liquid is large on the Kelvin scale, it is important not to be tied to a linear view of the Celsius scale. The temperature range from 1 to 2 K is much more significant than from 273 to 274 K, both in fractional terms and in terms of how physical processes occur over that range. Although ammonia is liquid at lower temperatures than water, the temperature range over which ammonia is liquid for relevant planetary surface pressures is greater than for water.

Considered in a cosmological context, water is a liquid over a temperature range present in only a very small number of objects in the universe. Assuming that a fluid is necessary for life, and accepting the premise that water has properties that make it well suited to support life in the cosmos, what do water's freezing and boiling points imply about the likelihood of its fluidity over a significant range of the space in the galaxy, or even around a star? This question has been examined recently by Ward and Brownlee,[1] who concluded that complex life (i.e., animal life) is distributed sparsely, perhaps extremely sparsely, in the cosmos precisely because it requires water as a liquid, and that liquid water is sparsely distributed in the cosmos. However, Kasting has raised questions about this conclusion.[2]

Despite the apparent match between terran metabolism and water, water does not seem to be unquestionably preferable as a biosolvent. For example, although much of terran metabolism exploits C═O molecules, the electrophilicity of carbon doubly bonded to nitrogen (C═N) is equally satisfactory for supporting carbon-carbon bond-forming reactions. Yet, with the exception of aromatic heterocycles such as the purines, C═N is hardly used in terran biochemistry, because most other compounds containing C═N units spontaneously hydrolyze in water to generate the corresponding C═O compound and the corresponding N-containing by-product. But if a solvent other than water were the matrix for life, and if that solvent tolerated C═N units, would life not have been able to evolve in that solvent to exploit C═N units just as effectively as terran life today exploits C═O units?

The ability of water to form strong hydrogen bonds disrupts the hydrogen bonding useful for supramolecular structures. Water does not support protein folding, because it disrupts hydrogen bonds that stabilize the fold. Indeed, an examination of the chemical literature for examples of work on self-organizing molecules indicates that chemists consciously trying to achieve this outcome rarely use water, precisely because it disrupts noncovalent directional bonding such as hydrogen bonding. The situation is similar for genetic molecules. The DNA double helix is joined by hydrogen bonds that in water are only barely stable because water offers opportunities for a single strand of DNA to form hydrogen bonds, not with its complementary strand, but with water itself. But DNA, because it has a repeating charge in its backbone, is not expected to work well in nonwater solvents. This implies that a genetic molecule in nonwater solvents would not be DNA, or not be a polyelectrolyte. Conversely, if one accepts the notion of repeating charges as universal in genetic molecules, then one might have to conclude that a somewhat polar solvent (like water) is necessary for life.

Nevertheless, certain features of terran metabolism might benefit from a biosolvent whose properties differ from water's. For example, the instability of C═N in water constrains the structure of metabolites in water. The compound HN═C═NH, an analog of O═C═O (carbon dioxide), immediately hydrolyzes in water to give urea ($H_2NCO\text{-}NH_2$), whose thermodynamic instability with respect to hydrolysis in water yields carbon dioxide and ammonia. Thus, water as a solvent requires that the dominant form of carbon at the +4 oxidation state be carbon dioxide.

Water and carbon dioxide, however, are a problematic pair, at least at terran sea-level atmospheric pressure. The carbon of carbon dioxide is a good electrophilic center. But carbon dioxide itself is poorly soluble in water (0.88 v/v at 293 K and 1 atm) and dissolves at pH 7 primarily in the form of the bicarbonate anion. Bicarbonate, however, has its electrophilic center shielded by the anionic carboxylate group and so is intrinsically unreactive as an electrophile. Thus, the metabolism of carbon dioxide is caught in a conundrum. The reactive form is insoluble; the soluble form is unreactive.

Terran metabolism has worked hard to manage this conundrum. The reactivity of the vitamin biotin was discussed nearly three decades ago in this context.[3] Biotin is metabolically costly, however, and cannot be used to manage carbon dioxide and its problematic reactivity in large amounts. Although the enzyme ribulose bisphosphate carboxylase attempts to manage the problem without biotin, the problematic reactivity of carbon dioxide competes with the problematic reactivity of dioxygen. Even in highly advanced plants, a sizable fraction of the substrate intended to capture carbon dioxide is destroyed through reaction with dioxygen.[4] Terran life, in nearly a billion years, has not found a compelling solution to this problem, which may be universal. Indeed, if we do encounter nonterran carbon-based life that lives in water, it will be interesting to see how it has come to manage the unfortunate properties of carbon dioxide.

6.2 IF NOT WATER, THEN WHAT SOLVENT?

Nature presents a large number of atomic and small molecular species that might be discussed as biosolvents. Table 6.1 lists some of these, together with their freezing and normal (i.e., at 1 atmosphere) boiling points. It is important to note another contribution of pressure to physical properties. The physical properties of the substances listed the Table 6.1 are described by a phase diagram that relates the state of a material (solid of various types, liquid, or gas) to temperature and pressure. Above a critical point in the phase diagram, the substance is a supercritical fluid, neither liquid nor gas. Table 6.2 shows the critical temperatures and pressures for some substances common in the solar system.

The properties of supercritical fluids are generally different from those of regular fluids. For example, supercritical water is relatively nonpolar and acidic. Further, the properties of a supercritical fluid, such as its density and viscosity, change with changing pressure and temperature, dramatically as the critical point is approached. Thus, carbon dioxide is not listed in Table 6.1 because it has no liquid phase at terran atmospheric pressure. Carbon dioxide has a critical temperature of 304.2 K and pressure of 73.8 atm, however. It is therefore a supercritical fluid above that pressure, and may even exist as a potential biosolvent for rocky planets having the approximate mass of Earth (or Venus).

TABLE 6.1 Freezing and Boiling Points (at 1 atm) of Some Solvents

Solvent	Freezing Point (K)	Boiling Point (K)
Ammonia	195	240
Dihydrogen	14	20
Dinitrogen	63	77
Ethane	101	184
Formamide	273	495
Helium	—	4
Hydrazine	275	387
Hydrogen cyanide	260	299
Hydrogen fluoride	190	293
Hydrogen sulfide	192	213
Methane	91	112
Neon	25	27
Sulfuric acid	283	563
Water	273	373

TABLE 6.2 Critical Temperature and Pressure for Selected Substances

Liquid	Critical Temperature (K)	Critical Pressure (atm)
Hydrogen	33.3	12.8
Neon	44.4	26.3
Nitrogen	126	33.5
Argon	151	48.5
Methane	191	45.8
Ethane	305	48.2
Carbon dioxide	305	72.9
Ammonia	406	112
Water	647	218

It is useful to divide the solvents into three groups: polar solvents that are not water, nonpolar solvents, and cryosolvents.

6.2.1 Polar Solvents That Are Not Water

6.2.1.1 Ammonia

Ammonia is analogous to water in many of its properties. Ammonia, like water, dissolves many organic compounds, including many polyelectrolytes. Preparative organic reactions are often done in ammonia in the laboratory. Ammonia, like water, is liquid over a wide range of temperatures (195 to 240 K at 1 atm). The liquid range is even broader at higher pressure. For example, at 60 atm ammonia is liquid from 196 to 371 K. Further, liquid ammonia may be abundant in the solar system. A large amount of the inventory of liquid ammonia in the solar system exists, for example, in clouds in the jovian atmosphere. However, as noted earlier by the committee, some view clouds as unlikely places to harbor life. However, if clouds are not transient and broken (as on Earth) but are rather more continuous (as on Venus), this view may need modification.

As compared with water, ammonia's increased ability to dissolve hydrophobic organic molecules suggests an increased difficulty in using the hydrophobic effect to generate compartmentalization in ammonia, relative to water. This in turn implies that the liposome, a compartment that works in water, generally will not work in liquid ammonia. Hydrophobic phase separation is possible in ammonia, however, albeit at lower temperatures. For example, Brunner reported that liquid ammonia and hydrocarbons form two phases, where the hydrocarbon chain contains from 1 to 36 CH_2 units.[5] Different hydrocarbons become miscible with ammonia at different temperatures and pressures. Thus, formation of ammonia-phobic and ammonia-philic phases, analogous to the hydrophobic and hydrophilic phases in water, useful for isolation would be conceivable in liquid ammonia at temperatures well below its boiling point at standard pressures.

The greater basicity of liquid ammonia must also be considered. The species that serve as acid and base in pure water are H_3O^+ and HO^-. In ammonia, NH_4^+ and NH_2^- are the acid and base, respectively. H_3O^+, with a pKa of -1.7, is about 11 orders of magnitude stronger (in water) as an acid than NH_4^+, with a pK_a of 9.2 (in water). Likewise, NH_2^- is about 15 orders of magnitude stronger as a base than HO^-.

The increased strength of the dominant base in ammonia, as well as the corresponding enhanced aggressivity of ammonia as a nucleophile, implies that ammonia would not support the metabolic chemistry found in terran life. Terran life exploits compounds containing the C═O carbonyl unit. In ammonia, carbonyl compounds are (at the very least) converted to compounds containing the corresponding C═N unit. Nevertheless, hypothetical reactions that exploit a C═N unit in ammonia can be proposed in analogy to the metabolic biochemistry that exploits the C═O unit in terran metabolism in water (Figure 6.1).[6] Given this adjustment, metabolism in liquid ammonia is easily conceivable.

Most interestingly, ammonia is a potent antifreeze for water. Recently recovered data from Titan suggest that that moon is periodically being resurfaced by a liquid having a viscosity comparable to that of a water-ammonia eutectic, which is liquid even in an environment that experiences methane rain. Water-ammonia eutectics, which

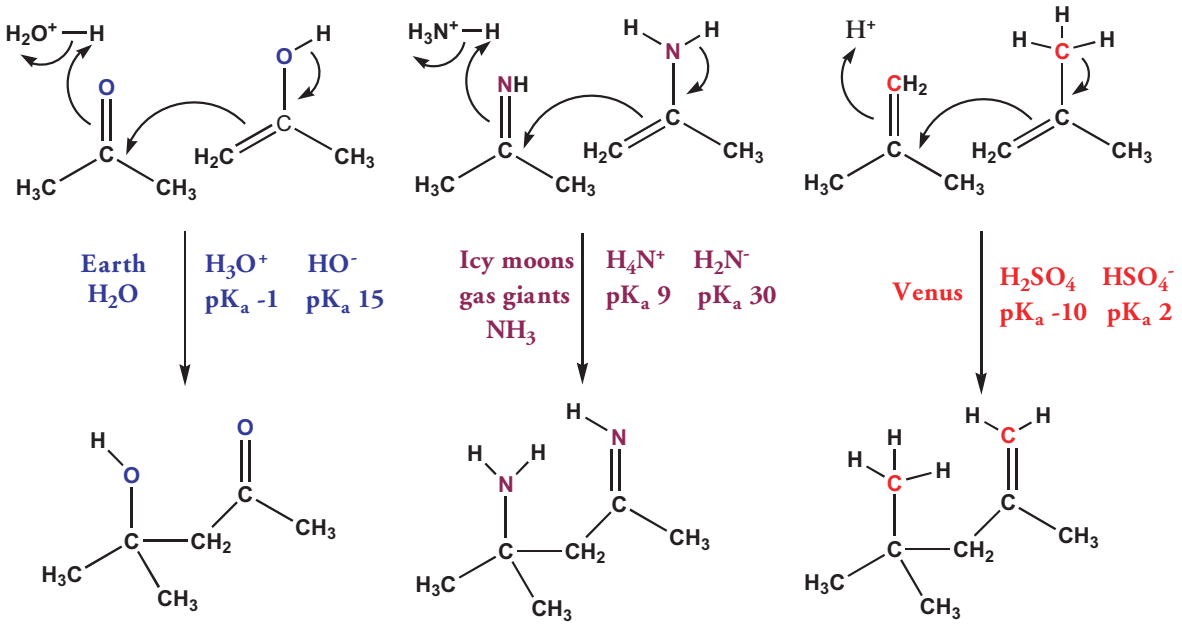

FIGURE 6.1 Different functional groups, but analogous mechanisms, could be used to form new C—C bonds in different solvents. In water, the C═O unit would provide the necessary reactivity. In ammonia, the C═N unit would provide the necessary reactivity. In sulfuric acid, the C═C unit is sufficient to provide the necessary reactivity.

are liquid even at the temperature of Titan, are a potential biosolvent. They are abundant in the cosmos, they have a wide temperature range of liquidity, and they are not bad as solvents.

6.2.1.2 Sulfuric Acid as a Possible Solvent

Ammonia is not the only polar solvent that might serve as an alternative to water. For example, sulfuric acid is a reasonably good solvent that supports chemical reactivity.[7] Sulfuric acid is known to exist above Venus,[8] where three cloud layers at 40 to 70 km are composed mostly of aerosols of sulfuric acid, about 80 percent in the upper layer and 98 percent in the lower layer. The temperature (about 310 K at about 50 km altitude, at about 1.5 atm) is consistent with stable carbon-carbon covalent bonds. Many authors have discussed the possibility of early life on Venus in its acidic environment.[9-13] The surface temperature of Venus is approximately 740 K. Sagan and Morowitz even considered organisms that float above the hot surface using hydrogen "float bladders"[14] analogous to those found in terran aquatic organisms, although others have raised questions about how to explain the prior evolution of a float bladder that would let an organism control its altitude.[a] Schulze-Makuch et al. argued for sample return from the venusian atmosphere to address the possibility of life there.[15]

Hypotheses about metabolism are abundant for the hypothetical life in acidic aerosols. In strong acid, the C═C bond is reactive as a base and can support a metabolism as an analog of the C═O unit. This type of chemical reactivity is exemplified in some terran biochemistry. For example, acid-based reactions of the C═C unit have been used by plants as they synthesize fragrant molecules.[16]

[a]The 2006 National Research Council letter report "Assessment of Planetary Protection Requirements for Venus Missions" (The National Academies Press, Washington, D.C.) provides arguments for why life is unlikely to have originated in the clouds of Venus.

Nor are sources of energy in short supply in the venusian atmosphere. For example, a venusian metabolism might exploit the relatively high flux of ultraviolet radiation in the venusian clouds.[17]

6.2.1.3 Formamide as a Possible Solvent

Formamide ($HCONH_2$) is another potential biosolvent. Formamide is formed by the reaction of hydrogen cyanide with water; both are abundant in the cosmos. Like water, formamide has a large dipole moment and is an excellent solvent for almost anything that dissolves in water, including polyelectrolytes. In particular, formamide is able to dissolve RNA, DNA, and proteins, as well as their precursors. Formamide is not reactive like water. Indeed, many species that are thermodynamically unstable in water with respect to hydrolysis, are stable in formamide.

Formamide is itself hydrolyzed by water, meaning that it persists only in a relatively dry environment, such as a desert. Desert environments recently proposed as being potential sites for the prebiotic synthesis of ribose[18] may hold formamide as well. Since formamide boils at ~400 K, a mixture of formamide and water, if placed in the desert, would lose its water over time and end up as a pool of formamide. Within this pool, many syntheses are thermodynamically favorable: polypeptides from amino acids, nucleosides from sugars and bases, nucleotides from nucleosides and inorganic phosphate, and RNA from nucleotides. Indeed, phosphate esters are also spontaneously synthesized. This includes ATP (from ADP and inorganic phosphate), nucleosides (from ribose borates and nucleobases), peptides (from amino acids), and others.[19-21]

6.2.2 Nonpolar Solvents

A variety of solvents have higher boiling points than that of water but do not have polar structures. The most accessible of these are the hydrocarbons, which come in a series from the smallest (methane) to higher homologs (ethane, propane, butane, and so on) and are abundant in the solar system. Methane, ethane, propane, butane, pentane, and hexane have boiling points of about 109, 184, 231, 273, 309, and 349 K, respectively, at standard terran pressure. Thus, at a mean surface temperature of 95 K, methane (which freezes at 90 K) would be liquid, implying that oceans of methane could cover the surface of Titan.

Many discussions of life on Titan have considered the possibility that water, normally frozen at the ambient temperature, might remain liquid following heating by impacts.[22] Life in this aqueous environment would be subject to the same constraints and opportunities as life in water. Water droplets in hydrocarbon solvents are, in addition, convenient cellular compartments for evolution, as Tawfik and Griffiths have shown in the laboratory.[23] An emulsion of water droplets in oil is obtainable by simple shaking. This could easily be a model for how life on Titan achieves the isolation necessary for Darwinian evolution, and it provides an interesting alternative for membranes, discussed in earlier chapters as a common feature of terran life.

Broad empirical experience shows that organic reactivity in hydrocarbon solvents is no less versatile than in water. Indeed, many terran enzymes are believed to catalyze reactions by having an active site that is not water-like. Further, with ethane as a solvent, a hypothetical form of life would be able to use hydrogen bonding more effectively; these bonds would have the strength appropriate for the low temperature. Further, hydrocarbons with polar groups can be hydrocarbon-phobic; acetonitrile and hexane, for example, form two phases. It is possible to conceive of liquid/liquid phase separation in bulk hydrocarbons that could achieve the isolation necessary for Darwinian evolution.

Because of its reactivity, water destroys hydrolytically unstable organic species. Thus, a hypothetical form of life in a Titan hydrocarbon ocean would be less subject to the hydrolytic deamination of its nucleobases, and would be able to guide reactivity more easily than life in water.

Thus the environment of Titan meets the absolute requirements for life. Titan is not at thermodynamic equilibrium. It has abundant carbon-containing molecules and heteroatoms and a fluid environment. Titan's temperature is low enough to permit a wide range of bonding, covalent and noncovalent. Titan undoubtedly offers other resources believed to be useful for catalysis necessary for life, including metals and surfaces.

This makes inescapable the conclusion that if life is an intrinsic property of chemical reactivity, life should exist on Titan. Indeed, for life not to exist on Titan, we would have to argue that life is not an intrinsic property

of the reactivity of carbon-containing molecules under conditions where they are stable. Rather, we would have to conclude that either life is scarce in these conditions or that there is something special, and better, about the environment that Earth presents (including its water).

6.2.3 Cryosolvents

Many of the most important potential solvents found in the solar system exist only in their gaseous form on Earth. They become liquids at temperatures that are (regarded by humans as being) low. Hence, they are known as cryosolvents. Low temperatures are, however, prominent throughout the cosmos, as are species that are liquid there. Therefore, cryosolvents cannot be dismissed as potential biosolvents.

6.2.3.1 Dihydrogen

The most abundant compound in the solar system is dihydrogen, the principal component (86 percent) of the upper regions of the gas giants Jupiter, Saturn, Uranus, and Neptune. The other principal component of the outer regions of the giant planets is helium (14 percent). Throughout most of the volume of gas giants where dihydrogen is stable, it is a supercritical fluid. For the gas giants, two radii can be defined. The first is the radius of the region where dihydrogen becomes supercritical. The second is where the temperature rises to a point where organic molecules are no longer stable; for this discussion, 500 K is chosen as that point. If the second radius is smaller than the first, then the gas giant has a habitable zone for life in supercritical dihydrogen. If the second radius is larger than the first, however, then the planet has no habitable zone.

If such a zone exists on Jupiter, it is narrow. Where the temperature is 300 K (clearly suitable for organic molecules), the pressure (about 8 atm) is still subcritical. At about 200 km down, where the jovian pressure is supercritical, the temperature rises above 500 K, approaching the upper limit where carbon-carbon bonds are stable.[24]

For Saturn, Uranus, and Neptune, the habitable zone is broader relative to the planetary radius. On Saturn, the temperature is about 300 K when dihydrogen becomes supercritical. On Uranus and Neptune, the temperature when dihydrogen becomes supercritical is only 160 K, a temperature at which organic molecules are stable.

The atmospheres of these planets convect, of course. To survive on Jupiter, any hypothetical life based on molecules containing carbon-carbon covalent bonds would have to avoid being moved by convection to positions in the atmosphere where they are not stable. This is, of course, not impossible. Even on Earth, life in the oceans must avoid being moved by convection from its particular habitable zone. Sagan and Salpeter presented a detailed discussion of what might be necessary for a "floater" to remain stable in the jovian atmosphere.[25]

Bains has recently discussed dinitrogen as a possible biocryosolvent.[26] His article also reviews the principal issues in using a cryosolvent. The most significant is the relative insolubility of substances in cryosolvents.

Thus, little is known about the behavior of organic molecules in supercritical dihydrogen as a solvent. In the 1950s and 1960s, various laboratories studied the solubility of organic molecules (e.g., naphthalene) in compressed gases, including dihydrogen and helium.[27,28] None of the environments examined in the laboratory explored high pressures and temperatures, however.

6.2.3.2 Dinitrogen

Bains considered the possibility of organic species being dissolved in dinitrogen, including the possibility that silicon-based species might have greater solubility in dinitrogen than carbon-based species.[29] However, the reactivity of silanes would make them unworkable as biopolymers on today's Earth, because water reacts with many silanes.

This is not the case for dinitrogen as a biosolvent. Dinitrogen is abundant in the cosmos, like water. Its lower freezing and boiling points, however, make it a liquid over a wider range of the cosmos. For example, liquid dinitrogen may be a bulk solvent on Triton, the largest moon of Neptune.

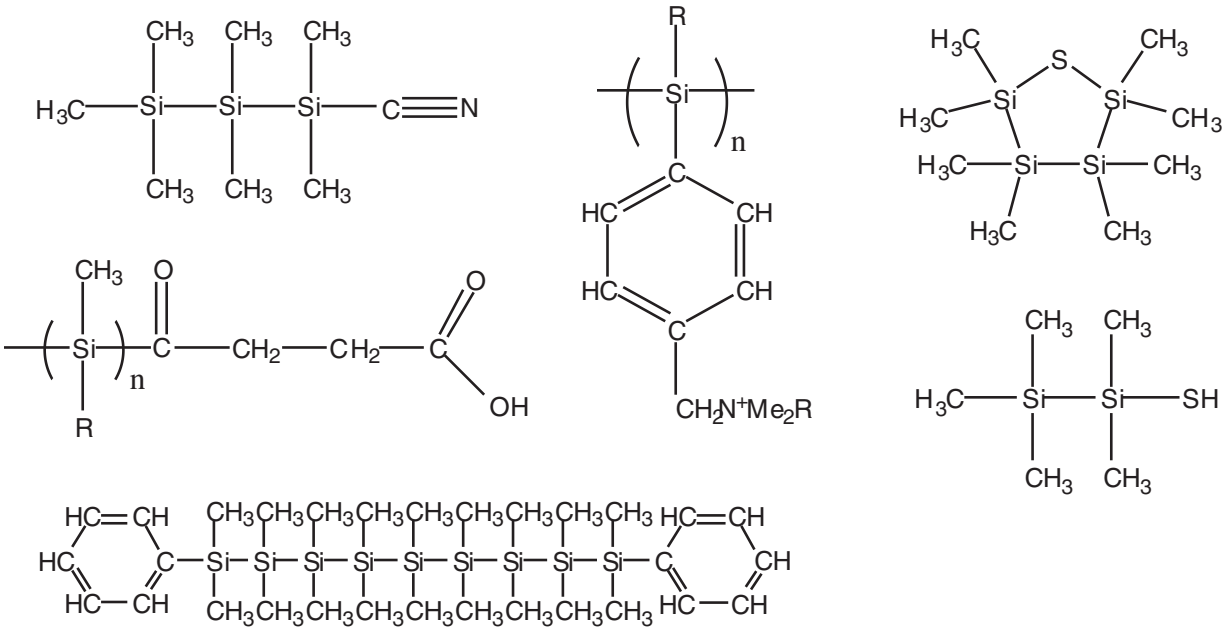

FIGURE 6.2 Structures of some synthetic polysilanes that have been described in the literature.

A range of silicon-containing compounds is known (see Figure 6.2). There is little question that polymeric diversity is possible using silicon, rather than carbon, as a scaffolding.

One difficulty with cryosolvents is the slow rate of chemical reactions. Here again, it is important not to be geocentric. Chemical reactions of the type known in water that occur between 273 and 373 K are indeed slow at 100 K. However, a wide range of chemical reactions that are too fast to be considered as part of terran metabolism—such as reactions involving the making and breaking of hydrogen bonds—become slower at cryotemperatures. At cryotemperatures, such reactions might be productively controlled by catalysts that could be under the control of genetic molecules in a hypothetical life form.

6.2.3.3 Other Supercritical Cryosolvents

As noted in Tables 6.1 and 6.2, many of the most abundant potential solvents in the solar system are supercritical in their most abundant environments.

Supercriticality in an environment does not, in itself, prohibit life. Some terran enzymes are known to be active in supercritical fluids.[30-32] Subsequent reviews can be found in Aaltonen and Rantakyla,[33] Kamat et al.,[34] and Aaltonen.[35] Although most of that work concerns supercritical carbon dioxide as the solvent, fluorinated hydrocarbons (HCF_3) and simple alkanes (e.g., ethane, propane) have also been reported,[36] providing a formal demonstration that terran-derived proteins can function in these media. Any enzyme adapted to the supercritical media would undoubtedly be different from those used in the studies cited.

Organic chemists have been attracted for a variety of reasons to supercritical media as an environment for performing reactions. These reasons include, especially for CO_2 and H_2O, the environmental friendliness of the medium. The fact that supercritical fluids can be removed without a residue is an advantage. Other advantages include the solubility of gases within supercritical mixtures, the high diffusion rates, and the variable and adjustable density, solvent power, and dielectric constant of the medium. Ordinary gases, such as O_2 and H_2, are miscible with

supercritical fluids, such as supercritical CO_2. That property removes a limitation that is well known in standard liquids, including water, in which reactions are limited by the poor solubility of gases in the standard liquid. Table 6.1 shows some of the solvents studied.

Supercritical water is a key species in mineralogy, as well as in the commercial synthesis of crystalline inorganic species, including alpha-quartz and gemstones.[37] It has rarely been used in organic chemistry, although there are examples of its use for the oxidation of methane, the degradation of biopolymers, and the dehydration of alkenes.[38] Its high critical temperature (647 K) implies that many organic species are unstable in supercritical water. Nevertheless, some reactions are known.[39]

6.3 STILL MORE EXOTIC HABITATS

6.3.1 Life in the Gas Phase

In the gas phase, chemistry is limited to molecules that are sufficiently volatile under the ambient conditions to deliver an adequate amount of material to the gas phase at moderate temperatures. Raising the temperature does not necessarily solve the problem. Although more molecules enter their gas phases as the temperature is increased, organic molecules generally cease to be stable at temperatures in excess of 500 K.

It is conceivable that in the vacuum of interstellar space, life exists through molecules at high dilution reacting in the gas phase. Grains have been suggested as a way of collecting organic species, perhaps with an outer layer that protects the inner layer from irradiation. The possible disadvantage of this, of course, is the difficulty in holding together the components of the interstellar life form. One advantage of such a life form would be that it need not be encumbered by the lifetime of a planetary system. Indeed, one can imagine that life in the gas phase might be associated with a galaxy and its energy flux for nearly the age of the universe.

6.3.2 Life in the Solid Phase

Species can diffuse through solids to give chemical reactions.[40] Diffusion through the solid phase is slow by terran standards. Nevertheless, given cosmic lengths of time, and the input of energy via cosmic rays or other high-energy particles, a biochemistry able to support Darwinian evolution can certainly be conceived.[41] Clathrates and solites, in which different molecules fit into exchangeable cages, might be relevant here.

Allamandola and Hudgins have considered the formation of complex organic species in ice matrices and provided a summary of the photochemical evolution on those ices found in the densest regions of molecular clouds, the regions where stars and planetary systems are formed.[42] Ultraviolet photolysis of these ices produces many new compounds, some of which have prebiotic possibilities. These compounds might have played a part in organic chemistry on early Earth.

Given that interstellar ices are the building blocks of comets and comets are thought to be an important source of the species that fell on primitive Earth, the structures of molecules in comets may be related to the origin of life. It is possible that organic materials formed in the solid ice phase of interstellar materials provided raw materials used for life originating solely on Earth. If so, the deep freeze of ice in the Oort cloud would have been an excellent place to store these, especially the unstable ones, awaiting delivery to a planet.

As an alternative, Allamandola and Hudgins considered the possibility that compounds made in ice might have initiated a Darwinian process within the comet. Indeed, a weird life form might reside within solids in the Oort cloud living in deeply frozen water, obtaining energy occasionally from the trail of free radicals left behind by ionizing radiation, and carrying out only a few metabolic transformations per millennium. While such a life form would presumably metabolize slowly, it would not necessarily be constrained by the lifetime of an associated star.

6.4 OPPORTUNITIES FOR RESEARCH

Alternative polar solvents offer potential solutions to paradoxes presented by replicator-first theories of the origin of life. That is particularly true for formamide. Formamide has been shown to be a suitable precursor of

adenine—preferable to hydrogen cyanide—especially in the presence of trace amounts of hydrogen cyanide. Nucleoside phosphates, thermodynamically understandable with respect to hydrolysis in water, can be synthesized in formamide. Furthermore, formamide is a liquid over wide ranges of temperature and pressure; it can form a liquid phase on Mars and can be dehydrated at low pressure, high temperature, or combinations of the two. RNA duplexes need not be unstable in formamide-water mixtures. Small-molecule (metabolism-first) theories of origins avoid such paradoxes but can be expected to be viable with respect to alternative solvents as well as water. That offers an opportunity for a broad research program to explore formamide as a potential prebiotic solvent—a simple task for Earth-based laboratory research.

The discovery of evidence of liquid water-ammonia eutectics on Titan provides a context for the potential for polar fluids outside what is normally regarded as the "habitable zone." The stay of the Cassini-Huygens mission on the surface of Titan was brief, but this moon of Saturn is the locale that is most likely to support exotic life. The committee believes that it is important to consider whether the planned missions to the solar system should be reordered to permit returning to Titan earlier than now scheduled.

Essentially nothing is known about the solubility of biomolecules in cryogenic solvents. Therefore, research into the possibility of life in cryogenic solvents needs to begin by making fundamental measurements and establishing a database describing the solubility of organic species in such solvents over a range of pressures and temperatures that are relevant to locales in the solar system.

Concepts of life in the gas phase are speculative, but questions related to organic chemistry in ices are experimentally approachable and are components of NASA missions. With sample return from comets imminent, NASA missions have the potential to generate fundamentally new insights into the kinds of organic compounds that might have been present on early Earth. Indeed, the committee concluded that the least expensive mission to the solar system that would have a high potential for fundamentally altering current understanding of how life originated would be the return to Earth of samples of cometary ice. Laboratory studies on Earth can provide sensible simulations of interstellar and solar system ice bodies that would be key to guiding these missions.

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7

Life Detection and Biomarkers

Prior chapters suggest some constraints on where carbon-based life might be found. In particular, environments that have a temperature greater than 500 K, lack a liquid solvent, and lack carbon are not likely locales for life related to the type we are familiar with on Earth. Not discussed above, however, are simple approaches to distinguishing between the products of Darwinian evolution and the products of inanimate processes. Even if organic molecules are necessary for life, their presence is clearly not a sufficient condition for the existence of life. Organic compounds are abundant in the cosmos, as meteorites show.

7.1 CHIRALITY AS A BIOMARKER

The vast preponderance of biologically formed chiral compounds are synthesized exclusively as one or other enantiomer. Enantiomers are isomers for which the left- and right-handed forms have the same physical behavior but respond differently in chemical reactions with other chiral molecules. See Box 7.1 for an explanation of stereoisomerism. In chemical processes, left- and right-handed molecules are generally formed in equal amounts, unless chiral reagents, catalysts, or surfaces are present to introduce a bias. Also, some organisms may synthesize the same chiral compound in different enantiomeric forms (e.g., bacterial peptide toxins). For the formation of regular structures when these monomers are assembled, however, it is generally more efficient to have the same handedness in all molecules. Thus, 100 percent of amino acids in terran proteins have the same absolute spatial relationship of the amino group, the acid group, and the side chain distinctive for the amino acid and are all designated “L”. Likewise, all of the sugars in nucleic acids have the opposite spatial relationship and are designated “D”. Enantiomeric excess (also called chirality, or handedness), the predominance of one enantiomer over the other for a given assembly of atoms, is therefore believed to be a universal feature of chemistry characteristic of living systems.

Once an organism dies and its biochemicals are released into the environment, their chiral purity (and optical activity) may or may not persist depending on the relative chemical stability of the bonds in the vicinity of the chiral center. Various natural chemical processes can lead to racemization, the formation of mixtures of the two enantiomers. While racemization may result in loss or corruption of a biological signature, the rate at which it happens can also have a practical application. The best known example is the dating of fossil organic matter on the basis of the degree of amino acid racemization.

BOX 7.1 Stereoisomerism

Molecules are three-dimensional. A carbon atom with four single bonds lies at the center of a tetrahedron. The atoms to which the carbon is bonded are at the vertices of the tetrahedron. Those atoms are in turn likely to be bonded to other atoms. If the chemical structures at the four corners all differ, however slightly, the mirror images of the tetrahedron will not be superimposable (Figure 7.1.1).

Mirror-image stereoisomers that are not superimposable are called enantiomers; stereoisomers that are not enantiomers are called diastereomers. Enantiomers possess chirality, or handedness, and when dissolved rotate the plane of polarized light when it is passed through the solution.

Life on Earth makes use of only a limited number of diastereomers of all those that are possible. Moreover, biotic processes display an enantiomeric excess; e.g., left-handed amino acids and right-handed sugars almost exclusively predominate in living systems.

Carbon atoms bearing four different substituents are said to be chiral centers. If a molecule has n chiral centers it will, in most cases, have 2^n stereoisomers. There will, for example, be 256 stereoisomers of a compound with eight chiral centers. Each will have exactly the same chemical formula and pattern of connectivity among its atoms (A is connected to B is connected to C and D, and so on). Only the arrangements of those atoms in space will differ, and there will be 256 variations. Life functions by using only a small subset of all possible stereoisomers.

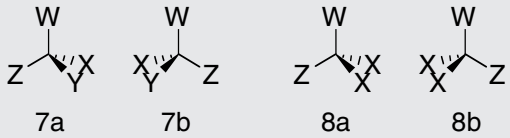

FIGURE 7.1.1 An illustration of stereoisomerism. In these depictions of tetrahedral carbon atoms, bonds represented by straight lines lie in the plane of the paper. Those represented by wedges project in front of the paper (the filled wedges) or to the rear (the broken wedges). W, X, Y, and Z represent different chemical groups, anything from a single atom (an H, for example) to a complex chemical substituent with many atoms in addition to the one that is bonded directly to the carbon atom. In structure 7, all four groups are different. The mirror images, 7a and 7b, cannot be rotated so that the structures are superimposable. In structure 8, by contrast, two of the groups are identical. If structure 8b is rotated 180° about its vertical axis, it can be superimposed on 8a (i.e., it is seen to be identical to 8a). Mirror images of tetrahedra will be nonsuperimposable only when all four vertices are different.

SOURCE: Reprinted from National Research Council, 2007, *Exploring Organic Environments in the Solar System,* The National Academies Press, Washington, D.C., p. 13.

Enantiomeric excess can be detected in a number of ways. Direct observation of optical activity, that is, determination of the specific rotation $[\alpha]_d$ of a compound, is cumbersome. Biochemical detection is also possible, although methodologies are generally specific to individual compounds or compound types. Indirect measurement through a chromatographic procedure, for example, gas chromatography or gas chromatography-mass spectrometry on a chiral stationary phase, has wide applicability and is very sensitive.

Chirality is often viewed as the unique province of biological systems. For this reason, researchers were surprised to find that several of the amino acids found in the Murchison meteorite were slightly enantiomerically

TABLE 7.1 Enantiomeric Enrichments for Amino Acids in the Murchison and Murray Meteorites

Compound	On Earth[a]	Enantiomeric Enrichment (%)	
		Murchison	Murray
2-Amino-2,3-dimethyl-pentanoic acid			
2S,3S/2R,3R	Unknown	7.6	1.0
2S,3S/2R,3S	Unknown	9.2	2.2
α-Methylnorleucine	Unknown	4.4	1.8
α-Methylnorvaline	Unknown	2.8	1.4
α-Methylvaline	Unknown	2.8	1.0
Isovaline	Rare	8.4	6.0
Norvaline	Rare	0.4	0.8
α-amino-n-butyric acid	Common	0.4	−0.4
Valine	Ubiquitous	2.2	−0.4
Alanine	Ubiquitous	1.2	0.4

[a]Natural abundance of the amino acid in Earth's biosphere.
SOURCE: Data from Pizzarello, S., and Cronin, J.R. 2000. Non-racemic amino acids in the Murray and Murchison meteorites. *Geochim. Cosmochim. Acta* 64:329-338.

enriched (Table 7.1). Especially notable were enantiomeric enrichments of 7 to 9 percent in three specific amino acids not known in forms of life currently on Earth, thus making contamination from terrestrial sources unlikely.

If the amino acids in Murchison were not generated by living processes, it is possible that enantiomeric enrichment can be achieved by processes that are independent of life. Several such processes are known in the laboratory, some of which involve crystallization, or autocatalysis, and/or some multiple steps requiring human intervention. It is conceivable that some of these processes involve selective degradation of one of the two enantiomers. This weighs against the usual thinking that enantiomeric enrichment is a unique signature of life.

However, the data on enantiomeric excess are also consistent with a biotic origin, The larger asteroids may have stayed clement inside for several hundreds of millions of years, raising the possibility that life, or some borderline form, emerged there. This would be the case if the Murchison meteorite originated in an asteroid (or a comet) that supported life based on α-methyl amino acids that preferred one enantiomer to the other. While few would argue so, this result is consistent with life having emerged in the asteroid belt.

7.2 THERMODYNAMIC RELATION OF METABOLIC INTERMEDIATES AS A BIOSIGNATURE

For a metabolic sequence to convert substrates into products, the system in which they occur must not be at equilibrium. When they are in equilibrium, the overall reaction must be coupled to another reaction where substrates and products are at disequilibrium. This is frequently phrased as a requirement that a metabolic pathway be energetically "downhill," or coupled to an energy source.

Many terran metabolic multistep pathways are run close to equilibrium for internal steps (Figure 7.1), with the first step being energetically downhill and a site of regulation. Required, however, is that the last step be energetically downhill, thereby pulling the reaction to completion.[1] This feature may be universal in metabolic pathways simply because it exploits most economically a surrounding chemical disequilibrium. Having large drops in free energy at every step in a pathway is wasteful.

This characteristic energetic relationship between a set of compounds that are intermediates in an evolved metabolism may be a universal biosignature. If an inventory of the small molecules in a suspected living system (on Titan, for example) reveals this characteristic energetic relationship, this may be evidence of Darwinian evolution acting to create an optimal metabolism.

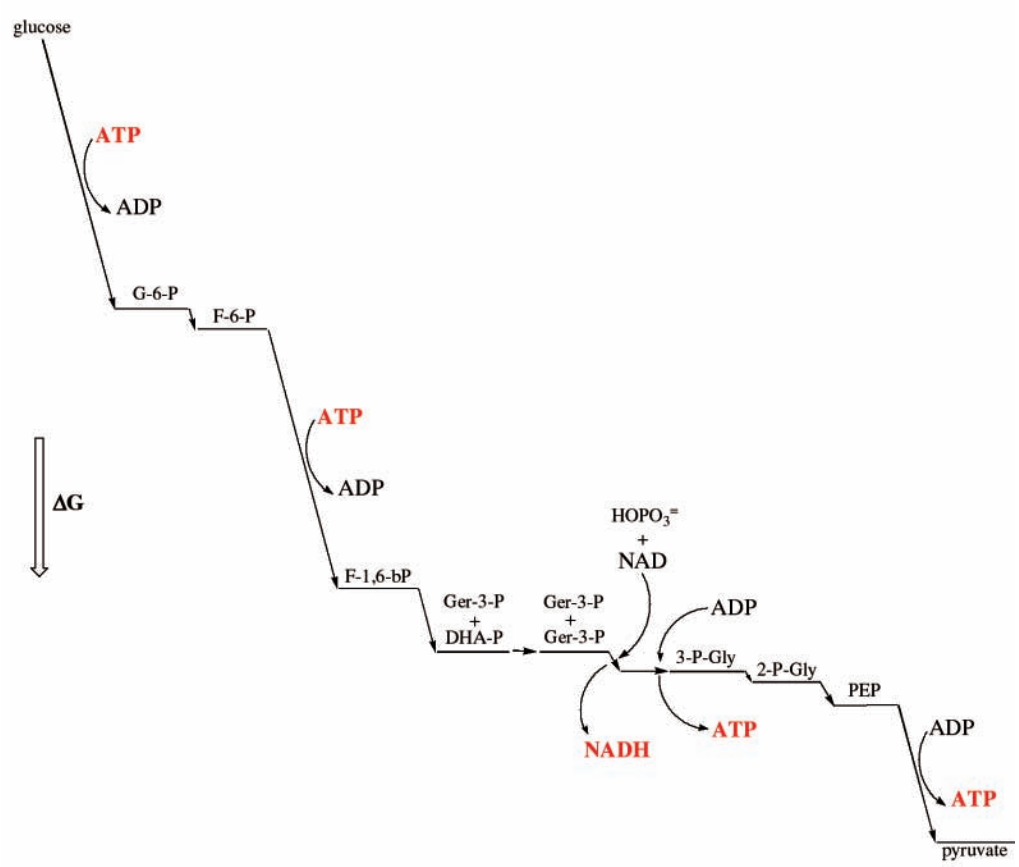

FIGURE 7.1 Physiological free energy diagram for the glycolysis reaction for one of the two glyceraldehyde-3-phosphate generated in glycolysis. G, glucose; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-bP, fructose-1,6-bisphosphate; Ger-3-P, glyceraldehyde-3-phosphate; DHA-P, dihydroxyacetone-phosphate; 3-P-Gly, 3-phosphoglycerate; 2-P-Gly, 2-phosphoglycerate; PEP, phosphoenolpyruvate. Vertical height is proportional to the change in free energy (ΔG).

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8

Conclusions and Recommendations

The statutory goals of the National Aeronautics and Space Administration (NASA)—sending missions to the solar system, understanding the origin and distribution of life in the galaxy, and contributing to the scientific development of the United States—remain. However, recent long-term planning has come to focus on human missions, first to the Moon, then to Mars, and then beyond. Human missions have both strengths and weaknesses. It is clear that an astronaut can improvise and explore in a fashion not possible for even the most sophisticated robot, including exploration for living systems. Balancing that advantage, in addition to the much greater cost per unit of data obtained, is the fact that human exploration is concomitant with human contamination. No discovery that we can make in our exploration of the solar system would have a greater impact on our view of our position in the cosmos or be more inspiring than the discovery of an alien life form, even a primitive one.

At the same time, it is clear that nothing would be more tragic in the American exploration of space than to encounter alien life and fail to recognize it either because of the consequences of contamination or because of the lack of proper tools and scientific preparation.

The committee's investigation makes clear that life is possible in forms different from those on Earth. Different specific biomolecules may be considered highly likely in extraterrestrial life. Different architectures at the microscopic and macroscopic levels must also be considered likely. Advocates of replicator-first theories hold that—with reasonable interactions between minerals, polar refractory solvents, and organic species—the spontaneous emergence of genetic biopolymers may be expected. Other scientists feel that the chances of such an event are infinitesimal but that other circumstances may suffice for the origin of life. Whichever group is correct, the likelihood of encountering some form of life in subsurface Mars and sub-ice Europa appears high. Life-detection strategies should be redesigned to maximize the possibility of successful detection of life by seeking intermediates common to the two theories.

Furthermore, life should be considered possible in aqueous environments that are extreme in their solute content, in their acidity or alkalinity, and in their temperature range, especially with ammonia as an antifreeze in low-temperature water-ammonia eutectics. The committee sees no reason to exclude the possibility of life in environments as diverse as the aerosols above Venus and the water-ammonia eutectics of Titan. It seems that life is less likely in more exotic solvents—such as liquid dinitrogen, liquid methane, and supercritical dihydrogen—but this conclusion is based on few data.

Given the importance of developing the understanding and capability to detect and recognize possible life forms in other planetary environments, the committee offers recommendations for three lines of research: labora-

tory studies, field studies, and space studies. The first two are clearly appropriate for both NASA and the National Science Foundation (NSF), and the third is likely to be solely in the purview of NASA.

8.1 LABORATORY STUDIES

Laboratory studies are needed that will help to elucidate the origin of life, especially studies that use information from NASA missions, the inventory of organic materials in the cosmos, and interactions between organic materials and minerals set in a planetary context. There is a need for basic research to understand interactions of organic and inorganic species in exotic solvents, including water under extreme conditions (as found on Venus, Mars, Europa, Enceladus, and elsewhere), water-ammonia eutectics at low temperatures (as possible on Titan), and liquid cryosolvents (as found on Triton and elsewhere). A need also exists for synthetic biology that constructs and studies molecular systems capable of Darwinian evolution that are different from standard DNA and RNA, especially those designed to improve understanding of the chemical possibilities that support Darwinian evolution. Such studies should include the search for self-sustaining energy-driven metabolic cycles. The committee's recommendations to NASA and NSF for specific kinds of laboratory studies are as follows.

- Origin-of-life studies, including prebiotic-chemistry and directed-evolution studies that address physiologies different from those of known organisms. Such alternate physiologies can include novel metabolisms and growth in extreme conditions that are not found on Earth but are found on other planets and moons. Some examples are growth in media with low ratios of water to organic solvents, the substitution of arsenic for phosphate, the use of carbon-silicon polymers, and the use of mineral catalysis instead of enzyme catalysis.
- Further studies of chirality, particularly studies focused on the hypothesis that specific environmental conditions can favor chiral selection, or on an alternative model that life with L-amino acids and D-sugars is better "fit," from an evolutionary perspective, to evolve into complex organisms.
- Work to understand the environmental characteristics that can affect the ability of organisms to fractionate key elements, including not only carbon but also sulfur, nitrogen, iron, molybdenum, nickel, and tungsten. An understanding of how life fractionates transition elements could provide an essential marker for past and present life and insight into their metabolic potential. Even weird carbon life will use elements for energy, such structural polymers as proteins or protein analogues, and oxidation and reduction reactions.

8.2 FIELD STUDIES

Many of the environments on Earth that have analogous environments on other planets and moons have not been adequately sampled. They include the deep subseafloor crust, the deep oligotrophic ocean, and the upper atmosphere. Therefore, the committee places particular emphasis on the search for organisms that have novel metabolisms that use novel energy sources, including black-body radiation or sources thought to be ancient but ubiquitous on other rocky planets. They include organisms that use hydrogen and sulfur as energy sources and iron (FeIII) as an electron acceptor. The focus should be on microbial ecosystems that do not depend on photosynthesis. There is also an opportunity to look at submarine hydrothermal vent environments as primordial and to design experiments to search for organisms that have relic genes and metabolisms that can reveal something about early life. The committee's recommendations to NASA and NSF for field studies are as follows:

- A search for remnants of an RNA world in extant extremophiles that are deeply rooted in the phylogenetic tree of life. They could include RNA genes that, unlike the common retrotransposons found in eukaryotes that are just "selfish genes," may have some function in the cell. The search should also include viruses from hyperthermophilic archaeans that have already been shown, in the study at Yellowstone, to be unlike anything that has been seen before and that have characteristics of all three domains of life.
- A search for organisms with novel metabolic and bioenergetic pathways, particularly pathways involved in carbon dioxide and carbon monoxide reduction and methane oxidation coupled with electron acceptors other than oxygen. Regardless of how weird carbon life might be, there will be a "unity of metabolism" in which all

organisms use the same carbon and energy sources. There will be a finite number of ways to use the carbon and energy sources, and science is not close to knowing this limit.

- A search for organisms that derive some of their catalytic activity from minerals rather than protein enzymes, including organisms that combine mineral and protein catalysis.
- A search for organisms from environments that are limited in key nutrients, including phosphorus and iron, and determination of whether they can substitute other elements, such as arsenic, for phosphorus. This effort would involve a search for adenosine tri-arsenate instead of adenosine triphosphate and for DNA with arsenic instead of phosphorus.
- A search for life that can extract essential nutrients—such as phosphorus, iron, and other metals—from rocks, such as pyrites and apatite.
- A search for anomalous gene sequences in conserved genes, particularly DNA- and RNA-modifying genes. The anomalous sequence in the *Nanoarchium* 16S rRNA gene may indicate that there are others in other extremophiles, and these may have some significance in the origin of genes and the RNA world.
- Study of the resistance of microorganisms that form biofilms on minerals to the harsh conditions of interplanetary transport.
- A search for life that stores its heredity in chemicals other than nucleic acids.

8.3 SPACE STUDIES

The laboratory and field studies that are recommended above are not expensive by any metric; progress can be made with consistent NASA support at the $20 million annual level. That is a small fraction of the cost of a single launch of a space shuttle or of the contribution over the years to the space station, and it is a nearly negligible fraction of the cost of a human mission to Mars. Laboratory and field studies are a necessary component of such a mission. The results obtained from such studies not only will provide an answer to the question, Why go to Mars? but also will be needed to prevent a human landing on Mars from vitiating a key discovery that might have the greatest of effects for science and society.

The committee's specific recommendations to NASA for space studies are as follows:

- Programs that combine the exploration of potential metabolic cycles with the synthetic biology of unnatural nucleic acid analogues and their building blocks and that use the results to guide the design of instruments. This may be one of the principal ways in which ground-based research in astrobiology can inform NASA missions of exploration.
- Astrobiology measurements that can potentially distinguish between life on Mars (and possibly other bodies) that arrived via material ejected from Earth (or vice versa) and life that emerged on another body independently of life on Earth. The scientific and societal effects of discovering a second genesis of life, as opposed to discovering another branch on the same tree of life, cannot be overstated.
- Inclusion in missions planned for Mars of instruments that detect lighter atoms, simple organic functional groups, and organic carbon to help distinguish between "replicator-first" and "metabolism-first" theories of the origin of life by identifying organic mixtures that differ sharply in composition from the nearby random collections identified in meteorites. Similar considerations should guide inclusion of small-organic-molecule detectors that could function on the surfaces of Europa, Enceladus, and Titan.
- Consideration, in view of the discovery of evidence of liquid water-ammonia eutectics on Titan and active water geysers on Saturn's moon Enceladus, of whether the planned missions to the solar system should be reordered to permit returning to Titan or Enceladus earlier than is now scheduled. The discovery of evidence of liquid water-ammonia eutectics on Titan provides a context for the potential for polar fluids outside what is normally regarded as the "habitable zone." The stay of the Cassini-Huygens mission on the surface of Titan was unfortunately brief; but this moon of Saturn is the locale that is arguably likely to support exotic life.

Finally, the committee calls attention to the importance of using remote sensing to detect and characterize extrasolar planets that could support alternative carbon-based life. In addition to looking for evidence of water in

such places, it will be valuable to look for evidence of plate tectonics, because that would indicate hydrothermal activity and the abiotic production of chemical energy and carbon sources that can support life. Studies of atmospheres of extrasolar planets will benefit from access to models of atmospheric conditions on planets that never have evolved oxygenic photosynthesis and that remain anaerobic. Earth did not accumulate oxygen during the first roughly 3 billion years, and it did not form an ozone layer until about 1.5 billion years ago. There is considerable emphasis on looking for contemporary Earth atmospheres that have oxygen and an ozone layer, but there should also be models of atmospheres with different anaerobic microbial ecosystems, atmospheres that might parallel the different stages in the evolution of Earth's atmospheres over 4 billion years, and atmospheric conditions that could indicate the presence of a tectonically active planet.

Appendixes

A

Glossary

Abiotic	Of or relating to nonliving things; independent of life or living organisms
Acidophile	An organism adapted to living in highly acidic environments
ADP	Adenosine diphosphate
Aerosol	A fine aerial suspension of liquid (mist, fog) or solid (dust, fume, smoke) particles
Albedo	The fraction of light that is reflected by a surface; commonly used in astronomy to describe the reflective properties of planets, satellites, and asteroids
Alkalophile	An organism that is adapted to living in highly alkaline environments
Amino acid	Any organic compound containing an amino (NH_2) and a carboxyl (COOH) group; there are 20 α-amino acids from which proteins are synthesized during ribosomal translation of mRNA
Anaerobe	An organism that lives and reproduces in the absence of dissolved oxygen, instead using oxidants such as iron and sulfur compounds in energy metabolism
Anoxic	Lacking oxygen
Aquaporin	A water channel that form pores in the membranes of cells and selectively conducts water molecules through the membrane, while preventing the passage of ions (such as sodium and potassium) and other small molecules
Archaea (Archaebacteria)	A group of unique microorganisms classified as bacteria that are related only distantly to eukaryotes and other prokaryotes
Aromatic hydrocarbon	A member of the class of hydrocarbons consisting of assemblages of cyclic conjugated carbon atoms and characterized by large resonance energies
Asteroid	A small, rocky body in orbit around the Sun, found mainly between the orbits of Mars and Jupiter

ATP	Adenosine triphosphate
Autotroph	An organism that can synthesize its own food—complex organic compounds—using simple inorganic sources, such as carbon dioxide
Biocatalysis	The process by which an enzyme expedites biochemical reactions without itself being changed
Biocryosolvent	A biological solvent for maintaining constant low temperature
Biofilm	An aggregate of microbes with a distinct architecture
Biopolymer	A large molecule having a repeating structural feature assembled by a living system from building blocks; proteins (built from amino acids) and DNA (built from nucleotides) are two examples
Biotic	Of or relating to living things; caused or produced by living organisms
Bolide	A detonating meteor fireball
Carbohydrate	A group of organic compounds that consist of carbon, hydrogen, and oxygen; carbohydrates can be simple sugars that consist of a single sugar molecule and cannot be further decomposed by hydrolysis, or complex sugars such as starch
Carbonaceous chondrite	A rare type of stony meteorite that is rich in carbon compounds and is thought to be relatively unaltered since the beginning of the solar system; its spectrum (and probably also its composition) closely resembles that of the C-type asteroids
Carbonate mineral	A mineral having the carbonate ion, such as calcite (calcium carbonate), siderite (iron carbonate), and magnesite (magnesium carbonate)
Catalyst	A substance that enhances the rate of reaction by providing a lower-energy alternative pathway
Chemolithoautotroph	An organism capable of generating metabolically useful energy by the oxidation of inorganic compounds
Chirality	Handedness; property of a molecule configured such that it cannot be superimposed on its mirror image
Chondrite	A stony meteorite that contains spherical bodies of pyroxene and olivine minerals
Codon	The basic unit of the genetic code; a set of any three adjacent bases in DNA or RNA
Colemanite	A natural white or colorless hydrated calcium borate, $Ca_2B_6O_{11} \cdot 5H_2O$
Cosmic rays	High-energy charged particles consisting of atomic nuclei, electrons, and protons and originating from the Sun and from energetic astrophysical processes (e.g., those associated with black holes, supernovas, and so on)
Cryosolvent	A solvent for maintaining constant low temperature
Cryptoendolith	An organism that lives within rocks on Earth's surface
Cytotoxic	Producing toxic effects on cells

Deamination	Removal of an amine group, usually by hydrolysis
Decarboxylation	Removal of a carbonyl radical, especially from amino acids and proteins
Deoxyribose	A sugar, $C_5H_{10}O_4$, that is a major constituent of DNA
DNA	Deoxyribonucleic acid; the genetic biopolymer of most terrean organisms
Electrophile	An electron-deficient ion or molecule that accepts a pair of electrons from its reaction partner to form a new bond
Enantiomer	One of an isometric pair of either crystalline forms or chemical compounds whose molecules are non-superimposable mirror images of each other; enantiomers are optically active and rotate the plane of polarized light
Endergonic	Requiring energy
Endosymbiosis	A mutually beneficial relationship between two organisms in which one lives inside the other
Eubacteria	The group of prokaryotes that are the true bacteria
Eukaryote	An organism having a membrane-bound nucleus and usually other organelles
Exogenous	Derived or originated externally; not arising within an organism
Exopolymer	A high-molecular-weight bipolymer, usually a carbohydrate, that is secreted by an organism into the environment
Exopolysaccharide	Polysaccharides that are secreted by an organism into the environment
Extrasolar	Being or originating outside the solar system
Extremophile	An organism adapted to living in conditions of extreme temperature, pressure, or chemical concentration
Fractionation	Separation of a complex mixture in successive stages into fractions, each of which is enriched in one of the components of the mixture
Free radical	A highly reactive chemical species carrying no charge and having a single unpaired electron in an orbital
Fullerene	Any of various cagelike molecules that constitute the third form of pure carbon (along with the forms diamond and graphite), whose prototype C_{60} (buckyball) is the roundest molecule that exists; fullerenes are a class of discrete molecules, soccerball-shaped forms of carbon with extraordinary stability (so named because their configuration suggests the shape of Buckminster Fuller's famous geodesic dome)
Gray (Gy)	Unit of absorbed dose of ionizing radiation corresponding to the absorption of 1 J per kg of absorbing material; 1 gray = 100 rads; on Earth a typical mid-latitude, sea-level natural background level is ~0.3 mGy per year, whereas on the surface of Europa the level is almost 1010 times higher, enough to kill humans in one minute of exposure)
Gyrase	A type II topoisomerase that introduces negative supercoils (or relaxes positive supercoils) into DNA by looping the template so as to form a crossing, then cutting one of the double helices and passing the other through it before resealing the break, changing the linking number by two in each enzymatic step
Halophilic	Requiring a high salt concentration for optimal growth

Heteroatom	Atom other than carbon in the structure of a heterocyclic compound
Heterocyclic compound	Compound in which the structure contains one or more rings of atoms with at least one atom being an element other than carbon
Heterotroph	An organism that uses only organic matter for energy and growth
Hydrophobicity	The tendency to repel water
Hyperthermophile	An organism adapted to living in high temperatures of 80°C or higher
Interstellar medium	The dust, molecular clouds, and neutral hydrogen that lie between the stars of this galaxy, generally in the plane of the Milky Way, but whose density is highly variable
Isomer	One of two or more substances that have the same chemical composition but differ in structural form
Isomerization	The process whereby a compound is changed into a isomeric form
Isosteres	Molecules or ions of similar size and the same number of valence electrons
Lipid	An organic molecule that is not soluble in water and often forms membranes
Liposome	An artificial microscopic vesicle consisting of an aqueous core enclosed in one or more phospholipid layers
Lysozome	A membrane-bound organelle in the cytoplasm of most cells containing various hydrolytic enzymes that function in intracellular digestion
Magnetotaxis	Coordinated movement of certain motile microorganisms in response to their sensing a magnetic field
Mesophilic	Preferring moderate temperatures
Metabolism	The processes or chemical changes in a cell by which food is built up (anabolism) into living protoplasm and by which protoplasm is broken down (catabolism) into simpler compounds with the exchange of energy
Metallicity	A measure of the proportion of an object's matter that is made up of chemical elements other than hydrogen and helium
Methanogen	An organism capable of producing methane from the decomposition of organic material
Monomer	A building block of a polymer, including a biopolymer; amino acids are monomers of polypeptides (proteins), and nucleotides are monomers of nucleic acids
Murchison (meteorite)	A carbonaceous chondrite, type II (CM2), suspected to be of cometary origin due to its high water content (12 percent)
Nucleophile	A molecule that contributes a pair of electrons to its reaction partner to form a new bond
Nucleoside	Glycosylamine made by attaching a nucleobase to a ribose or deoxyribose ring (for example, cytidine, uridine, adenosine, guanosine, thymidine, and inosine)
Nucleosynthesis	The process by which heavier chemical elements are synthesized from hydrogen nuclei in the interiors of stars
Oligonucleotide	A short polymer of 2 to 20 nucleotides

Oligotroph	A microorganism specifically adapted to grow under conditions of low nutrient supply
Oligotrophic	Term used to describe lakes or oceans that lack carbon and energy sources for photosynthetic organisms
Organic	Of or relating to any covalently bonded compound containing carbon atoms; derived from living systems; more recently, given the fact that all known living systems contain carbon in reduced form (i.e., not carbonate), "organic" has come to mean "containing reduced carbon"
Oxaloacetate	A salt or ester of oxaloacetic acid
Oxidation/reduction	The change in the oxidation state of atoms or ions due to the "loss" or "gain" of electrons
Panspermia	The theory that microorganisms or biochemical compounds from outer space are responsible for originating life on Earth and possibly in other parts of the universe where suitable atmospheric conditions exist
PCR	Polymerase chain reaction
Peptide	Any of various natural or synthetic compounds containing two or more amino acids linked by the carboxyl group of one amino acid and the amino group of another
Peptidoglycan	A polymer found in the cell walls of prokaryotes that consists of polysaccharide and peptide chains in a strong molecular network
Peridotite	Any of a group of igneous rocks composed mainly of olivine and various pyroxenes and having a granitelike texture
Phosphide	A compound of phosphorus and a more electropositive element or radical
Phosphorylation	Chemical process in which a phosphate group is added to an organic molecule
Photolysis	The decomposition of a substance into simpler units as a result of the action of light
Phylogenetic	Relating to evolutionary history
Piezophile	An organism adapted to living in high-pressure environments, such as hydrothermal vents
Pigment	A colored molecule
Polycyclic aromatic hydrocarbons (PAHs)	A class of very stable organic molecules that are made up of only carbon and hydrogen; are flat, with each carbon having three neighboring atoms, much like graphite; and are a standard product of combustion
Polyelectrolyte	An electrolyte, such as a protein or polysaccharide, that has a high molecular weight
Polysaccharides	A class of high-molecular-weight carbohydrates that can be broken down to monosaccharides on hydrolysis
Prebiotic	Occurring before life appeared on Earth
Protein	Any of a group of complex organic compounds, consisting essentially of com-

	binations of amino acids in peptide linkages, that contain carbon, hydrogen, oxygen, nitrogen, and usually sulfur
Protonated	Having protons added to a base
Protoplanetary disk	The disk of dust and gas surrounding a star out of which planets form
Psychrophilic	Requiring low temperatures for growth
Radiolysis	The breakdown of molecules as a result of exposure to ionizing radiation
Retrotransposon	A transposon copied from RNA with the use of reverse transcriptase
Ribosome	A minute, round particle composed of RNA and protein found in the cytoplasm of living cells and active in the synthesis of proteins
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
Serpentinization reaction	A hydrothermal reaction by which magnesium-rich silicate minerals are converted into or replaced by serpentine minerals
Siderophore	A molecular receptor that binds and transports iron
Silane	Any of a group of silicon hydrides having the general formula SiH that are analogous to the paraffin hydrocarbons
Sphalerite	The primary ore of zinc, occurring in usually yellow-brown or brownish-black crystals or cleavage masses, essentially ZnS with some cadmium, iron, and manganese
Stereochemistry	The branch of chemistry concerned with the study of how atoms or molecules are affected by their three-dimensional spatial arrangement, e.g., the study of stereoisomers
Synthetic biology	Engineering of biological components or systems that are not known to nature or the reengineering of existing biological components
Tagish Lake (meteorite)	A unique carbonaceous chondrite collected very soon after falling to Earth in a remote part of northwestern Canada in January 2001
Thermophile	An organism adapted to living in high-temperature environments
Thioester	Compound resulting from the bonding of sulfur with an acyl group with the general formula R-S-CO-R′
Tholin	A term used in planetary science to refer generally to organic heteropolymers; the reddish tar-like organic residue created in simulations of the action of ultraviolet radiation on gases typically found in planetary environments
Thymidine	A nucleoside, $C_{10}H_{14}N_2O_5$, composed of thymine and deoxyribose
Vesicle	A microscopic volume defined by a boundary structure; examples include self-assembled vesicles bounded by a membranous lipid bilayer, and small cavities formed in volcanic rock by entrapment of a gas bubble during solidification
Vitrification	The process of liquid water moving directly into the glassy state without ice crystal formation

B

Biographies of Committee Members and Staff

JOHN A. BAROSS, *Chair*, is a professor in the School of Oceanography and the Center for Astrobiology and Evolution at the University of Washington. His research specialty is the ecology, physiology, and taxonomy of microorganisms from hydrothermal vents and subseafloor environments. Dr. Baross has particular interests in the microbiology of extreme environments and in the significance of submarine hydrothermal vent environments for the origin and evolution of life and for the possibility of life on other planets in similar settings. He is a fellow of the American Academy of Microbiology, an associate member of the National Academy of Sciences, and a member of the American Society for Microbiology, the American Chemical Society, the American Geophysical Union, and the International Society for the Study of the Origin and Evolution of Life. He previously served on several National Research Council (NRC) committees, including service as co-chair of the Committee on the Origins and Evolution of Life (2000-2002) and the Committee for a Review of Programs to Determine the Extent of Life in the Universe (2001-2002). Dr. Baross also served as a member of the Steering Group for the Workshop on Size Limits of Very Small Microorganisms (1998-1999), the Task Group on Sample Return from Small Solar System Bodies (1997-1998), and the Ad Hoc Task Group on Planetary Protection (1991-1992).

STEVEN A. BENNER is a distinguished fellow at the Foundation for Applied Molecular Evolution. He was previously a professor in the Department of Chemistry at the University of Florida. Dr. Benner's research involves various facets of biochemistry and bioorganic studies with emphases on bioinformatics, experimental paleobiochemistry, nucleic acid chemistry, small-molecule evolution, astrobiology, and nanotechnology. He has lectured on such topics as "Genomic Sequences as Organic Molecules: An Evolutionary Approach to Understanding What They Do," "Reconstructing the Chemical Past: Experimental Paleobiochemistry," and "Redesigning Nucleic Acids: Obtaining Molecular Evolution in the Laboratory." Dr. Benner has served on several NRC committees, including the Committee on the Astrophysical Context of Life (2003-2004), the Committee on the Origins and Evolution of Life in the Universe (2001-2004), and the Committee for a Review of Programs to Determine the Extent of Life in the Universe (2001-2002).

GEORGE D. CODY is a geologist and member of the Senior Research Staff in the Geophysical Laboratory at the Carnegie Institution of Washington. His research interests include abiotic organic synthesis, the organic geochemistry of biomacromolecules, and the structure and chemistry of extraterrestrial macromolecules. His most recent research focuses on coupling programs in experimental organic geochemistry with theoretical or computational

chemistry with the ultimate goal of highlighting subtle but important long-range controls in local molecular reactivity. Dr. Cody was awarded an Enrico Fermi Scholarship in the Chemistry Division at Argonne National Laboratory in 1994 and a Japanese Society for the Advancement of Science fellowship in 1996. He was elected vice chair of the Origins of Life Gordon Conference for 2003, and subsequently chair, in 2005. Dr. Cody also served as co-organizer of the Living Planet Symposium held at the Carnegie Institution of Washington in 2002. He served as a member of the NRC Task Group on Exploring Organic Environments in the Solar System (2000-2004).

SHELLEY D. COPLEY is a professor of molecular, cellular and developmental biology at the University of Colorado at Boulder. Her research interests center on the molecular evolution of enzymes and metabolic pathways and protein structure-function relationships. Dr. Copley is a member of the Council of Fellows of the University of Colorado's Cooperative Institute for Research in Environmental Sciences. Dr. Copley served on the NSF Molecular Biochemistry Panel (1999-2003), was co-chair for the Gordon Conference on Enzymes, Coenzymes, and Metabolic Pathways (2004), and currently serves on the National Institutes of Health Genetic Variation and Evolution Study Section.

NORMAN R. PACE is a professor in the Department of Molecular, Cellular, and Developmental Biology at the University of Colorado. Dr. Pace is an internationally recognized expert in nucleic acids and associated enzymes. His studies of ribosomal RNA structures have set new standards for the definition of phylogenetic relationships among organisms. His research interests include RNA enzymes, RNA processing, macromolecular structure, molecular evolution, and microbial ecology. Dr. Pace formerly served as a member of the Board of Scientific Counselors for the National Center for Biotechnology Information, National Library of Medicine. He is a member of the National Academy of Sciences and a fellow of the American Association for the Advancement of Science and the American Academy of Microbiology. In addition, he has served on several NRC committees, including the Committee on the Origins and Evolution of Life in the Universe (2000-2002), the Committee for a Review of Programs to Determine the Extent of Life in the Universe (2001-2002), and the Steering Group for the Workshop on Size Limits of Very Small Microorganisms (1998-1999).

JAMES H. SCOTT leads the geobiology group within the Department of Earth Sciences at Dartmouth College. He analyzes carbon, nitrogen, and sulfur isotope ratios to understand how physiological and biochemical processes that occur in microorganisms affect the surrounding geochemistry, and vice versa. Recently, he has been focusing on the isotopic fractionation associated with specific biochemicals such as amino acids, lipids, and nucleotides, which may reveal key pathways that communities of microorganisms use in metabolism. He was formerly a member of the senior staff in the Geophysical Laboratory at the Carnegie Institution of Washington. He has presented papers at numerous scientific conferences, including ASLO, Under Represented Minorities Program, Presentation Session, Santa Fe, N. Mex., 1992; Carnegie Institution of Washington, Washington, D.C. (Geophysical Laboratory, Fall-Winter Seminar Series), 1999 and 2001; and the American Chemical Society, San Diego, Calif., 2001. In addition, he has presented papers at the (1) International Conference on High Pressure Bioscience and Biotechnology, Dortmund, Germany 2002, (2) Living Planet Seminar at the Carnegie Institution of Washington, Washington, D.C., 2002, (3) Carnegie Centennial Symposium (Connecting the Earth's Physical and Biological Components), Carnegie Institution of Washington, Washington, D.C., 2002, and (4) Gordon Research Conference on the Origins of Life in Ventura, Calif., 2005. His academic awards and distinctions include a 1989 SROP-CIC internship, University of Wisconsin (UW)-Milwaukee; a 1991 REU-NSF internship, Center for Great Lakes Studies, UW-Milwaukee; a 1992-1995 AOP pre-doctoral fellow award; a 1993 MBL scholarship, Microbial Diversity Summer Course, MBL, Woods Hole, Mass.; and the 2001 NASA Astrobiology Institute Director's Travel Scholarship.

ROBERT SHAPIRO is professor emeritus and senior research scientist in the Department of Chemistry at New York University. His research has centered on the chemistry of nucleic acids, with emphasis on the reactions of DNA and RNA with carcinogens and mutagens. Dr. Shapiro is author or co-author of over 110 publications, primarily in the area of DNA chemistry. In particular, he and his co-workers have studied the ways in which environmental chemicals can damage our hereditary material, causing changes that can lead to mutations and cancer.

Dr. Shapiro is also the author or co-author of books that include *Origins: A Skeptic's Guide to the Creation of Life on Earth and Life Beyond Earth.* His research has been supported by numerous grants from the National Institutes of Health, Department of Energy, National Science Foundation, and other organizations. Dr. Shapiro is the recipient of the 2004 Trotter Prize in Information, Complexity and Inference and he has received a National Institutes of Health Career Development Award. He is a member of the American Chemical Society, the American Society for Biochemistry and Molecular Biology, and the New York Academy of Sciences. Although Dr. Shapiro had no prior NRC committee experience, he participated in the formulation of the current study by virtue of the key role he played in the April 2002 Weird Life Workshop sponsored by the SSB/BLS Committee on the Origins and Evolution of Life.

MITCHELL L. SOGIN is director of the Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory. His research interests emphasize molecular phylogeny and the evolution of eukaryotic ribosomal RNAs. He is a member of the American Society of Microbiology, the Society of Protozoologists, the International Society of Evolutionary Protozoologists, the Society for Molecular Biology and Evolution, the American Association for the Advancement of Science, and the American Society for Cell Biology. Dr. Sogin is a former member of the Space Studies Board, an associate fellow of the Canadian Institute for Advanced Research, a division lecturer for the American Society of Microbiology, a recipient of the Stoll Stunkard Award from the American Society of Parasitologists, a fellow of the American Academy of Microbiology, and a visiting Miller Research Professor at the University of California at Berkeley. He was a member of the NRC Committee on Preventing the Forward Contamination of Mars.

JEFFREY L. STEIN is currently a Kauffman Fellow at Sofinnova Ventures. Dr. Stein has held a number of senior scientific and management positions in the biopharmaceutical industry. He was a founder, director, executive vice president, and chief scientific officer of Quorex Pharmaceuticals. Prior to co-founding Quorex, he was principal scientist at Diversa Corporation, where he founded and led both the small-molecule discovery team and the microbial diversity group. Dr. Stein was formerly a principal investigator at the Agouron Institute in La Jolla, Calif., where he pioneered the cloning and expression of multi-gene small-molecule pathways from microbial genomes. Additionally, he currently holds research positions in the Center for Marine Biotechnology and Biomedicine and the Marine Biology Research Division at the University of California, San Diego (UCSD). Dr. Stein received his Ph.D. from UCSD and conducted postdoctoral research as an Alexander Hollaender Distinguished Postdoctoral Fellow at Caltech in the laboratory of Melvin Simon.

ROGER SUMMONS is a professor in the Department of Earth, Atmospheric, and Planetary Sciences at the Massachusetts Institute of Technology. He is also a member of the Earth System Initiative, under which he and his team work in collaboration with microbiologists and ecologists to identify and study environmentally and geologically significant processes that are mediated by microorganisms. His laboratory research focuses on the biogeochemistry of microbial ecosystems, chemistry of biomarkers-molecular fossils, isotopic biosignatures, geochemistry of petroleum, and co-evolution of life and Earth's surface environment. Dr. Summons is a fellow of the Royal Australian Chemical Institute and a fellow of the Australian Academy of Science. His NRC experience includes membership on the Committee on the Origins and Evolution of Life (2003-2006).

JACK W. SZOSTAK is the Alexander Rich Distinguished Investigator at Massachusetts General Hospital and a professor of genetics at Harvard Medical School. A distinguished molecular biologist, Dr. Szostak has made groundbreaking contributions in several different areas of biology, most recently to the understanding of the origins of biological catalysis. He has contributed more than 100 articles to scientific journals. He served as co-chair of the Nucleic Acids Gordon Research Conference in 1993 and of the Keystone Symposium on RNA in 1996, and he was the Harvey Society Lecturer in 1998. Dr. Szostak was awarded, along with Gerald Joyce, the National Academy of Sciences Award in molecular biology in 1994 and the Hans Sigrist Prize from the University of Bern in 1997. He participated in the SSB's workshop "Research Issues Regarding Alternative Life Forms (Weird Life)."

Dr. Szostak is a member of the National Academy of Sciences and a fellow of the American Academy of Arts and Sciences. He has served as the co-chair of two NRC committees: the Origins and Evolution of Life in the Universe and the Astrophysical Context of Life.

Staff

DAVID H. SMITH, *Study Director*, joined the staff of the Space Studies Board in 1991. He is the senior staff officer and study director for a variety of NRC activities, including the Committee on Planetary and Lunar Exploration, the Committee on the Origins and Evolution of Life, the Mars Astrobiology Task Group, the Mars Architecture Assessment Task Group, the Committee on the Limits of Organic Life in Planetary Systems, the Task Group on Organic Environments in the Solar System, the Nuclear Systems Committee, and the proposed Lunar Science Strategy Committee. He also organizes the SSB's summer intern program and supervises most, if not all, of the interns. He received a B.Sc. in mathematical physics from the University of Liverpool in 1976 and a D.Phil. in theoretical astrophysics from Sussex University in 1981. Following a postdoctoral fellowship at Queen Mary College, University (1980-1982) he held the position of associate editor and, later, technical editor of Sky and Telescope. Immediately prior to joining the staff of the Space Studies Board, Dr. Smith was a Knight Science Journalism Fellow at the Massachusetts Institute of Technology (1990-1991).

JOSEPH K. ALEXANDER, senior program officer, served previously as director of the Space Studies Board (1999-2005), deputy assistant administrator for science in the Environmental Protection Agency's Office of Research and Development (1994-1998), associate director of space sciences at NASA Goddard Space Flight Center (1993-1994), and assistant associate administrator for space sciences and applications in the NASA Office of Space Science and Applications (1987-1993). Other positions have included deputy NASA chief scientist and senior policy analyst at the White House Office of Science and Technology Policy. Mr. Alexander's own research work has been in radio astronomy and space physics. He received B.S. and M.A. degrees in physics from the College of William and Mary.

ROBERT L. RIEMER joined the staff of the National Research Council in 1985. He served as Senior Program Officer for the two most recent decadal surveys of astronomy and astrophysics and has worked on studies in many areas of physics and astronomy for the Board on Physics and Astronomy (where he served as associate director from 1988 to 2000) and the Space Studies Board. Prior to joining the NRC, Dr. Riemer was a senior project geophysicist with Chevron Corporation. He received his Ph.D. in experimental high-energy physics from the University of Kansas-Lawrence and his B.S. in physics and astrophysics from the University of Wisconsin-Madison.

CATHERINE A. GRUBER is an assistant editor with the Space Studies Board (SSB). She joined the SSB as a senior program assistant in 1995. Ms. Gruber first came to the NRC in 1988 as a senior secretary for the Computer Science and Telecommunications Board and has also worked as an outreach assistant for the National Academy of Sciences-Smithsonian Institution's National Science Resources Center. She was a research assistant (chemist) in the National Institute of Mental Health's Laboratory of Cell Biology for 2 years. She has a B.A. in natural science from St. Mary's College of Maryland.

RODNEY N. HOWARD joined the Space Studies Board as a senior project assistant in 2002. Before joining SSB, most of his vocational life was spent in the health profession—as a pharmacy technologist at Doctor's Hospital in Lanham, Maryland, and as an interim center administrator at the Concentra Medical Center in Jessup, Maryland. During that time, he participated in a number of Quality Circle Initiatives that were designed to improve relations between management and staff. Mr. Howard obtained his B.A. in communications from the University of Baltimore County in 1983. He plans to begin coursework next year for his master's degree in business administration.