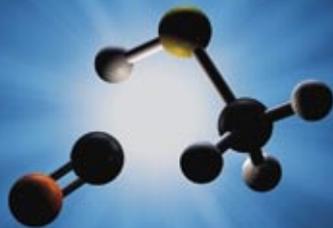


A Simpler
ORIGIN
for **LIFE**



The sudden appearance of a large self-copying molecule such as RNA was exceedingly improbable. Energy-driven networks of small molecules afford better odds as the initiators of life

Extraordinary discoveries inspire extraordinary claims. Thus, James Watson reported that immediately after he and Francis Crick uncovered the structure of DNA, Crick “winged into the Eagle (pub) to tell everyone within hearing that we had discovered the secret of life.” Their structure—an elegant double helix—almost merited such enthusiasm. Its proportions permitted information storage in a language in which four chemicals, called bases, played the same role as 26 letters do in the English language.

Further, the information was stored in two long chains, each of which specified the contents of its partner. This arrangement suggested a mechanism for reproduction: The two strands of the DNA double helix parted company, and new DNA building blocks that carry the bases, called nucleotides, lined up along the separated strands and linked up. Two double helices now existed in place of one, each a replica of the original.

The Watson-Crick structure triggered an avalanche of discoveries about the way living cells function today. These insights also stimulated speculations about life’s origins. Nobel laureate H. J. Muller wrote that the gene material was “living material, the present-day representative of the first life,” which Carl Sagan visualized as “a primitive free-living naked gene situated in a dilute solution of organic matter.” (In this context, “organic” specifies compounds containing bound carbon atoms, both those present in life and those playing no

part in life.) Many different definitions of life have been proposed. Muller’s remark would be in accord with what has been called the NASA definition: life is a self-sustained chemical system capable of undergoing Darwinian evolution.

Richard Dawkins elaborated on this image of the earliest living entity in his book *The Selfish Gene*: “At some point a particularly remarkable molecule was formed by accident. We will call it the *Replicator*. It may not have been the biggest or the most complex molecule around, but it had the extraordinary property of being able to create copies of itself.” When Dawkins wrote these words 30 years ago, DNA was the most likely candidate for this role. Later, researchers turned to other possible molecules as the earliest replicator, but I and others think that this replicator-first model of the origin of life is fundamentally flawed. We prefer an alternative idea that seems much more plausible.

When RNA Ruled the World

COMPLICATIONS to the DNA-first theory soon set in. DNA replication cannot proceed without the assistance of a number of proteins—members of a family of large molecules that are chemically very different from DNA. Both are constructed by linking subunits together to form a long chain, but whereas DNA is made of nucleotides, proteins are made of amino acids. Proteins are the handymen of the living cell. Enzymes, proteins’ most famous subclass, act as expeditors, speeding up chemical pro-

An earlier, longer version of this story was posted on www.sciam.com. Feedback about that version helped to shape the article that appears here.

cesses that would otherwise take place too slowly to be of use to life. Proteins used by cells today are built following instructions encoded in DNA.

The above account brings to mind the old riddle: Which came first, the chicken or the egg? DNA holds the recipe for protein construction. Yet that information cannot be retrieved or copied without the assistance of proteins. Which large molecule, then, appeared first—proteins (the chicken) or DNA (the egg)?

plate an RNA world, containing only RNA molecules that serve to catalyze the synthesis of themselves.... The first step of evolution proceeds then by RNA molecules performing the catalytic activities necessary to assemble themselves from a nucleotide soup.” In this vision, the first self-replicating RNA that emerged from nonliving matter carried out the various functions now executed by RNA, DNA and proteins.

A number of additional clues support

tinguish it from the assertion that RNA merely arose before DNA and proteins.

The Soup Kettle Is Empty

THE RNA-FIRST HYPOTHESIS faces a tremendously challenging question: How did that first self-replicating RNA arise? Enormous obstacles block Gilbert’s picture of RNA forming in a non-living nucleotide soup.

RNA’s building blocks, nucleotides, are complex substances as organic molecules go. Each contains a sugar, a phosphate and one of four nitrogen-containing bases as sub-subunits. Thus, each RNA nucleotide contains nine or 10 carbon atoms, numerous nitrogen and oxygen atoms and the phosphate group, all connected in a precise three-dimensional pattern. Many alternative ways exist for making those connections, yielding thousands of plausible nucleotides that could readily join in place of the standard ones but that are not represented in RNA. That number is itself dwarfed by the hundreds of thousands to millions of stable organic molecules of similar size that are not nucleotides.

The idea that suitable nucleotides might nonetheless form draws inspiration from a well-known experiment published in 1953 by Stanley L. Miller. He applied a spark discharge to a mixture of simple gases that were then thought to represent the atmosphere of the early earth and saw that amino acids formed. Amino acids have also been identified in the Murchison meteorite, which fell in Australia in 1969. Nature has apparently been generous in providing a supply of these particular building blocks. By extrapolation of these results, some writers have presumed that *all* life’s building blocks could be formed with ease in Miller-type experiments and were present in meteorites. This is not the case.

Amino acids, such as those produced in experiments like Miller’s, are far less complex than nucleotides. Their defining features are an amino group (a nitrogen and two hydrogens) and a carboxylic acid group (a carbon, two oxygens and a hydrogen), both attached to the same carbon. The simplest of the 20 amino acids used to build natural proteins contains

Inanimate nature provides us with a variety of mixtures of small molecules as potential incubators for life.

A possible solution appeared when attention shifted to a new champion—RNA. This versatile class of molecule is, like DNA, assembled of nucleotide building blocks but plays many roles in our cells. Certain RNAs ferry information from DNA to ribosomes, structures (which themselves are largely built of other kinds of RNA) that construct proteins. In carrying out its various duties, RNA can take the form of a double helix that resembles DNA or of a folded single strand, much like a protein.

In the early 1980s scientists discovered ribozymes, enzymelike substances made of RNA. A simple solution to the chicken-and-egg riddle now appeared to fall into place: life began with the appearance of the first self-copying RNA molecule. In a germinal 1986 article, Nobel laureate Walter Gilbert wrote in the journal *Nature*: “One can contem-

the idea that RNA appeared before proteins and DNA in the evolution of life. For example, many small molecules, called co-factors, play a role in enzyme-catalyzed reactions. These co-factors often carry an attached RNA nucleotide with no obvious function. Such structures have been considered “molecular fossils,” relics descended from the time when RNA alone, without DNA or proteins, ruled the biochemical world.

This clue and others, however, support only the conclusion that RNA preceded DNA and proteins; they provide no information about the origin of life, which may have involved stages prior to the RNA world in which other living entities ruled supreme. Confusingly, researchers use the term “RNA world” to refer to both notions. Here I will use the term “RNA first” for the claim that RNA was involved in the origin of life, to dis-

Overview/Origin of Life

- Theories of how life first originated from nonliving matter fall into two broad classes—replicator first, in which a large molecule capable of replicating (such as RNA) formed by chance, and metabolism first, in which small molecules formed an evolving network of reactions driven by an energy source.
- Replicator-first theorists must explain how such a complicated molecule could have formed before the process of evolution was under way.
- Metabolism-first proponents must show that reaction networks capable of growing and evolving could have formed when the earth was young.

blocks, whether nucleotides or a simpler substitute, their spontaneous assembly into a replicator involves implausibilities that dwarf those required for the preparation of the soup. Let us presume that the soup of building blocks has somehow been assembled, under conditions that favor their connection into chains. They would be accompanied by hordes of defective units, the inclusion of which in a nascent chain would ruin its ability to act as a replicator. The simplest kind of flawed unit would have only one “arm” available for connection to a building block, rather than the two needed to support further growth of the chain.

An indifferent nature would theoretically combine units at random, producing an immense variety of short, terminated chains, rather than the much longer one of uniform backbone geometry needed to support replicator and catalytic functions. The probability of this latter process succeeding is so vanishingly small that its happening even once anywhere in the visible universe would count as a piece of exceptional good luck.

Life with Small Molecules

NOBEL LAUREATE Christian de Duve has called for “a rejection of improbabilities so incommensurably high that they can only be called miracles, phenomena

that fall outside the scope of scientific inquiry.” DNA, RNA, proteins and other elaborate large molecules must then be set aside as participants in the origin of life. Inanimate nature instead provides us with a variety of mixtures of small molecules with which to work.

Fortunately, an alternative group of theories that can employ these materials has existed for decades. The theories use a thermodynamic, rather than a genetic, definition of life, under a scheme put forth by Sagan in the *Encyclopedia Britannica*: a localized region that increases in order (decreases in entropy) through cycles driven by an energy flow would be considered alive. This small-molecule approach is rooted in the ideas of Soviet biochemist Alexander Oparin. Origin-of-life proposals of this type differ in specific details; here I will list five common requirements (and add some ideas of my own).

1. A boundary is needed to separate life from nonlife. Life is distinguished by its great degree of organization, yet the second law of thermodynamics requires that the universe move in a direction in which disorder, or entropy, increases. A loophole, however, allows entropy to decrease in a limited area, provided that a greater increase occurs outside the area. When living cells grow and multiply, they convert chemical energy or radia-

tion to heat. The released heat increases the entropy of the environment, compensating for the decrease in living systems. The boundary maintains this division of the world into pockets of life and the nonliving environment in which they must sustain themselves.

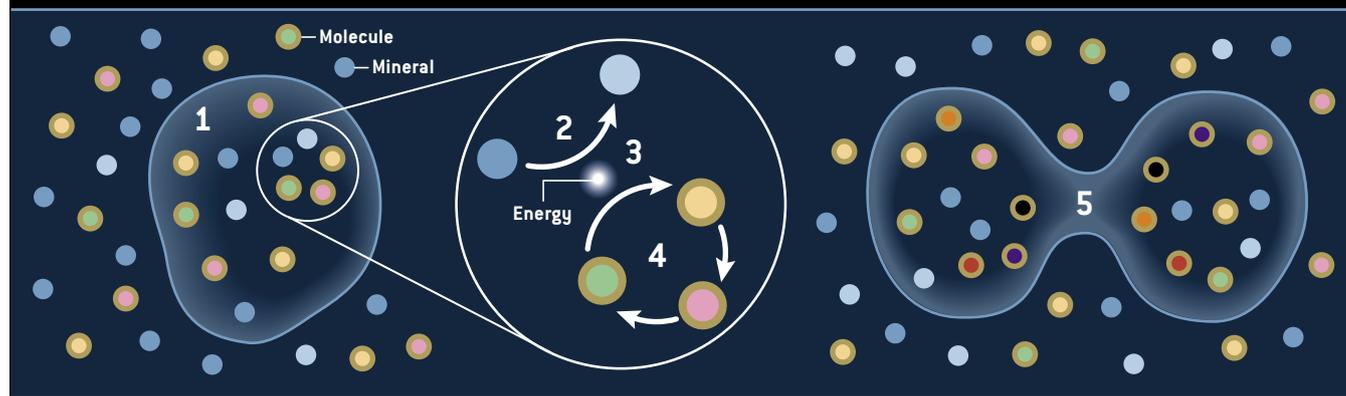
Today sophisticated double-layered cell membranes, made of chemicals classified as lipids, separate living cells from their environment. When life began, some natural feature probably served the same purpose. In support of this idea, David W. Deamer of the University of California, Santa Cruz, has observed membranelike structures in meteorites. Other proposals have suggested natural boundaries not used by life today, such as iron sulfide membranes, rock surfaces (in which electrostatic interactions segregate selected molecules from their environment), small ponds and aerosols.

2. An energy source is needed to drive the organization process. We consume carbohydrates and fats, combining them with oxygen that we inhale, to keep ourselves alive. Microorganisms are more versatile and can use minerals in place of the food or the oxygen. In either case, the transformations that are involved are called redox reactions. They entail the transfer of electrons from an electron-rich (or reduced) substance to

FIVE REQUIREMENTS FOR METABOLISM FIRST

At least five processes must occur for small molecules to achieve a kind of life—here defined as the creation of greater order in localized regions by chemical cycles driven by an energy flow. First, something must create a boundary to separate the living region from the nonliving environment (1). A source of energy must be available, here depicted as a mineral (blue) undergoing a heat-producing reaction (2). The released energy

must drive a chemical reaction (3). A network of chemical reactions must form and increase in complexity to permit adaptation and evolution (4). Finally, the network of reactions must draw material into itself faster than it loses material, and the compartments must reproduce (5). No information-storing molecule (such as RNA or DNA) is required; heredity is stored in the identity and concentration of the compounds in the network.



an electron-poor (or oxidized) one. Plants can capture solar energy directly and adapt it for the functions of life. Other forms of energy are used by cells in specialized circumstances—for example, differences in acidity on opposite sides of a membrane. Yet others, such as radioactivity and abrupt temperature differences, might be used by life elsewhere in the universe.

3. A coupling mechanism must link the release of energy to the organization process that produces and sustains life. The release of energy does not necessarily produce a useful result. Chemical energy is released when gasoline is burned within the cylinders of an automobile, but the vehicle will not move unless that energy is used to turn the wheels. A mechanical connection, or coupling, is required. Every day, in our own cells, each of us degrades pounds of a nucleotide called ATP. The energy released by this reaction serves to drive processes necessary for our biochemistry that would otherwise proceed too slowly or not at all. Linkage is achieved when the reactions share a common intermediate, and the process is sped up by the intervention of an enzyme. One assumption of the small-molecule approach is that coupled reactions and primitive catalysts sufficient to get life started exist in nature.

4. A chemical network must be formed to permit adaptation and evolution. We come now to the heart of the matter. Imagine, for example, that an energetically favorable redox reaction of a mineral drives the conversion of an organic chemical, A, to another one, B, within a compartment. I call this key transformation a driver reaction, because it serves as the engine that mobilizes the organization process. If B simply reconverts back to A or escapes from the compartment, we would not be on a path that leads to increased organization. In contrast, if a multistep chemical pathway—say, B to C to D to A—reconverts B to A, then the steps in that circular process (or cycle) would be favored to continue operating because they replenish the supply of A, allowing the continuing useful discharge of energy by the mineral reaction [see box on page 53].

What Readers Want to Know

In Scientific American's blog, Robert Shapiro answered questions raised by readers of the Web version of this article. An edited selection follows.

Does the metabolism-first hypothesis point to a single origin or multiple independent origins of life? —JR

A: Multiple origins seem more viable with the metabolism-first scenario. Gerald Feinberg and I discussed the possibility of alien life (life not based on RNA, DNA and other biochemistry familiar to us) in our 1980 book, *Life beyond Earth*. Researchers at a conference hosted by Paul Davies at Arizona State University in December 2006 concluded that alien life may even exist, undetected, on this planet. The great majority of microorganisms that can be observed under a microscope cannot be grown in conventional culture media and remain uncharacterized. Alien microbes may also exist in habitats on the earth that are too extreme for even the hardest forms of our familiar life.

Why do we have to demonstrate metabolism first in a reaction vessel? Can't we simulate it in software? —Dave Evanoff

A: Stuart Kauffman, Doron Lancet and others have used computer simulations to illustrate the feasibility of self-sustaining reaction cycles. Such simulations have not specified the exact chemical mixtures and reaction conditions needed to establish self-sustaining chemical networks. We do not yet know all the reaction pathways open to mixtures of simple organic compounds, let alone their thermodynamic constants. Even if such data were available, most chemists would not be convinced by a computer simulation but would demand an experimental demonstration.

The fact that all biological molecules are of one handedness needs some explanation. —John Holt

A: If the mineral transformation that powered the reaction cycle I discuss in my article were selective for only one mirror-image form of chemical A, then the product B and other members of the cycle might also occur in only one mirror-image form. Control of handedness, or chirality, becomes crucial when small chiral molecules are linked together to form larger ones. A modern enzyme may contain 100 linked amino acids, all of the same handedness (so-called L-amino acids). If a D-amino acid were substituted for its mirror-image L-form at a sensitive site within the enzyme, then the enzyme's shape would change and its function might be lost.

Branch reactions will occur as well, such as molecules converting back and forth between D and another chemical, E, that lies outside the ABCD cycle. Because the cycle is driven, the E-to-D reaction is favored, moving material into the cycle and maximizing the energy release that accompanies the driver reaction.

The cycle could also adapt to changing circumstances. As a child, I was fascinated by the way in which water, released from a leaky hydrant, would find a path downhill to the nearest sewer. If falling leaves or dropped refuse blocked that path, the water would back up until another route was found around the obstacle. In the same way, if a change in the acidity or in some other environmental circumstance should hinder a step in the

pathway from B to A, material would back up until another route was found. Additional changes of this type would convert the original cycle into a network. This trial-and-error exploration of the chemical "landscape" might also turn up compounds that could catalyze important steps in the cycle, increasing the efficiency with which the network used the energy source.

5. The network must grow and reproduce. To survive and grow, the network must gain material faster than it loses it. Diffusion of network materials out of the compartment into the external world is favored by entropy and will occur to some extent. Some side reactions may produce gases, which escape, or form tars, which will drop out of so-

lution. If these processes together should exceed the rate at which the network gains material, then it would be extinguished. Exhaustion of the external fuel would have the same effect. We can imagine, on the early earth, a situation where many start-ups of this type occur, involving many alternative driver reactions and external energy sources. Finally, a particularly hardy one would take root and sustain itself.

A system of reproduction must eventually develop. If our network is housed in a lipid membrane, physical forces may split it after it has grown enough. (Freeman Dyson of the Institute for Advanced Study in Princeton, N.J., has described such a system as a “garbage bag world” in contrast to the “neat and beautiful scene” of the RNA world.) A system that

functions in a compartment within a rock may overflow into adjacent compartments. Whatever the mechanism may be, this dispersal into separated units protects the system from total extinction by a local destructive event. Once independent units were established, they could evolve in different ways and compete with one another for raw materials; we would have made the transition from life that emerges from nonliving matter through the action of an available energy source to life that adapts to its environment by Darwinian evolution.

Changing the Paradigm

SYSTEMS OF THE TYPE I have described usually have been classified under the heading “metabolism first,” which implies that they do not contain a mecha-

nism for heredity. In other words, they contain no obvious molecule or structure that allows the information stored in them (their heredity) to be duplicated and passed on to their descendants. Yet a collection of small items holds the same information as a list that describes the items. For example, my wife gives me a shopping list for the supermarket; the collection of grocery items that I return with contains the same information as the list. Doron Lancet of the Weizmann Institute of Science in Rehovot, Israel, has given the name “compositional genome” to heredity stored in small molecules, rather than a list such as DNA or RNA.

The small-molecule approach to the origin of life makes several demands on nature (a compartment, an external energy supply, a driver reaction coupled to that supply, a chemical network that includes that reaction, and a simple mechanism of reproduction). These requirements are general in nature, however, and are immensely more probable than the elaborate multistep pathways needed to form a molecule that is a replicator.

Over the years, many theoretical papers have advanced particular metabolism-first schemes, but relatively little experimental work has been presented in support of them. In those cases where experiments have been published, they have usually served to demonstrate the plausibility of individual steps in a proposed cycle. The greatest amount of new data has perhaps come from Günter Wächtershäuser and his colleagues at Munich Technical University. They have demonstrated parts of a cycle involving the combination and separation of amino acids in the presence of metal sulfide catalysts. The energetic driving force for the transformations is supplied by the oxidation of carbon monoxide to carbon dioxide. The researchers have not yet demonstrated the operation of a complete cycle or its ability to sustain itself and undergo further evolution. A “smoking gun” experiment displaying those three features is needed to establish the validity of the small-molecule approach.

The principal initial task is the identification of candidate driver reactions—small-molecule transformations (A to B

An RNA-First Researcher Replies

Steven A. Benner of the Westheimer Institute for Science and Technology in Gainesville, Fla., argues that RNA-first models are alive and well.

Even as some declare that the RNA-first model of life's origin is dead because RNA arising spontaneously is fantastically improbable, research is lending support to the model.

Let me first acknowledge that most organic molecules when hit with energy (such as lightning or heat from volcanoes) become something resembling asphalt, more suitable for paving roads than sparking life. But metabolism-first models, to the extent that they have been supported with *any* real chemicals, must also deal with this paradox: molecules reactive enough to participate in metabolism are also reactive enough to decompose. There are no easy solutions.

Like many others, my research group has returned to the scientific imperative: actually do laboratory research to learn about how RNA might have arisen on the earth.

The sugar ribose, the “R” in RNA, provides an object lesson in how a problem declared “unsolvable” may instead merely be “not yet solved.” Ribose long remained “impossible” to make by prebiotic synthesis (reactions among mixtures of molecules that could plausibly have existed on a prebiotic earth) because it contains a carbonyl group—a carbon atom twice bonded to an oxygen atom. The carbonyl group confers both good reactivity (the ability to participate in metabolism) and bad reactivity (the ability to form asphalt). A decade ago Stanley L. Miller concluded that the instability of ribose stemming from its carbonyl group “preclude[s] the use of ribose and other sugars as prebiotic reagents.... It follows that ribose and other sugars were not components of the first genetic material.”

But prebiotic soups need soup bowls made of appropriate minerals, not Pyrex beakers. One attractive “bowl” is found today in Death Valley. In a primordial Death Valley, the environment was alternately wet and dry, rich in organic molecules from planetary accretion and (most important) full of minerals containing boron. Why care about boron? Because boron stabilizes carbohydrates such as ribose. Further, if borate (an oxide of boron) and organic compounds abundant in meteorites are mixed and hit with lightning, good quantities of ribose are formed from formaldehyde and the ribose does not decompose.

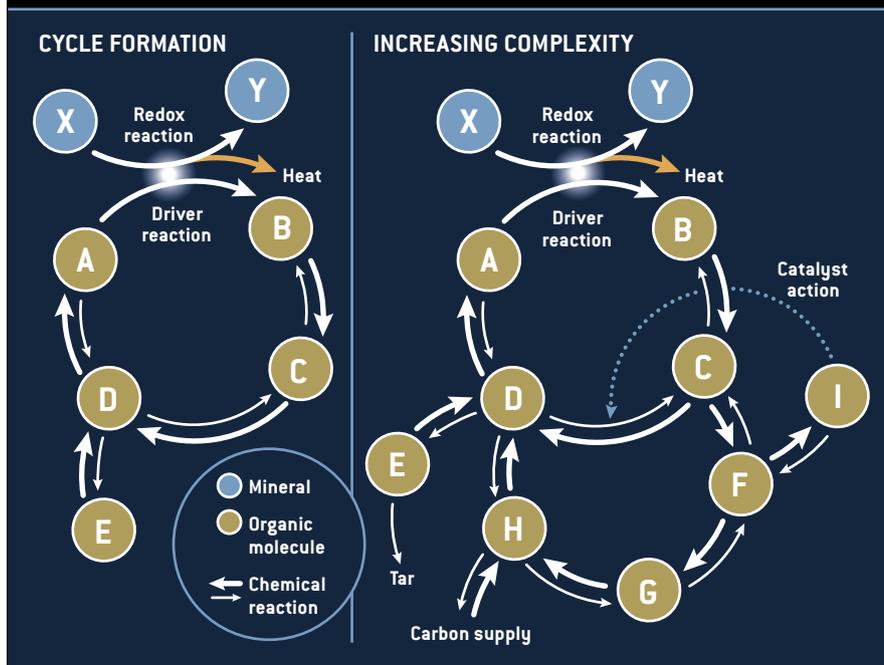
The fact that such a simple solution can be found for a problem declared “unsolvable” does not mean that the first form of life definitely used RNA to do genetics. But it should give us pause when advised to discard avenues of research simply because some of their problematic pieces have not yet been solved.

EVOLUTION OF CHEMICAL NETWORKS

The metabolism-first hypothesis requires the formation of a network of chemical reactions that increases in complexity and adapts to changes in the environment.

CYCLE FORMATION: An energy source (here the so-called redox reaction converting mineral X to mineral Y) couples to a reaction that converts the organic molecule A to molecule B. Further reactions (B to C, C to D...) form a cycle back to A. Reactions involving molecular species outside the cycle (E) will tend to draw more material into the cycle.

INCREASING COMPLEXITY: If a change in conditions inhibits a reaction in the cycle (for example, C to D), then other paths can be explored. Here a bypass has been found by which C is converted to D through intermediates F, G and H. Another solution would be the incorporation into the reaction network of a catalyst (I) whose action (dotted line) unblocks the C to D transformation. To survive, the evolving network must draw in carbon-containing materials from the environment more rapidly than it loses them by diffusion and side reactions, such as the formation of tars that settle out of the solution.



in the preceding example) that are coupled to an abundant external energy source (such as the oxidation of carbon monoxide or a mineral). Once a plausible driver reaction has been identified, there should be no need to specify the rest of the system in advance. The selected components (including the energy source), plus a mixture of other small molecules normally produced by natural processes (and likely to have been abundant on the early earth), could be combined in a suitable reaction vessel. If an evolving network were established, we would expect the concentration of the participants in the network to increase and alter with time. New catalysts that increased the rate of key reactions might appear, whereas irrelevant materials would de-

crease in quantity. The reactor would need an input device (to allow replenishment of the energy supply and raw materials) and an outlet (to permit removal of waste products and chemicals that were not part of the network).

In such experiments, failures would be easily identified. The energy might be dissipated without producing any significant changes in the concentrations of the other chemicals, or the chemicals might be converted to a tar, which would clog the apparatus. A success might demonstrate the initial steps on the road to life. These

steps need not duplicate those that took place on the early earth. It is more important that the general principle be demonstrated and made available for further investigation. Many potential paths to life may exist, with the choice dictated by the local environment.

An understanding of the initial steps leading to life would not reveal the specific events that led to the familiar DNA-RNA-protein-based organisms of today. Still, because we know that evolution does not anticipate future events, we can presume that nucleotides first appeared in metabolism to serve some other purpose, perhaps as catalysts or as containers for the storage of chemical energy (the nucleotide ATP continues to serve this function today). Some chance event or circumstance may have led to the connection of nucleotides to form RNA. The most obvious function of modern RNA is to serve as a structural element that assists in the formation of bonds between amino acids in the synthesis of proteins. The first RNAs may have served the same purpose, but without any preference for specific amino acids. Many further steps in evolution would be needed to “invent” the elaborate mechanisms for replication and specific protein synthesis that we observe in life today.

If the general small-molecule paradigm were confirmed, then our expectations of the place of life in the universe would change. A highly improbable start for life, as in the RNA-first scenario, implies a universe in which we are alone. In the words of biochemist Jacques Monod, “the universe was not pregnant with life nor the biosphere with man. Our number came up in the Monte Carlo game.”

The small-molecule alternative, however, is in harmony with the views of biologist Stuart Kauffman: “If this is all true, life is vastly more probable than we have supposed. Not only are we at home in the universe, but we are far more likely to share it with as yet unknown companions.”



Additional coverage—including commentaries, answers to questions, links to further reading and the opportunity to post your own comments—can be found at www.sciam.com/ontheweb