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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.

February 12, 2007 | By [Robert Shapiro](#) |

Extraordinary discoveries inspire extraordinary claims. Thus James Watson reported that, immediately after they had uncovered the structure of DNA, Francis 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 twenty six 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, that was subsequently illustrated in many biochemistry texts, as well as on a tie that my wife bought for me at a crafts fair: The two strands of the DNA double helix parted company. As they did so, new DNA building blocks, 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.



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The Watson-Crick structure triggered an avalanche of discoveries about the way in which 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 material containing bound carbon atoms. Organic chemistry, a subject sometimes feared by pre-medical students, is the chemistry of carbon compounds, 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 of life: 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. As we shall see, several other replicators have now been suggested.

When RNA Ruled the World

Unfortunately, complications 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. Proteins, like DNA, are constructed by linking subunits, amino acids in this case, together to form a long chain. Cells employ twenty of these building blocks in the proteins that they make, affording a variety of products capable of performing many different tasks--proteins are the handymen of the living cell. Their most famous subclass, the enzymes, act as expeditors, speeding up chemical processes that would otherwise take place too slowly to be of use to life.

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 in getting life started--proteins (the chicken) or DNA (the egg)?

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 structures (which themselves are largely built of other kinds of RNA) that construct proteins. In carrying out its various duties, RNA can take on the form of a double helix that resembles DNA, or of a folded single strand, much like a protein. In 2006 the Nobel prizes in both chemistry and medicine were awarded for discoveries concerning the role of RNA in editing and censoring DNA instructions. Warren E. Leary could write in the *New York Times* that RNA "is swiftly emerging from the shadows of its better-known cousin DNA."

For many scientists in the origin-of-life field, those shadows had lifted two decades earlier with the discovery of ribozymes, enzyme-like 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 RNA molecule. In a germinal 1986 article, Nobel Laureate Walter Gilbert of Harvard University wrote in the journal *Nature*: "One can contemplate 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 non-living matter carried out the functions now executed by RNA, DNA and proteins.

A number of additional clues seemed to support the idea that RNA appeared before proteins and DNA in the evolution of life. Many small molecules, called cofactors, play a necessary role in enzyme-catalyzed reactions. These cofactors often carry an attached RNA nucleotide with no obvious function. These structures have been considered "molecular fossils," relics descended from the time when RNA alone, without DNA or proteins, ruled the biochemical world. In addition, chemists have been able to synthesize new ribozymes that display a variety of enzyme-like activities. Many scientists found the idea of an organism that relied on ribozymes, rather than protein enzymes, very attractive.

The hypothesis that life began with RNA was presented as a likely reality, rather than a speculation, in journals, textbooks and the media. Yet the clues I have cited only support the weaker 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. Just the same, and despite the difficulties that I will discuss in the next section, perhaps two-thirds of scientists publishing in the origin-of-life field (as judged by a count of papers published in 2006 in the journal *Origins of Life and Evolution of the Biosphere*) still support the idea that life began with the spontaneous formation of RNA or a related self-copying molecule. Confusingly, researchers use the term "RNA World" to refer to both the strong and the weak claims about RNA's role prior to DNA and proteins. Here, I will use the term "RNA first" for the strong claim that RNA was involved in the origin of life.

The Soup Kettle is Empty

The attractive features of RNA World prompted Gerald Joyce of the Scripps Research Institute and Leslie Orgel of the Salk Institute to picture it as "the molecular biologist's dream" within a volume devoted to that topic. They also used the term "the prebiotic chemist's nightmare" to describe another part of the picture: How did that first self-replicating RNA arise? Enormous obstacles block Gilbert's picture of the origin of life, sufficient to provoke another Nobelist, Christian De Duve of Rockefeller University, to ask rhetorically, "Did God make RNA?"

RNA's building blocks, nucleotides, are complex substances as organic molecules go. They each contain a sugar, a phosphate and one of four nitrogen-containing bases as sub-subunits. Thus, each RNA nucleotide contains 9 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 RNA nucleotides are familiar to chemists because of their abundance in life and their resulting commercial availability. In a form of molecular vitalism, some scientists have presumed that nature has an innate tendency to produce life's building blocks preferentially, rather than the hordes of other molecules that can also be derived from the rules of organic chemistry. This idea drew inspiration from a well known experiment published in 1953 by Stanley Miller. He applied a spark discharge to a mixture of simple gases that were then thought to represent the atmosphere of the early Earth. Two amino acids of the set of 20 used to construct proteins were formed in significant quantities, with others from that set present in small amounts. (A description of the Miller experiment and the chemical structures of an amino acid and a nucleotide can be found in "The Origin of Life on the Earth," by L. E. Orgel; *Scientific American*,

October 1994.) In addition, more than 80 different amino acids, some present and others absent from living systems, have been identified as components of 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* of life's building could be formed with ease in Miller-type experiments and were present in meteorites and other extraterrestrial bodies. This is not the case.

A careful examination of the results of the analysis of several meteorites led the scientists who conducted the work to a different conclusion: inanimate nature has a bias toward the formation of molecules made of fewer rather than greater numbers of carbon atoms, and thus shows no partiality in favor of creating the building blocks of our kind of life. (When larger carbon-containing molecules are produced, they tend to be insoluble, hydrogen-poor substances that organic chemists call tars.) I have observed a similar pattern in the results of many spark discharge experiments.

Amino acids, such as those produced or found in these experiments, 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 used to build natural proteins contains only two carbon atoms. Seventeen of the set contain six or fewer carbons. The amino acids and other substances that were prominent in the Miller experiment contained two and three carbon atoms. By contrast, no nucleotides of any kind have been reported as products of spark discharge experiments or in studies of meteorites, nor have the smaller units (nucleosides) that contain a sugar and base but lack the phosphate.

To rescue the RNA-first concept from this otherwise lethal defect, its advocates have created a discipline called prebiotic synthesis. They have attempted to show that RNA and its components can be prepared in their laboratories in a sequence of carefully controlled reactions, normally carried out in water at temperatures observed on Earth. Such a sequence would start usually with compounds of carbon that had been produced in spark discharge experiments or found in meteorites. The observation of a specific organic chemical in any quantity (even as part of a complex mixture) in one of the above sources would justify its classification as "prebiotic," a substance that supposedly had been proved to be present on the early Earth. Once awarded this distinction, the chemical could then be used in pure form, in any quantity, in another prebiotic reaction. The products of such a reaction would also be considered "prebiotic" and employed in the next step in the sequence.

The use of reaction sequences of this type (without any reference to the origin of life) has long been an honored practice in the traditional field of synthetic organic chemistry. My own PhD thesis advisor, Robert B. Woodward, was awarded the Nobel Prize for his brilliant syntheses of quinine, cholesterol, chlorophyll and many other substances. It mattered little if kilograms of starting material were required to produce milligrams of product. The point was the demonstration that humans could produce, however inefficiently, substances found in nature. Unfortunately, neither chemists nor laboratories were present on the early Earth to produce RNA.

I will cite one example of prebiotic synthesis, published in 1995 by *Nature* and featured in the *New York Times*. The RNA base cytosine was prepared in high yield by heating two purified chemicals in a sealed glass tube at 100 degrees Celsius for about a day. One of the reagents, cyanoacetaldehyde, is a reactive substance capable of combining with a number of common chemicals that may have been present on the early Earth. These competitors were excluded. An extremely high concentration was needed to coax the other participant, urea, to react at a sufficient rate for the reaction to succeed. The product, cytosine, can self-destruct by simple reaction with water. When the urea concentration was lowered, or the reaction allowed to continue too long, any cytosine that was produced was subsequently destroyed. This destructive reaction had been discovered in my laboratory, as part of my continuing research on environmental damage to DNA. Our own cells deal with it by maintaining a suite of enzymes that specialize in DNA repair.

The exceptionally high urea concentration was rationalized in the *Nature* paper by invoking a vision of drying lagoons on the early Earth. In a published rebuttal, I calculated that a large lagoon would have to be evaporated to the size of a puddle, without loss of its contents, to achieve that concentration. No such feature exists on Earth today.

The drying lagoon claim is not unique. In a similar spirit, other prebiotic chemists have invoked freezing glacial lakes, mountainside freshwater ponds, flowing streams, beaches, dry deserts, volcanic aquifers and the entire global ocean (frozen or warm as needed) to support their requirement that the "nucleotide soup" necessary for RNA synthesis would somehow have come into existence on the early Earth.

The analogy that comes to mind is that of a golfer, who having played a golf ball through an 18-hole course, then assumed that the ball

could also play itself around the course in his absence. He had demonstrated the possibility of the event; it was only necessary to presume that some combination of natural forces (earthquakes, winds, tornadoes and floods, for example) could produce the same result, given enough time. No physical law need be broken for spontaneous RNA formation to happen, but the chances against it are so immense, that the suggestion implies that the non-living world had an innate desire to generate RNA. The majority of origin-of-life scientists who still support the RNA-first theory either accept this concept (implicitly, if not explicitly) or feel that the immensely unfavorable odds were simply overcome by good luck.

A Simpler Replicator?

Many chemists, confronted with these difficulties, have fled the RNA-first hypothesis as if it were a building on fire. One group, however, still captured by the vision of the self-copying molecule, has opted for an exit that leads to similar hazards. In these revised theories, a simpler replicator arose first and governed life in a "pre-RNA world." Variations have been proposed in which the bases, the sugar or the entire backbone of RNA have been replaced by simpler substances, more accessible to prebiotic syntheses. Presumably, this first replicator would also have the catalytic capabilities of RNA. Because no trace of this hypothetical primal replicator and catalyst has been recognized so far in modern biology, RNA must have completely taken over all of its functions at some point following its emergence.

Further, the spontaneous appearance of any such replicator without the assistance of a chemist faces implausibilities that dwarf those involved in the preparation of a mere nucleotide soup. Let us presume that a soup enriched in the building blocks of all of these proposed replicators has somehow been assembled, under conditions that favor their connection into chains. They would be accompanied by hordes of defective building blocks, the inclusion of which would ruin the ability of the chain to act as a replicator. The simplest flawed unit would be a terminator, a component that had only one "arm" available for connection, rather than the two needed to support further growth of the chain.

There is no reason to presume that an indifferent nature would not combine units at random, producing an immense variety of hybrid short, terminated chains, rather than the much longer one of uniform backbone geometry needed to support replicator and catalytic functions. Probability calculations could be made, but I prefer a variation on a much-used analogy. Picture a gorilla (very long arms are needed) at an immense keyboard connected to a word processor. The keyboard contains not only the symbols used in English and European languages but also a huge excess drawn from every other known language and all of the symbol sets stored in a typical computer. The chances for the spontaneous assembly of a replicator in the pool I described above can be compared to those of the gorilla composing, in English, a coherent recipe for the preparation of chili con carne. With similar considerations in mind Gerald F. Joyce of the Scripps Research Institute and Leslie Orgel of the Salk Institute concluded that the spontaneous appearance of RNA chains on the lifeless Earth "would have been a near miracle." I would extend this conclusion to all of the proposed RNA substitutes that I mentioned above.

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 provides us with a variety of mixtures of small molecules, whose behavior is governed by scientific laws, rather than by human intervention.

Fortunately, an alternative group of theories that can employ these materials has existed for decades. The theories employ a thermodynamic rather than a genetic definition of life, under a scheme put forth by Carl Sagan in the Encyclopedia Britannica: A localized region which 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 the Soviet biologist Alexander Oparin, and current notable spokesmen include de Duve, Freeman Dyson of the Institute for Advanced Study, Stuart Kauffman of the Santa Fe Institute, Doron Lancet of the Weizmann Institute, Harold Morowitz of George Mason University and the independent researcher Günter Wächtershäuser. I estimate that about a third of the chemists involved in the study of the origin of life subscribe to theories based on this idea. Origin-of-life proposals of this type differ in specific details; here I will try to list five common requirements (and add some ideas of my own).

(1) A boundary is needed to separate life from non-life. 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 radiation to heat at the same time. 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. David W. Deamer of the University of California, Santa Cruz, has observed membrane-like structures in meteorites. Other proposals have suggested natural boundaries not used by life today, such as iron sulfide membranes, mineral 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, and combine 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 involve the transfer of electrons from an electron rich (or reduced) substance to 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. Here I will consider redox reactions as the energy source.

(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 my automobile, but the vehicle will not move unless that energy is used to turn the wheels. A mechanical connection, or coupling, is required. Each day, in our own cells, each of us degrades pounds of a nucleotide called ATP. The energy released by this favorable reaction serves to drive processes that are less favorable but necessary for our biochemistry. Linkage is achieved when the reactions share a common intermediate, and the process is speeded 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 naturally-occurring mineral is linked to the conversion of an organic chemical A to another one B within a compartment. The favorable, energy releasing, entropy-increasing reaction of the mineral drives the A-to-B transformation. I call this key transformation a driver reaction, for 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. By contrast, if a multi-step 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 because they replenish the supply of A, allowing the continuing discharge of energy by the mineral reaction.

If we visualize the cycle as a circular railway line, the energy source keeps the trains traveling around it one way. Each station may also be the hub for a number of branch lines, such as one connecting station D to another station, E. Trains could travel in either direction along that branch, depleting or augmenting the cycle's traffic. Thanks to the continual depletion of A, however, material is drawn from D to A. The resulting depletion of D in turn tends to draw material from E to D. In this way, material is "pulled" along the branch lines into the central cycle, 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 utilized the energy source.

(5) The network must grow and reproduce. To survive and grow, the network must gain material at a rate that compensates for the paths that remove it. Diffusion of network materials out of the compartment into the external world is favored by entropy and will occur to some extent, especially at the start of life when the boundary is a crude one established by the environment rather than one of

the highly effective cell membranes available today after billions of years of evolution. Some side reactions may produce gases, which escape, or form tars, which will drop out of solution. 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 startups 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, then physical forces may split it, after it has grown enough. (Freeman Dyson 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 mineral may overflow into adjacent compartments. Whatever the mechanism may be, this dispersal into separated units protects the system from total extinction by a localized 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 mechanism 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. However 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 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 upon nature (a compartment, an external energy supply, a driver reaction coupled to that supply, and the existence of a chemical network that contains that reaction). These requirements are general in nature, however, and are immensely more probable than the elaborate multi-step pathways needed to form a molecule that can function as 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 the Technische Universität München. They have demonstrated portions 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. They 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 in the example before) 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, while irrelevant materials would decrease in quantity. The reactor would need an input device to allow replenishment of the energy supply and raw materials, and an outlet to permit the 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 simply 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. However, 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 still serves this function today). Some chance event or circumstance may have led to the connection of nucleotides to form RNA. The most obvious function of RNA today 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 implausible start for life, as in the RNA-first scenario, implies a universe in which we are alone. In the words of the late 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 unknown companions."

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