

The RNA World: Life before DNA and Protein*

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Introduction

All of the life that is known, all organisms that exist on Earth today or are known to have existed on Earth in the past, are of the same life form: a life form based on DNA and protein. It does not necessarily have to be that way. Why not have two competing life forms on this planet? Why not have biology as we know it and some other biology that occupies its own distinct niche? Yet that is not how evolution has played out. From microbes living on the surface of antarctic ice to tube worms lying near the deep-sea hydrothermal vents, all known organisms on this planet are of the same biology.

Looking at the single known biology on Earth, it is clear that this biology could not have simply sprung forth from the primordial soup. The biological system that is the basis for all known life is far too complicated to have arisen spontaneously. This brings us to the notion that something else, something simpler, must have preceded life based on DNA and protein. One suggestion that has gained considerable acceptance over the past decade is that DNA and protein-based life was preceded by RNA-based life in a period referred to as the 'RNA world'.

Even an RNA-based life form would have been fairly complicated – not as complicated as our own DNA- and protein-based life form – but far too complicated, according to prevailing scientific thinking, to have arisen spontaneously from the primordial soup. Thus, it has been argued that something else must have preceded RNA-based life, or even that there was a succession of life forms leading from the primordial soup to RNA-based life. The experimental evidence to support this conjecture is not strong because, after all, the origin of life was a

* Edited transcript of a public lecture entitled 'The Dawn of Biology Current Views Concerning the Origins of Life', presented at the Scripps Institution of Oceanography, La Jolla, California, 16 May 1991.

historical event that left no direct physical record. However, based on indirect evidence in both the geologic record and the phylogenetic record of evolutionary history on earth, it is possible to reconstruct a rough picture of what life was like before DNA and protein.

What is Life?

It is useful, at the outset, to consider what is meant by the word 'life'. This word has a vague popular meaning, making it difficult to provide a rigorous scientific definition that will satisfy all audiences. The popular definition of 'life' might be stated simply as: 'that which is squishy'. Life, after all, is protoplasmic and cellular. It is made up of cells and organic stuff and is undeniably squishy. A more mechanistic popular definition might be: 'life is that which eats and procreates'. In a very broad sense, living organisms turn food into offspring. They metabolize food and use the energy derived from the food to produce offspring, that is, to produce more life. Among biologists and biochemists a current working definition of 'life' is: 'a self-sustained chemical system capable of undergoing darwinian evolution'. This is the definition that I shall adopt for the following discussion. 'Life' is that which evolves in a darwinian sense.

Darwinian evolution occurs as a result of three physical processes: amplification, mutation and selection. Amplification involves the replication of a prototype, or, more precisely, the replication of a genetic description of a prototype. Mutation is a process that introduces variation during replication of the prototype. Selection involves choosing among the various replicates to establish a new prototype, which is then used to begin another round of amplification, mutation and selection.

How does terrestrial biology embody these three processes? It relies on DNA to provide a genetic description of the prototype. The DNA contains instructions describing, in effect, how to build and operate the organism. These instructions are copied from DNA to RNA. The RNA then acts as a messenger to carry the instructions to a complex cellular apparatus, the ribosome, where the instructions are interpreted to produce proteins. The resulting assemblage of functional proteins might be called the prototype or, more formally, the 'phenotype' of the organism.

Biology carries out amplification by replicating the genetic description of phenotype, that is, by replicating the DNA. Mutations occur during the replication process, so that the DNA copies resemble, but are not identical with, the parental DNA. No two copies are exactly alike. Mutations that exist in the DNA copies are interpreted by the ribosome as altered instructions for the production of proteins. This results in somewhat altered proteins that may have altered function.

Some functional variations will be more useful than others, and it is the variations that are most favorable, together with the DNA that describes them, that are selected to begin the next round of amplification, mutation, and selection. And so it goes, round after round, generation after generation. The power of darwinian evolution, and the success of life on earth, are attributable to the very large number of repetitions of this cycle that can occur. Biology on Earth has undergone trillions of rounds of amplification, mutation and selection. These events, played out on a global scale, constitute the natural history of our planet.

However, as alluded to previously, this is all too complicated if one is thinking about the prebiotic Earth. It is not so difficult to imagine how an instruction in DNA could be copied over to an RNA messenger. But it is very hard to imagine how that message could be translated into protein without the aid of a complex biochemical apparatus such as the ribosome. If one is considering a time prior to the origins of life on Earth, then a translation apparatus would not yet have been invented. It would require a great many rounds of darwinian evolution for a functional entity as complicated as a ribosome to develop.

Life Based on RNA

So how does the game get started? What is the solution to what is often referred to as 'the chicken-and-egg problem'; the egg being the genetic instructions contained within DNA and the chicken being an expression of phenotype at the level of protein function? An important insight that has taken hold in recent years stems from the observation that, like proteins, RNA can have complex function. Biological phenotype derives from the function of cellular enzymes, and these enzymes may be comprised of either protein or RNA. A discovery that revolutionized our understanding of biology, for which Thomas Cech and Sidney Altman shared the 1989 Nobel Prize in Chemistry, is that RNA can be both a carrier of genetic instructions and an agent that exhibits enzymatic function (Kruger *et al.*, 1982; Guerrier-Takada *et al.*, 1983).

Why not, therefore, have a life form that is based solely on RNA, in which RNA is at once both the instructional molecule, the genotype, and the functional molecule, the phenotype? RNA as an instructional molecule can be amplified, subject to mutational error, to produce progeny copies of variable composition. RNA as a functional molecule can be subject to a selection process, such that those individuals that are best able to solve problems imposed by the environment are chosen as the prototypes to begin the next round of amplification, mutation and selection.

RNA is a polymer made up of subunits, termed 'nucleotides'. The subunits

are of four types: adenosine (A), guanosine (G), cytosine (C) and uracil (U). It is the specific ordering of the subunits within the polymer, for example A-U-G-U-C-A-A-G-U . . . , that constitutes the genetic information. An RNA molecule can assume a well-defined structure in water, based on the particular ordering of the subunits that it contains. This structure, in turn, causes the molecule to exhibit particular functional properties.

What is the evidence that an RNA-based life form actually existed on this planet prior to the emergence of DNA and protein-based life? First, it is known that RNA can function as a genetic molecule. There are a number of viruses in existence today that utilize RNA, rather than DNA, as their genetic material. There is no known example of a free-standing RNA-based organism, which would constitute an extant RNA-based life form. All of the known RNA viruses are parasites of DNA- and protein-based organisms and thus must be considered part of our own biology. However, the existence of RNA viruses demonstrates that RNA genomes can exist. A second piece of evidence comes from the work of Leslie Orgel and colleagues, who have shown that, in a purely chemical system, an RNA molecule can be made to copy itself (von Kiedrowski, 1986; Zielinski & Orgel, 1987). The copying process is intolerant of mutations, and thus these RNA molecules do not begin to evolve. But, again, this is a demonstration of the principle that RNA can be a carrier of amplifiable genetic information. A third piece of evidence favoring the possibility of an RNA-based life form is the discovery that RNA can function as an enzyme. There are now many known examples of RNA enzymes in biology (for reviews see Cech, 1987, 1993). This establishes the fact that RNA can be a functional molecule as well as a genetic molecule, meaning that it has the wherewithal to provide the chemical basis for darwinian evolution. This is not proof, however, that such a situation actually existed.

If it really did happen, if there was a time when life on Earth was based on RNA before it gave way to DNA and proteins, then one might expect to see remnants of the prior RNA-based life form within the succeeding DNA- and protein-based life form. What, then, is the role of RNA in our present life form? It seems to be involved in just about everything, especially as concerns the most central, most highly conserved, most primitive aspects of cellular function. RNA is a messenger, carrying genetic instructions from DNA to the protein-synthesizing machinery. RNA is an integral part of the protein-synthesizing machinery itself, drawing in the protein subunits in response to genetic instructions and carrying out the process by which the subunits are joined to form mature proteins. RNA is also involved in editing and splicing various bits of genetic information, to properly arrange the genetic instructions prior to translation. RNA is even needed for the replication of DNA. When DNA is copied, the process is initiated by the

production of a partial RNA copy, which is then extended to give DNA. In summary, RNA is involved in nearly all of the informational processes of the cell.

What about function? Except for the few oddball RNA enzymes that have been discovered over the past decade, the bulk of cellular function is, after all, carried out by proteins. On the other hand, a close look at the protein enzymes reveals that over half of them, and nearly all of the protein enzymes that are involved in the most fundamental aspects of cellular metabolism, rely on 'coenzymes.' Coenzymes do more than assist proteins in carrying out biochemical reactions; they play a crucial role in the mechanism of the reaction. Remarkably, almost all of the known coenzymes contain components of RNA. They typically consist of one or two nucleotides, together with some chemical attachment. Thus, at the level of function, even protein-based function, the components of RNA are found to be intimately involved.

Finally, consider the two main differences between RNA and DNA: (1) RNA contains the sugar component ribose, while DNA contains the sugar deoxyribose; (2) one of the subunits of RNA is uridine, while the comparable subunit of DNA is methyluridine or thymidine. The way in which biological organisms generate these two different forms is to first produce the RNA version and then, as a last step, convert the RNA version to the DNA version. For example, the deoxyribose sugar of DNA is first produced as ribose, which is incorporated into nucleotide subunits and, at the last step, modified to give deoxyribose. Why not have it the other way around? Why not produce deoxyribose first and ultimately convert it to ribose? One explanation is that RNA existed prior to DNA, so that a means of producing ribose was already in place before there was a need to produce deoxyribose. Similarly, the thymidine subunit of DNA is first produced as uridine, which is ultimately converted to thymidine, again suggesting the primacy of RNA.

If we accept the notion that RNA-based life preceded DNA- and protein-based life, then it is reasonable to wonder when the RNA world first came into existence and when it gave way to DNA and protein. Both the genetic and functional properties of RNA require that it be dissolved in water. Thus, until the newly formed Earth had cooled to the point that liquid water was available, it would not have been possible for RNA-based life to exist. Attempting to set the time frame for the disappearance of the RNA world, one can search the geologic and phylogenetic record for the earliest evidence of DNA- and protein-based life. Between these two endpoints lies the window of opportunity for the existence of the RNA world (Joyce, 1991).

The Prebiotic Earth

The story begins roughly 4.6 billion years ago, generally agreed to be about the time when our solar system was formed. This number is based on isotope dating of lunar rocks returned from the Apollo missions and of fallen asteroids. Prior to 4.6 billion years ago the pre-solar system consisted of a swirling cloud of gas and dust, collapsing under its own gravitational forces. At the center of this giant cloud, the Sun began to form. At varying distances from the center, matter condensed to form planetesimals – solid objects only a few kilometers in diameter. These in turn coalesced to form planets, although the details of this process are somewhat unclear. In any case, within 100 million years, roughly 4.5 billion years before the present, the formation of the Earth was largely complete (Stevenson, 1983).

Over the next 300 million years, from 4.5 to 4.2 billion years ago, the young Earth continued to accrete material, picking up stray planetesimals and debris that lay in its orbital path. As this material impacted the Earth, its kinetic energy was transferred to the planet in the form of buried heat. As a counterbalance, the Earth underwent convective cooling, causing much of the buried heat to be radiated back to space. Eventually, however, the Earth reached a size at which convection could no longer keep pace with the amount of heat being buried, and planetary temperatures began to rise. These conditions made it impossible for liquid water to exist, which in turn made it impossible for RNA-based life to exist.

When was it first possible that there was liquid water on Earth? There are two ways of looking at this problem. On the one hand, suppose that water was present from the time of initial accretion. Then it would have been especially difficult for the planet to maintain a moderate temperature, because water vapor in the atmosphere would act to promote a 'greenhouse effect'. Water vapor, like carbon dioxide, is a greenhouse gas that reflects some of the Earth's radiated heat back to the surface. This would diminish the amount of buried heat that could be radiated to space, resulting in increased surface temperatures, which in turn would cause still more water to evaporate and enter the atmosphere. The culmination of this positive feedback loop would be a runaway greenhouse effect, causing all of the surface water to evaporate and the Earth to be covered by a global magma ocean (Kasting, 1988; Zahnle *et al.*, 1988). Thus, if water was present from the beginning, the dawn of the RNA world could not have occurred until after the Earth's crust had cooled to the point that liquid water could be present again.

On the other hand, suppose that water was not present from the beginning and instead was delivered to the planet at a later time by impacting comets (Chyba, 1987). If water did not arrive until later, then there would not have been a runaway greenhouse effect. Of course, until the water did arrive, RNA-based life would have been impossible. Thus, in either case, the RNA world could not have arisen

until some time after the Earth's orbit had been cleared of debris, perhaps 4.2 billion years ago.

However, there is another problem, first pointed out by Maher & Stevenson (1988). Even after planetary accretion was complete, meteors and asteroids continued to intersect the Earth's orbit, occasionally striking the surface. The effect of an impacting meteor or asteroid can be devastating. For example, there was a major impact event at the Cretaceous–Tertiary boundary, 65 million years ago, that is thought to have been responsible for the extinction of the dinosaurs (Alvarez *et al.*, 1980). This so-called 'K–T impactor' is believed to have been an asteroid roughly 50–75 km in diameter that produced a crater about 200–300 km across, spewing vast amounts of vaporized rock into the atmosphere (Sharpton *et al.*, 1992, 1993). This had a profound effect on global climate, and turned out to be devastating for the dinosaurs and other organisms that were unable to evolve quickly enough to compensate for the changes. The K–T impactor was a rare event that occurred recently on the geologic timescale. During the early history of the Earth, however, such events are thought to have been much more common. Asteroids the size of the K–T impactor are thought to have hit the earth about once every 50 000 years at a time 4.2 billion years before the present (Maher & Stevenson, 1988). These events became progressively less frequent over the next half-billion years. But at the time when liquid water first became available and RNA-based life might have just begun to gain a foothold, it would not have been long before a devastating impact event occurred.

What is devastating for a dinosaur may not be so devastating for a microbe or an RNA-based organism. But there also were less frequent, though truly massive, impact events that would have made the K–T event seem like a summer hailstorm. On the basis of extrapolation from the lunar impact record, and taking into account the larger cross-sectional area and greater gravitational pull of the Earth, Maher and Stevenson estimate that massive impact events were occurring with distressing frequency on the early Earth. Impacting bodies having a diameter of 250 km or greater, producing a crater at least 850 km across, were occurring roughly every million years 4.2 billion years ago. An event of this magnitude would be expected to completely sterilize the Earth. Such an event would have been devastating to RNA.

Imagine you are an RNA-based life form, just beginning to evolve into something interesting, when along comes one of these devastating events. Maher and Stevenson appropriately term this phenomenon 'impact frustration' because what could be more frustrating to life? It is important to note that the models of impact frustration must be taken qualitatively. The data concerning the cratering history of the moon are not as complete as one would like. Furthermore, the estimated interval of one million years between global sterilizing events represents an

average. There may have been intervals that were considerably longer and allowed life to evolve adaptive countermeasures to survive the next big one. Perhaps life could survive at the bottom of the ocean near the deep-sea hydrothermal vents or developed the ability to enter a protected dormant state until the environmental upheaval had subsided.

In any case, as time went on, such devastating events became progressively less frequent, so that by 4.0 billion years ago they were occurring 'only' about once every ten million years. That may have provided enough time for life to evolve an effective survival strategy. By 3.8 billion years ago, massive impacts were becoming quite rare, occurring perhaps every hundred million years. This is the time, from 4.0 to 3.8 billion years ago, that RNA-based life would have enjoyed its first reasonable opportunity for survival.

Life before RNA

Just because environmental conditions made it possible for RNA to exist does not mean that in fact an RNA-based life form was present. One must consider the three chemical components of RNA: the ribose sugar, the phosphate connector and the nucleotide base (A, G, C or U), and ask whether these components would have been available on the primitive Earth. This is a challenging problem in prebiotic chemistry, but one that has largely been solved. For example, the chemistry needed to produce ribose under plausible prebiotic conditions is reasonably well understood. The availability of phosphate, while a bit more problematic, does not seem to be an insurmountable problem. Of the four nucleotide bases, A and G are expected to have formed quite readily, while C and U would probably have been present in far lower quantities. Thus, it appears that all of the components of RNA would have been present, at least to some extent, on the prebiotic Earth.

A far more difficult problem is one of specificity: explaining why the components of RNA should be assembled to the exclusion of other closely related compounds. The chemistry that produces ribose would be expected to yield many other sugars as well. The chemistry that leads to A and G would provide a variety of related molecules. Attachment of both the phosphate and nucleotide base to ribose would be complicated by hundreds of side-reactions, making it difficult to see how RNA could stand out in the crowd.

Perhaps there were special conditions, in at least one locale on the primitive Earth, that allowed preferential synthesis of ribose over all other sugars, and of A, G, C and U over all other related compounds. For example, Albert Eschenmoser and colleagues have shown that there is a favored route to ribose, provided one begins with the appropriate set of starting materials (Müller *et al.*,

1990). But even if there was a pure solution of all the components of RNA, there remains the difficult task of properly assembling these components to form RNA. Again, one is forced to appeal to a special set of conditions that would allow all of the pieces to come together in just the right way.

There is another problem with RNA, a problem that cannot be resolved by appealing to some special set of conditions. RNA, like most biological molecules, has a handedness. The ribose sugar of RNA can exist in either a left-handed or right-handed form. All of the ribose on the prebiotic Earth would have existed as an equal mixture of the two forms. By a quirk of chemistry, it turns out that the left- and right-handed versions of ribose are excellent mimics of each other, so much so that they spoil each other's ability to replicate (Joyce *et al.*, 1984). Without replication there is no evolution, and without evolution there is no way to devise a biochemical solution to the handedness problem. Biological organisms have solved the problem by ensuring that all of the ribose (and later deoxyribose) that they utilize is of the right-handed form. Biological enzymes themselves have a handedness and are able to distinguish between the left- and right-handed version of ribose when producing RNA. But handedness is a property of life, so that exclusion of the 'wrong'-handed form of ribose would not have been possible until after life had originated.

Faced with the difficulty of assembling the components of RNA under prebiotic conditions, and especially with the problem of handedness, many scientists have come to the conclusion that life did not begin with RNA. What might have come before RNA is open to conjecture. It has been suggested that RNA was preceded by a molecule that lacked handedness, or at least did not face the problem of one hand inhibiting the other (Joyce *et al.*, 1987). It is difficult to assess the plausibility of these theories. The experimental evidence for the RNA world is already scanty and largely circumstantial; the evidence as to what came before RNA is virtually non-existent.

In summary, although it may have been possible for an RNA-based life form to exist on the Earth roughly 4.0 billion years ago, some other life form must have come before RNA. We do not know the chemical nature of that preceding life form or, in fact, whether there were several successive life forms that preceded RNA. This is at present a highly active area of research.

The Antiquity of DNA and Protein

In order to gauge the time of transition from RNA- to DNA- and protein-based life, one can look to the fossil record for the earliest evidence of DNA- and protein-based organisms. In this regard, stromatolites, which are sedimentary

rocks of biogenic origin, have provided some of the oldest evidence for life on earth. Stromatolites can be thought of as 'living rocks'. On their surface exists a complex community of microbial life, including numerous forms of bacteria and fungi. Over time, these organisms trap sediment and organic debris, eventually polluting their local environment. The organisms then tend to migrate upward through the debris to re-establish a surficial community where they can flourish again. This cycle of growth, pollution and migration is repeated many times, ultimately giving rise to stromatolites, which have a characteristic laminated appearance.

Stromatolites are remarkable not only for the complex series of biological events that leads to their formation, but also for the fact that they appear virtually unchanged throughout the geologic record over the past 3.5 billion years. Modern stromatolites, such as those being formed on the west coast of Baja California or in Shark's Bay, Western Australia, are extraordinarily similar in appearance to specimens found in South Africa that have been dated as 2.3 billion years old. This similarity applies not only to their gross morphology, but also to their fine structure, such as the tracks left by migrating organisms and the remnants of cellular debris (Walter, 1983). The continuity of life, as represented by the stromatolites, extends deep into the geologic record. The oldest known stromatolites, found in Western Australia, have been dated at 3.56 ± 0.03 billion years before the present.

Modern stromatolites are produced by DNA- and protein-based organisms. One might imagine that the very oldest stromatolites were produced by RNA-based organisms. But it would require an astonishing coincidence for the RNA version and DNA/protein version of stromatolites to be nearly identical. Stromatolites reflect the physical and behavioral properties of an entire community of organisms. Surely RNA-based life would have done things somewhat differently than DNA- and protein-based life. Thus, the most parsimonious hypothesis is that all of the known stromatolites derive from DNA- and protein-based organisms. This places the boundary for the transition from RNA to DNA and proteins at a time prior to 3.56 billion years ago.

The oldest direct fossil evidence of life on Earth comes from the work of William Schopf and colleagues (Schopf & Packer, 1987; Schopf, 1993). They have obtained microfossils, i.e. fossils of microscopic organisms, dated at 3.46 billion years before the present. These fossil organisms are very similar in appearance to more recent examples of cyanobacteria that occur throughout the geologic record. Again, the continuity of form seen in microfossils over the past 3.5 billion years argues for the continued existence of DNA- and protein-based life over that same period of time. The time frame for the RNA world seems to be constrained to the half-billion year interval between 3.5 and 4.0 billion years ago.

The geologic record extends a bit further than the time of the oldest known stromatolites and microfossils. The oldest well-characterized rocks are 3.77 billion years old, found in the Isua region of southwestern Greenland. These rocks have undergone metamorphism at extremely high temperature and as a result are not expected to contain fossil evidence of life. However, the organic carbon in these rocks is very slightly enriched in the isotope ^{12}C relative to ^{13}C , and this observation has been taken by some as indirect evidence of life (Schidlowski *et al.*, 1983; Schidlowski, 1988).

Biological organisms that fix carbon, i.e. convert carbon dioxide to sugar, do so with the help of protein enzymes. These enzymes tend to discriminate among the various isotopes of carbon, preferentially incorporating ^{12}C while excluding ^{13}C . Organic debris of biogenic origin tends to be slightly enriched in ^{12}C , as is true, for example, of the material obtained from the 3.56 billion year old stromatolites discussed above. The very slight ^{12}C enrichment of the 3.77 billion year old rocks from Greenland is a soft call at best. It has been argued that the reason that the enrichment is so slight is because intense metamorphism has allowed partial re-equilibration of the carbon isotopes (Schidlowski, 1988). In effect, this is arguing that the carbon isotope evidence for life is lacking, but is lacking in just the way one would expect if life had been present. It is fair to say that there is no substantive claim for life, let alone DNA- and protein-based life, older than 3.56 billion years.

A New Approach

There is another approach to the problem of the existence of the RNA world. If one believes that an RNA-based life form is possible, then why not make one in the laboratory? This approach is not meant to diminish the importance of scientific issues such as: Where did ribose come from? Why was ribose the preferred sugar? Where did the nucleotide bases come from? Why were particular bases chosen for RNA? How were the components of RNA joined together? How was the handedness problem resolved? When did RNA first begin to replicate? How did it survive massive impact events? How did life make the transition from RNA to DNA and protein? But a research biochemist knows how to obtain the components of RNA: they can be bought from a chemical supply house! These components are available as pure compounds having only the proper handedness. They can be assembled in the laboratory to produce RNA.

The challenge is to devise RNA molecules that have the ability to direct their own replication. Replication should be made to occur with occasional mutations, so that the progeny copies resemble, but are not identical with, their parents.

Selection would be expected to occur automatically, favoring those molecules that replicate most rapidly under the prevailing reaction conditions.

Progress is occurring along these lines. It is now possible to amplify, mutate, and select large populations of RNA molecules in the laboratory (Joyce, 1989). So far, these RNA molecules have not demonstrated the ability to replicate themselves; it is up to the experimenter to carry out RNA amplification. But RNA evolution can be made to occur, leading to the development of new and interesting RNAs whose functional properties conform to the demands of the experimenter (Beaudry & Joyce, 1992; Bartel & Szostak, 1993; Lehman & Joyce, 1993). This laboratory process cannot be called 'life' because it is not a *self-sustained* chemical system capable of undergoing darwinian evolution. It requires the active intervention of the experimenter. However, it is probably only a matter of time, to be measured in years rather than decades, before a self-sustained RNA-based evolving system can be demonstrated in the laboratory. This would be a case in which a DNA- and protein-based life form, namely a human biochemist, gives rise to an RNA-based life form, an interesting reversal of the sequence of events that occurred during the early history of life on Earth.

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